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Starting from 1984, the name of the journal was changed to "Acta Pharmaceutica Turcica" and became a journal for national and international manuscripts, in all fields of the pharmaceutical sciences in both English and Turkish. (1984-1995, edited by Prof. Dr. Kasım Cemal GÜVEN, 1995-2001, edited by Prof. Dr. Erden GÜLER, 2002-2011, edited by Prof. Dr. Kasım Cemal GÜVEN)

Since 2006, the journal has been publishing only in English with the name, "Acta Pharmaceutica Sciencia" that represents internationally excepted high level scientific standards.

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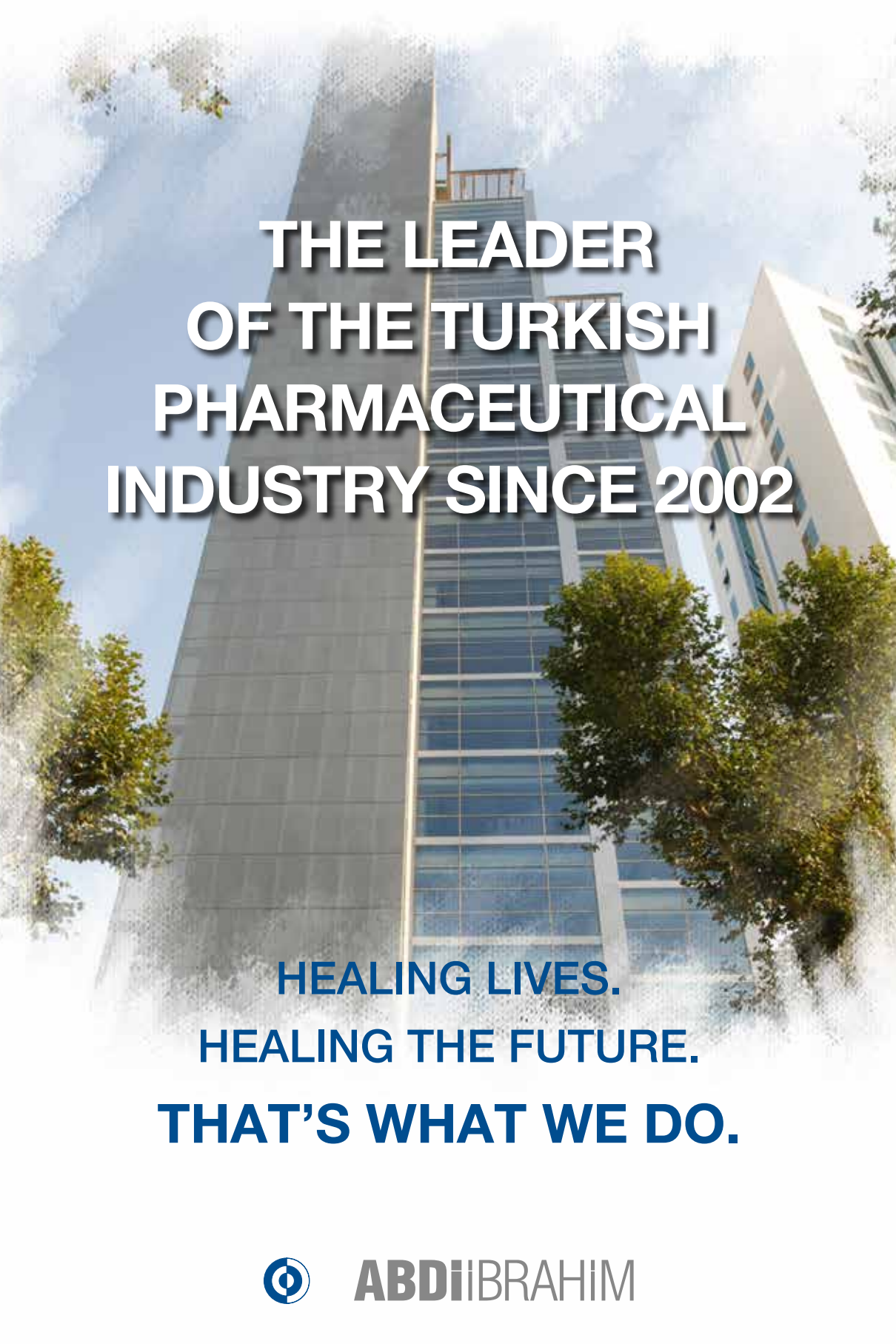
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Anti-inflammatory and Hypoglycemic Activities of Alpha-pinene

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ABSTRACT

The aim of this study is to investigate the anti-inflammatory and hypoglycemic activities of alpha-pinene, and to find the median lethal dose (LD_{50}) level in mice.

Lethal dose levels of alpha-pinene were investigated using a probit analysis method. For the anti-inflammatory activity measurement seven different groups were established and alpha-pinene was administered in four different doses: 0.05, 0.10, 0.25 and 0.50 mL/kg. For the evaluation of hypoglycemic activity six different groups, consisting of diabetic and healthy mice, were established.

The strongest anti-inflammatory activity of alpha-pinene was observed with a 0.50 mL/kg dosage. The median effective dose (ED_{50}) value of alpha-pinene was found to be 0.039 mL/kg. In diabetic mice α-pinene showed significant levels of hypoglycemic activity at the 2nd and 24th hours. LD_{50} level of α-pinene was determined to be 2.076 mL/kg.

As a result, we conclude that alpha-pinene is a molecule that displays hypoglycemic and anti-inflammatory activities.

Keywords: Alpha-pinene, anti-inflammatory activity, hypoglycemic activity, mice, rats.

INTRODUCTION

Foeniculum vulgare Miller, (family Umbelliferae) is an annual, biennial or perennial aromatic herb, depending on the variety, which has been known since antiquity in Europe and Asia Minor. The leaves, stalks and seeds (fruits) of the plant are edible[1]. Extracts of *Foeniculum vulgare* Miller (fennel) seeds are used as an anti-inflammatory agent in Turkish traditional medicine[2]. The anti-inflammatory, hypoglycemic and hepatoprotective effects of fennel were dem-

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onstrated in previous studies [3-7]. The volatile components of fennel seed extracts are *trans*-anethole, fenchone, methylchavicol, limonene, alpha-pinene, camphene, β-pinene, β-myrcene, α-phellandrene, 3-carene, camphor, and cisanethole [8]. The major components of the fennel seed essential oil are alpha-pinene, limonene, fenchone, methychavicol and *trans*-anethole[6].

In our previous work, we demonstrated the anti-inflammatory and hypoglycemic activities of volatile oil extract of fennel[3-7]. In order to determine the fennel component(s) responsible for these activities and to determine its lethal dose levels, this study investigates alpha-pinene, a major component of volatile oil extract of fennel.

METHODOLOGY

Animals

Sprague-Dawley rats and Mus musculus Swiss albino mice were maintained in the animal house. The animals were housed in standard cages with pelleted food and water *ad libitum*, at room temperature (22±2 °C) with a 12h light-dark cycle. Ethical approval was obtained from the Animal Ethics Committee.

Chemicals

(1R)-(+)-α-pinene ($C_{10}H_{16}$), lambda-carrageenan Type IV, indomethacin and alloxan were obtained from Sigma (Steinheim, Germany), and glibenclamide was obtained from Nobel (Istanbul, Turkey).

Acute toxicity

Swiss albino mice were randomly assigned to nine groups with six animals in each group. The control group was treated with isotonic saline solution (ISS) (0.9% NaCl), and the other eight groups were treated with alpha-pinene given intraperitoneally (ip) by Hamilton and insulin injectors in increasing dosages of 0.05, 0.10, 0.20, 0.40, 0.80, 1.60, 2.40 and 3.20 mL/kg body weight. The mortality in each cage was assessed 72 h after administration of alpha-pinene. The percentage mortalities were converted to probits. Regression lines were fitted by the method of least squares and confidence limits for the LD_1, LD_{10}, LD_{50}, LD_{90} and LD_{99} values were calculated by the method of Litchfield & Wilcoxon[9] and Kouadio et al[10].

Anti-inflammatory activity

The method of Winter et al was used with slight modification[11]. Forty-two rats were divided into seven groups of six animals each. The rats were starved for 12 h and deprived of water only during the experiment. Water deprivation was em-

ployed to ensure uniform hydration and to minimize variability in edematous response. Inflammation of the hind paw was induced by injecting 0.05 mL of fresh lambda carrageenan (phlogistic agent) into the subplantar surface of the right hind paw. The control group-I was given ISS (0.1 mL) and the control group-II was given ethyl alcohol (0.1 mL). The third group (reference group) received the anti-inflammatory agent indomethacin (3 mg/kg, ip), while the remaining four groups received alpha-pinene at doses of 0.05, 0.10, 0.25 and 0.50 mL/kg, i.p by Hamilton injector[12]. The doses utilized in the current study were chosen according to LD_1 value (LD_1 = 0.744 mL/kg).

Foot volume was measured by a displacement technique using a plethysmometer (Ugo Basile 7140 plethysmometer, Italy), immediately before and three hours after the 0.05 mL of fresh lambda carrageenan injection. The percentage inhibition of the inflammatory reaction was determined for each animal by comparison with controls and calculated by the formula[10]:

$$I\ \% = [(1-(dt/dc)] \times 100$$

where *dt* is the difference in paw volume in the drug-treated group and *dc* the difference in paw volume in the control group.

Preparation of alloxan diabetic mice

Diabetes was induced by i.p. injections of 150 mg/kg alloxan monohydrate prepared in ISS three times with 48 h intervals. Before injections were given mice were starved for 18 h[13]. Seven days after the last injection, fasting blood glucose levels were measured and mice with fasting blood glucose levels of 200 mg/dL and over were taken into the study[14].

Hypoglycemic activity in normal and diabetic mice

Diabetic animals were randomly divided into three groups of six animals each. Group I mice received 0.1 mL ISS i.p. The animals in group II were treated orally with 3.0 mg/kg glibenclamide, a hypoglycemic agent, used as reference. Group III received i.p. injection of 0.25 mL/kg alpha-pinene by Hamilton injector. The same protocol described above was applied in three groups of normal mice. Fasting blood glucose levels were measured after 18 h of fasting just before the treatment and 1, 2, 4 and 24 h after the treatment using the glucose oxidase peroxidase method (Abbott, United Kingdom).

Statistical analysis

Results were reported as mean ± standard error of mean (SEM). The total variation was analyzed by performing a one-way analysis of variance (ANOVA). Least Significant Difference (LSD) test, Dunnet test and Tukey's HSD (Honestly

Significant Difference) test were used for determining significance. Probability levels of less than 0.05 were considered significant. The medium effective dose (ED_{50}) value was calculated by non-linear regression analysis (SigmaPlot 2004 for Windows Version 9.01).

RESULTS AND DISCUSSION

Acute toxicity

The lethal doses of alpha-pinene are presented in Table 1. The intraperitoneal medium lethal dose (LD_{50}) value for the total number of animals was found to be 2.076 mL/kg.

Table 1. Lethal doses of (1R)-(+)-αpinene ($C_{10}H_{16}$).

Lethal doses	Dose (mL/kg)	95% confidence limits	
		Lower (mL/kg)	Upper (mL/kg)
LD_1	0.744	0.012	1.242
LD_{10}	1.179	0.111	1.663
LD_{50}	2.076	1.246	3.180
LD_{90}	3.655	2.630	32.295
LD_{99}	4.497	3.023	86.301

Anti-inflammatory activity

Table 2 shows the anti-inflammatory effects of intraperitoneally administered alpha-pinene on carrageenan induced paw edema in rats. Alpha-pinene showed significant anti-inflammatory effect in two doses studied (0.025 mL/kg and 0.50 mL/kg); peak response was obtained with 0.50 mL/kg alpha-pinene (60.33% decrease in inflammation) and 0.05 mL/kg alpha-pinene caused a lesser degree of inhibition of the inflammation (18.97%). Compared to the controls, the greatest anti-inflammatory activity was observed in the indomethacin group, with a 87.44% regression of the inflammation. Compared to indomethacin group, alpha-pinene group had significantly lower anti-inflammatory effects at all doses. At 0.05 mL/kg dose the alpha-pinene group showed significantly lower anti-inflammatory activity compared to the 0.50 mL/kg dose. The medium effective dose (ED_{50}) value of alpha-pinene was found to be 0.039 mL/kg.

Table 2. Effects of alpha-pinene on rat paw edema.

Groups	Dose	Paw edema (mL %)	Inhibition (%)
Control-I (ISS)	0.1 mL	1.043 ± 0.127	-
Control-II (ethyl alcohol)	0.1 mL	0.988 ± 0.112	-
Indomethacin	3 mg/kg	[ab] 0.024 ± 0.061	87.44
Alpha-pinene	0.05 mL/kg	[c] 0.845 ± 0.109	18.97
Alpha-pinene	0.10 mL/kg	[c] 0.672 ± 0.051	35.57
Alpha-pinene	0.25 mL/kg	[abc] 0.617 ± 0.073	40.83
Alpha-pinene	0.50 mL/kg	[abcd] 0.413 ± 0.069	60.34
F value		*17.750*	
p value		*0.000*	

Data is presented as mean ± standard error of the mean (n=6).
ED_{50}: 0.039 mL/kg.
Post-hoc Tukey's HSD and Dunnet tests:
a : $p<0.05$ compared to control-I (ISS) group,
b : $p<0.05$ compared to control-II (ethyl alcohol) group,
c : $p<0.05$ compared to indomethacin group,
d : $p<0.05$ compared to alpha-pinene 0.05 mL/kg.

Hypoglycemic activity

The fasting blood glucose levels of the alloxan diabetic mice are presented in Table 3. Table 4 demonstrate the levels of fasting blood glucose in normal mice. It was determined that alpha-pinene significantly decreased fasting blood glucose levels at the 2nd and 24th hours. It was observed that alpha-pinene significantly increased fasting blood glucose levels in healthy mice at the 1st and 2nd hours.

Table 3. Effects of alpha-pinene on fasting blood glucose levels in diabetic mice.

Groups	Fasting blood glucose (mg/dL)				
	Before treatment	**1st hour**	**2nd hour**	**4th hour**	**24th hour**
Control (ISS)	337.2±23.4	318.4±25.3	308.0±34.2	225.0±34.4	205.4±19.3
Glibenclamide	267.3±37.7	197.8±47.3	[a] 150.5±39.7	[a] 101.8±10.6	[a] 90.1±15.4
Alpha-pinene	290.4±17.4	306.1±37.0	[a] 196.1±26.3	[b] 170.7±25.5	[a] 137.6±31.4
F values	*1.539*	*2.827*	*5.291*	*5.786*	*6.865*
p values	*0.247*	*0.091*	*0.018*	*0.014*	*0.009*

Data is represented as mean ± standart error of the mean.
Post-hoc LSD test:
a: p<0.05 compared to ISS group.
b: p<0.05 compared to glibenclamide group.

Table 4. Effects of alpha-pinene on fasting blood glucose levels in healthy mice.

Groups	Fasting blood glucose (mg/dL)				
	Before treatment	**1st hour**	**2nd hour**	**4th hour**	**24th hour**
Control (ISS)	91.50±12.8	72.75±7.2	60.50±4.1	61.25±4.1	54.50±3.0
Glibenclamide	[a] 68.75±01.3	59.25±4.8	59.00±3.6	53.25±2.9	49.75±2.0
Alpha-pinene	[b] 96.0±03.8	[ab] 102.3±10.8	[ab] 79.6±4.2	[b] 76.6±7.3	73.5±11.7
F values	*4.274*	*6.019*	*8.155*	*4.059*	*2.060*
P values	*0.042*	*0.017*	*0.007*	*0.048*	*0.174*

Data is represented as mean ± standart error of the mean.

Post-hoc LSD test:

a: $p<0.05$ compared to ISS group.

b: $p<0.05$ compared to glibenclamide group.

In this work, the LD_{50} dose of alpha-pinene, a major component of the essential oil of *Foeniculum vulgare* Mill. was determined to be 2.076 mL/kg.

The current study clearly demonstrated the in vivo anti-inflammatory effect of alpha-pinene at doses of 0.25 mL/kg and 0.50 mL/kg. The ED_{50} dose of alpha-pinene related to its anti-inflammatory activity was found to be 0.039 mL/kg for its.

It has been reported that *Bupleurum fruticescens*, Salvia species and Helichyrisum species essential oils had anti-inflammtory effects. The anti-inflammatory activity shown by the essential oil can be attributed to the two major components-alpha-pinene and beta-caryophyllene[15-17]. The results of these studies are consistent with those of the current study. Zhou et al. reported that alpha-pinene inhibits the nuclear translocation of NF-kappa B induced by lipopolysaccharide (LPS) in THP-1 cells[18]. The transcription factor NF-kappa B plays a pivotal role in the activation of multiple inflammatory molecules[19]. Kim et al claimed that alpha-pinene exhibits anti-inflammatory activity through the suppression of mitogen-activated protein kinases (MAPKs) and the nuclear factor-kappa B (NF-κB) pathway in mouse peritoneal macrophages[20]. It can be suggested that alpha-pinene shows its anti-inflammatory effect through the NF-kappa B. Rufino et al. reported that at noncytotoxic concentrations, (+)-α-pinene elicited the most potent inhibition of the IL-1β-induced inflammatory and catabolic pathways, namely, NF-κB and JNK activation and the expression of the inflammatory (iNOS) and catabolic (MMP-1 and -13) genes in human chondrocytes[21].

It is found that alpha-pinene increased fasting blood glucose levels in healthy mice significantly at the 1st and 2nd hours. However, since these values are within normal limits the increments in fasting blood glucose levels induced by alpha-pinene, these observed values are considered to have no clinical significance. In addition, the results show that alpha-pinene caused mild hypoglycemic activity in the diabetic mice at the 2nd and 24th hours investigating. There is no study of the hypoglycemic activity of alpha-pinene in the literature. For this reason, it is not possible to comment about the mechanism of its the hypoglycemic activity. Further investigations must be conducted to reveal the mechanisms of the anti-inflammatory and hypoglycemic activities of alpha-pinene.

In conclusion, the current study shows that alpha-pinene had anti-inflammatory and hypoglycemic activities in vivo.

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On the Relationship Between Serum Apelin Levels and Some Parameters Related to Oxidative Stress and Energy Metabolism in Obese and Non-Obese

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ABSTRACT

Adipose tissue plays an important role in energy balance by secretion of various adipokines. Obesity is excessive fat accumulation in the body. The aim of this study is to examine the association of apelin, an adipokine secreted from fat tissue, with antioxidant system, glucose and lipid parameters in obese and control cases. After approval has been taken from the Ethics Committee, 61 obese and 24 control people were included in the study. The ages in the study group ranges between 18 and 75. The body mass index (BMI) is >24.9 in obese people, and it ranges between 18.5 - 24.9 in control cases. Apelin was calculated using the ELISA method, total oxidant and total antioxidant levels were calculated using the colorimetric method, fasting blood glucose, insulin, TG, LDL and HDL levels were calculated using the photometric method, insulin resistance was calculated using the HOMA-IR method. Serum apelin levels were not found to differ between obese and control groups ($p>0.05$). Total antioxidant level (TAS) decreased in the obese group ($p<0.001$) while total oxidant level (TOS) remained the same. The Oxidative Stres Index (OSI) increased ($p<0.001$) in the obese group. In the obese group, glucose and insulin resistance were high ($p<0.05$) while serum insulin levels were the same as in the control group ($p>0.05$). In our study group, lipid parameters did not exhibit any difference in the obese and control groups. There was a significant relationship at $p<0.05$ level between triglyceride values in the control and obese case groups, while there were no significant differences among other lipid parameters.

As a result; we think that further studies are needed to understand the effect of apelin, which is released from fat tissue and is active in energy metabolism.

Keywords: Obesity, apelin, oxidative stress, adipose tissue

INTRODUCTION

Studies show that health expenditures for obesity are higher than health expenditures for smoking.[1,2] The pathophysiology of obesity is related to a large number of factors secreted from enlarged fat cells. Increased release of free fatty acid increases fatty acid intake into liver and peripheral tissues. Insulin degradation in the liver is diminished and the level of insulin in the circulation increases.

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Storage of fatty acids in the liver plays a role in the development of insulin resistance. In this way, other organs start to be affected by the change in insulin metabolism and undesirable metabolic changes occur.[3,4] For many years, adipose tissue was known as an inert energy store, where triglycerides were stored and, when needed, released fatty acids into the blood. However, especially since the last 20 years, it has been shown that fat tissue works as an active endocrine organ, is metabolically important and that the cytokines (adipokines) released in here are signal transmitting molecules.[1,2,5]

Adipokines released from adipose tissue circulate like hormones and perform various functions in tissues of the immune system, brain, liver and adipose tissue. Pathologies such as obesity, Type 2 diabetes, inflammation and cardiovascular diseases are seen in dysregulation of adipokines.[6] In recent years, it is known that in the relationship between inflammation and Type 2 diabetes and insulin resistance, adipose tissue has an effective function. Various adipokines, such as leptin and apelin, released from adipose tissue as well as from other tissues, can be activated by complex mechanisms in fasting regulation and energy balance. Insulin resistance refers to impaired biologic response to insulin that is either externally applied or internally secreted. Insulin sensitivity is influenced by many factors such as age, weight, especially abdominal body fat, physical activity and drug intake. Fat distribution for cardiovascular diseases is a major risk factor. With a competitive inhibition, free fatty acid (FFA) prevents glucose from entering into the cell. FFA slowing of peripheral glucose utilization causes insulin resistance and hyperinsulinemia.[2,3,7-9]

Apelin is synthesized in adipose and other tissues in humans and rats and there are apelin receptors in various tissues.[10] Apelin, a cytokine that joined the adipokine family in 1998, is also considered as a hormone. It has isoforms in various organs. It also exists in the plasma. It is known to have endocrine and neurotransmitter effects Apelin expression in fat cells is suppressed by starvation and has similar effects on insulin following feeding.[11,12] It has been reported that apelin gene expression in fat tissue is stimulated by insulin and TNF-α Apelin is both synthesized and secreted in fat tissue.[13] Experimental animal studies have shown increased skeletal glucose use and reduced plasma glucose levels after apelin injection. Increased apelin with hyperinsulinemia was observed in obese subjects.[14-16]

There are studies reporting that the antioxidant capacity is impaired in obesity.[17,18] Sudden or excessive oxygen entrance into the organism due to various metabolic reasons, antioxidant defense system insufficiency or oxidant-antioxidant balance to gravitate towards the oxidant side, which then leads to occurrence of oxidative stress. This situation could also be caused by formation of free radicals or insufficient antioxidant activities, either of which indicates increase of free fatty acids among active molecules.[19-22]

The purpose of this study is to examine apelin, which is defined as an important adipokine, considered as a significant health problem that has gradually been more frequently encountered in the world, in terms of diabetes, insulin resistance and dyslipidaemia and portray how these parameters work with total oxidant/antioxidant status.

MATERIALS AND METHODS

This study was supported by İstanbul Medipol University Scientific Research Projects by the project no: 86770134-604/101.

Study Design and Data Collection

Included in the study were 24 control groups made up of 13 male and 11 female persons and 61 patient groups made up of 37 male and 24 female persons, all of which applied to the Medipol University's Mega Hospital Laboratory between September-October 2015. The people with $18.5 \leq BMI \leq 24.9$; kg/m^2 were considered normal weight and the people with $BMI > 30$ kg/m^2 were considered obese, and these persons were accordingly split into two groups (Table 1).

Table 1. The demographic measurement values of control and obese group.

	Control Group (Mean±SD)	Obese Group (Mean±SD)
Height(m)	1,70±0,05	1,70±0,07
Age (year)	37,6±11,22	48,74±12,5
Body weight (kg)	69,5±6,98	96,88±16,6
BMI (kg/m^2)	23,52±0,89	33,76±6,15

BMI=Body Mass Index

Study Exclusion Criteria

Exclusion criteria were; being younger than 18 years and older than 75 years, smoking hypertension, cardiac diseases, osteoarthrosis, cancer, polycystic ovary disease and inflammatory and infectious diseases. The study started after the approval of Medipol University Ethics Board. All the subjects were informed about the study and their approved consent forms were received.

Blood Collection and Storage

After 8 hours of fasting, the blood drawn into yellow capped flat tubes was centrifuged at 2400 rpm for 10 minutes in Medipol University Biochemistry Laboratory to separate serum. The separated serum was moved into eppendorf tubes and then stored there at -80 °C until the date of the study.

Methods Used

Apelin was calculated using the ELISA method, total oxidant and total antioxidant levels were calculated using the colorimetric method, glucose, insulin, TG, LDL, HDL levels were calculated using the photometric method, insulin resistance was calculated using the HOMA-IR method and oxidative stress index was calculated using the formula given below: OSI= [(TOS, μmol H_2O_2 equivalent/l) / (TAS, μmol Trolox equivalent/l)] x100.

Statistical Analysis

SPSS software was used in the study and variables were defined through standard deviation. Mann-Whitey U test was used in comparing averages which do not exhibit a normal distribution and Student t test was used in comparing averages which exhibit a normal distribution. One-Way ANOVA was used to compare averages of more than two groups and to interpret the differences between the subgroups in variables exhibiting differences. *P*-value <0.05 were accepted as significant.

RESULTS

The study findings are summarized in table 2.

Table 2. Laboratory findings of control and obese group.

	Control group (Mean±SD)	Obese group (Mean±SD)	*P**
Apelin (pg/mL)	916,29±139,07	902±132	0,676
TAS (μmolTroloxEquiv./L)	0,92±0,11	0,81±0,13	<0,05
TOS (μmol H_2O_2 Equiv./L)	26,96±5,49	28,26±6,31	0,386
OSI(AU)	2,92±0,62	3,56±0,86	<0,05
FBG(mg/dL)	103,44±15,87	151,96±69,05	<0,05
Insulin(μU/ml)	10,83±3,86	17,21±20,4	0,072
Insulin Resistance	2,76±1,00	6,40±7,46	0,05
HDL(mg/dL)	53,22±17,82	47,65±13,52	0,149
LDL(mg/dL)	119,35±34,45	121,98±37,88	0,768
TG(mg/dL)	126,14±82,92	158,13±85,36	0,033
TC(mg/dL)	195,46±41,36	198,35±40,99	0,771

*P-value <0.05 for t test. Abbreviations: TAS, total antioxidant response; TOS, total oxidant status; OSI, oxidative stress index value; AU, Arbitrary unit; FBG; fasting blood glucose; TC, total cholesterol

There was no significant difference between apelin values in the control and obese case groups. When apelin's relation to other parameters was studied; no significant correlation was observed between the control and obese group in terms of any parameters.

TAS values were significantly lower in the obese group than in the control group ($p<0.05$). When TAS relation to other parameters was studied; TAS and BMI ($r: -0.45$, $p < 0.05$) and TAS and OSI ($r: -0.47$, $p < 0.05$) showed weak negative correlations in all cases.

There was no significant difference between total oxidant values in the control and obese case groups. When the relationship of TOS with other parameters were studied, a positive relation was found between TOS and OSI ($r: 0.77$, $p< 0.05$) TOS and TG ($r: 0.52$, $p < 0.05$) TOS and insulin ($r: 0.34$, $p<0.05$), TOS and HbA_1c ($r: 0.23$, $p< 0.05$), a negative correlation was observed between TOS values and HDL ($r: -0.34$, $p < 0.05$).

OSI values were significantly higher in the obese group compared to the control group ($p<0.05$). When the relationship of OSI with other parameters were studied, a positive relation was found between OSI and TG ($r: 0.33$, $p< 0.05$) OSI and insuline ($r: 0.37$, $p< 0.05$).

FBG values of the obese group are higher than that of the control group ($p<0.05$). No significant difference was found between insulin values of the control and obese case groups but insulin resistance was seen in the obese group ($p< 0.05$).

When the relationship of FBG with other parameters were studied, a positive relation was found between FBG and TG ($r: 0.31$, $p < 0.05$) FBG and HbA_1c ($r: 0.75$, $p< 0.05$) a negative correlation was observed between FBGvalues and HDL ($r: -0.23$, $p< 0.05$).

TG values were significantly higher in the obese group compared to the control group ($p<0.05$) while there were no significant differences among other lipid parameters and a positive correlation was observed between TG values and HbA_1c ($r: 0.29$, $p< 0.05$).

DISCUSSION

Obesity is the result of the accumulation of triglycerides in adiposites that is, the intake of excess calories from the energy used. Fatty tissue is active in insulin sensitivity and energy balance by releasing various adipokines as an endocrine organ. Obesity-related pathologies are seen because excess fat tissue, also referred to as adipose tissue, causes modifications in adipokines. Dyslipidemia seen in obesity is one of the issues that are emphasized nowadays because it

leads to cardiovascular diseases and atherosclerosis. Having the waist circumference and waist / hip ratio above the reference values causes the android obesity, which leads to impaired adipokine balance. Obesity is examined in every direction, considering the future of the world and our country.[23,24]

Apelin is synthesized and secreted in some tissues as well as in fat tissue. Apelin upregulation has been observed in obese and hyperinsulinemic humans and mice. Despite these facts, however, there are still many unknowns. Do we have opelin in all body cells? Or can it be effective in regulation only in certain organs? This is one of the unknowns of apelin.[25,26,27]

Based on this information, we measured the apelin levels in the obese people we included in our study group. We also measured FBG, insulin level, insulin resistance, TG, TC, HDL-C and LDL-C levels in order to make evaluations in terms of carbohydrate metabolism and lipid metabolism. We measured TAS, TOS and OSI levels to understand the antioxidant and oxidant levels of the cases we included in the study group.

In 61 obese cases and 24 control groups included in our study group, apelin values did not show a significant difference despite FBG and insulin resistance were high.

The most characteristic feature of obesity and Type 2 diabetes, which develops with obesity, is that the insulin resistance develops and the insulin resistance increases parallel to the body mass index. Adipokines, such as apelin released from fat tissue, are effective in energy balance and glucose metabolism, attracting attention to increased adipokines with fat mass.

Cavallo et al. measured serum Apelin levels in 119 Type 2 diabetes, 113 Type 1 diabetes and 137 non-diabetic groups by Elisa method and found apelin levels higher in Type 2 diabetic patients rather than in Type 1 diabetic patients.[14] Researchers found a significant decrease in serum apelin levels after bariatric surgery in obese subjects with Type 2 diabetes in the same study group. The results from researchers' work indicate that the relationship between Type 2 diabetes and apelin is related to glucose balance, but not to obesity and other metabolic abnormalities. The researchers found no association between HOMA-IR and apelin-12 amount in the serum. In our study, apelin levels were not changed. Cavallo et al. reported that apelin levels were increased in relation to glucose level, not obesity.[14] This is consistent with our study.

Guo et al. reported that apelin activates P13-kinase phosphodiesterase leading to decreased apelin in pancreatic β cells, while Erdem at al. reported that serum apelin levels in newly diagnosed diabetes patients decreased.[15,27] Similarly, there was a decrease in serum apelin levels in Type 2 diabetes in Chinese population.[28]

On the other hand, children with Type 1 diabetes had high serum apelin levels.[29]

Yue et al. reported that apelin is a necessary adipokine in insulin sensitivity, but studies on the effect of apelin on impaired glucose metabolism are contradictory.[30] Boucher et al. found that apelin levels were high in hyperinsulinemic mice, whereas apelin levels were normal in persons with normal insulin levels.[31] In our study, the insulin values in the obese group were within the reference ranges and there was no change in apelin levels. This is consistent with Boucher et al. findings.[31]

The regulation of energy metabolism is a complex mechanism. The imbalances in this mechanism cause diseases that obesity causes. Apelin is one of the peptides that regulate energy metabolism. Heinnonen et al. reported that apelin levels correlated positively with BMI.[12] In our study, BMI was significantly different in normal and control groups, but there was no correlation between apelin levels and BMI index.

Dray et al. have shown that apelin injection in rats has a strong glucose-lowering effect and that it increases glucose utilization in skeletal muscle and adipose tissue.[16] In summary, researchers have found that apelin improves glucose tolerance and increases glucose utilization in obese and insulin resistant rats. With this feature apelin appears to be a promising adipokine in the correction of insulin resistance.

In the Söylemez et al. study, 87 individiduals selected among applications to cardiology polyclinic were separated as normal weight (group 1, n=29) based on BMI between 19-25 kg/m^2, overweight (group 2, n=29) based on BMI between 25-30 kg/m^2 and obese (group 3, n=29) based on BMI over 30kg/m^2.[32] Plasma leptin levels correlated with TAS, TOS and OSI, but no relation was found between adiponectin levels.

Bircan et al. showed that apelin-13 applied after renal ischemia/reperfusion increased the antioxidant enzyme activity in a dose dependent manner, prevented the lipid oxidation and improved the renal functions.[33] Based on these results, researchers thought that apelin could decrease oxidative stres.

In our study, the TAS values of the obese group were low and the OSI values were found to be higher than those of the control group, but there was no significant correlation between apelin values and TAS, TOS and OSI values.

In conclusion, we think that further studies are needed to understand the effect of apelin, which is released from fat tissue and is active in energy metabolism.

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ABDİİBRAHİM

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UMCA SOLUSYON BİLEŞİMİ: Her 100 g çözelti etkin madde olarak 80 g Pelargonium sidoides kökü sıvı ekstresi, çözücü ve koruyucu olarak etanol ve gliserol içermektedir. TIBBİ ÖZELLİKLERİ: Umca Pelargonium sidoides'in kökünden elde edilen bir öz içermektedir. Pelargonium sidoides'ten elde edilen özün bronşit, sinüzit, anjin (boğaz ağrısı), viral enfeksiyonlara bağlı burun akıntısı ve farenjit olgularında etkili olduğu saptanmıştır. Aynı zamanda bağışıklık sistemini güçlendirici antiviral özelliklere sahiptir. Ayrıca bazı bakterilere karşı antibakteriyel etkisinin yanısıra antioksidan özelliklerine sahiptir. Bunun dışında, organizmanın bağışıklık sistemini güçlendirdiği ve solunum yolu mukozasındaki titrek tüylerin vurum sıklığını artırarak balgam söktürücü etkiye sahip olduğu da bildirilmiştir. Bu nedenle Umca, değişik akut ve kronik enfeksiyonların özellikle de üst solunum yolları enfeksiyonları ve kulak-burun-boğaz enfeksiyonlarının tedavisi için uygundur. Umca uygulaması ile öksürük, ateş, boğaz ağrısı, halsizlik-yorgunluk gibi yakınmalarda hızlı bir iyileşme sağlanabilmektedir. ÖNERİLEN KULLANIM YERİ: Umca, akut ve kronik enfeksiyonlar, özellikle de solunum yolları enfeksiyonları (örneğin soğuk algınlığı ve bronşit gibi) ve kulak-burun-boğaz enfeksiyonları (örneğin sinüzit, anjin, rinofarenjit gibi) tedavisine yardımcıdır. Umca öksürük, ateş, boğaz ağrısı, halsizlik-yorgunluk gibi yakınmaların tedavisine yardımcıdır. KULLANILMAMASI GEREKEN DURUMLAR: Umca'nın içinde bulunan maddelerden herhangi birine karşı aşırı hassasiyeti olanlarda kullanılmamalıdır. Kanama eğiliminin yüksek olduğu hastalarda veya kan pıhtılaşması önleyen ilaçlar alanlarda kullanılmamalıdır. Yeterli klinik deneyim olmadığı için, ağır karaciğer ve böbrek rahatsızlıkları olanlarda kullanılmamalıdır. UYARILAR/ÖNLEMLER: Umca 1 yaşın altındaki bebeklerde önerilmemektedir. Gebeler veya emziren anneler tarafından kullanımı önerilmemektedir. Etanol içermesi nedeniyle araç ve makine kullanımında dikkatli olunmalıdır. YAN ETKİLER/ADVERS ETKİLER: Enfeksiyon durumlarında örneğin akut bronşitte karın ağrısı, mide yanması, bulantı ve ishal gibi yakınmalar görülebilir. Nadiren, bu yakınmalar Umca kullanımına bağlı olabilir. Nadir vakalarda, hafif diş eti veya burun kanaması görülebilir. Umca'nın içinde bulunan maddelere karşı aşırı hassasiyeti olanlarda çok nadiren aşırı duyarlılık reaksiyonlar gelişebilir. Bu tür reaksiyonlarda yüzde ödem (şişlik), nefes darlığı ve kan basıncında düşüş görülebilir ve ürünün ilk alımından sonra gelişebilir. Böyle bir durumda derhal doktora başvurulmalıdır. İstenmeyen bir etki görüldüğü zaman Sağlık Bakanlığı Türkiye Farmakovijilans Merkezi (TÜFAM)'ne bildiriniz. İLAÇ ETKİLEŞİMLERİ VE DİĞER ETKİLEŞİMLER: Kumarin türevleri ile birlikte kullanılması durumunda, kan pıhtılaşmasını engelleyici etkide bir artış meydana gelebilir. Bu nedenle koagülasyonu inhibe eden ilaçlar ile birlikte kullanılmamalıdır. GÜNLÜK KULLANIM ŞEKLİ VE DOZU: Yetişkinler ve 12 yaş üzeri çocuklarda günde 3 defa 30 damla, 6-12 yaş arası çocuklarda günde 3 defa 20 damla, ve 1-5 yaş arası çocuklarda günde 3 defa 10 damla şeklinde kullanılır. Damlalar, yemeklerden 30 dakika önce bir miktar sıvı ile birlikte içilmelidir. Hastalığın nüksetmemesi için, hastalığın belirtileri hafiflemesini takiben ilacın kullanımına birkaç gün daha devam edilmesi önerilir. Umca şişesi açıldıktan sonra oda ısında muhafaza edildiği takdirde 6 ay boyunca kullanılabilir. TİCARİ TAKDIM ŞEKLİ: 20 ve 50 ml'lik kendinden damlalıklı cam şişelerde. İZİN SAHİBİ: Abdi İbrahim İlaç San. ve Tic. A.Ş. Reşitpaşa Mah. Eski Büyükdere Cad. No:4 34467 Maslak / Sarıyer / İSTANBUL İZİN TARİH VE NUMARASI: 11.03.2008-2008/10 ÜRETİM YERİ & LİSANS SAHİBİ: Dr. Willmar Schwabe GmbH & Co. KG Willmar-Schwabe Straße 4, 76227, Karlsruhe / ALMANYA PAREKENDE SATIŞ FİYATI: 20 mL solusyon 35 TL, 50 mL solusyon 59 TL "25°C'nin altındaki oda sıcaklığında saklayınız." "Çocukların göremeyeceği, erişemeyeceği yerlerde ve ambalajında saklayınız." BU ÜRÜNÜN TİBBİ YARARI GELENEKSEL KULLANIMA VE LİTERATÜRE DAYANMAKTADIR. TIBBİ MÜSTAHZAR (İLAÇ) OLARAK DEĞERLENDİRİLMEMİŞTİR. SADECE ECZANELERDE SATILIR.

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Formulation of Microemulsions for Dermal Delivery of Cephalexin

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ABSTRACT

Introduction: Cephalexin monohydrate (CEM) is mostly used because of its activity against both the gram-positive and gram-negative microorganisms for infections. Microemulsions offer numerous advantages for dermal delivery of drugs.
Objective: The objective of the present study was to prepare novel CEM loaded microemulsions and to characterize formulations, to evaluate their *in vitro* release profiles and antibacterial activities.

Method: CEM loaded formulations [0.02% (w/w)] were characterized according to their droplet size, zeta potential, PDI, pH, electrical conductivity and viscosity. In addition, in vitro drug release studies and antibacterial activity tests were performed.

Results: The developed CEM loaded microemulsions (M1 and M2) achieved narrow droplet size distribution (152.75±4.85 and 128.05±9.22), low PDI (0.364±0.05 and 0.489±0.06), suitable pH (5.28-4.84) and conductivity (342±4.472-374±5.477 μS/cm). Zeta potential was measured as 0.209±0.041 and 0.141±0.024 mV. $M1_{CEM}$ showed 100% release at the 7^{th} hour and was provided almost the same zone diameter as CEM solution when evaluated for antibacterial activity.

Conclusion: Overall, it was concluded that microemulsions might be beneficial in improving dermal delivery of CEM for the treatment of skin and soft tissue infections.

Keywords: Cephalexin, microemulsion, dermal delivery, *in vitro* release, antibacterial activity.

INTRODUCTION

Human skin is the largest organ of a human being, therefore it takes the attention of scientists as being an important target site for the application of drugs [1]. Topical drug delivery is an important way of treating especially local diseases due to features of these systems such as being restricted to the affected area, therefore, reducing systemic side effects and being easy to stop treatment in a

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proper time in case of a severe side effect [1]. On the other hand, dermal drug delivery is a promising way of treatment because of large surface area of skin and being easy to access and the administration route is non-invasive therefore improved patient compliance [2].

In recent years, colloidal drug delivery systems especially nanocarriers range up to 500 nm have increased the attention of scientists for dermal drug delivery [3]. Microemulsions (MEs) are one of the colloidal drug delivery systems which its concept was introduced to the literature in early 1940s [4]. MEs are clear, thermodynamically stable and optically isotropic mixtures which has low viscosity and consist of oil, water, surfactant and co-surfactant [4–8]. The main benefit of MEs is being in need of less energy whilst forming. In order MEs to be formed, a highly fluid interfacial film and latter, low interfacial tension which occurs between colloidal and the external phase are required [5].

For dermal drug delivery, the main advantages of microemulsions can be divided into three groups. First, they can be a potential drug carrier system in order to dissolve both hydrophilic and lipophilic drugs therefore show increased thermodynamic activity towards skin itself. Secondly, by the effect of permeation enhancers involved in microemulsion formulation, Stratum Corneum (SC) can be destroyed and subsequently the flux of drugs via skin increases. Latter, the affinity of a drug to the internal phase can be easily modified in favor of partitioning into the SC, therefore permeation rate of drug can be improved [9,10].

The most commonly occurring infections in people are skin infections[11]. Microbial invasions into skin and the soft tissues underneath the skin are defined as Skin and Soft Tissue Infections (SSTIs). They show variable presentations, etiologies and severities. The main obstacle of SSTIs is to conveniently differentiate those cases that have severe presentations which require immediate attention and intervention from those that are less severe. SSTIs can be produced by the extremely diverse ecology of organisms localized on the skin. The clinical manifestations of SSTIs are the sum of two-step process. The first step is microbial invasion to the host and the second step is interaction with host defences [12]. Gram-positive species such as *Staphylococcus epidermidis*, Corynebacterium species, *S aureus* and *Streptococcus pyogenes* are generally the typical flora which colonize the skin. *S. aureus* and *Streptococcus pyogenes* are the main reason for SSTIs.

Cephalosporins, are the most widely used for treatment of skin infections because of their safety profiles[13]. Cephalexin monohydrate (CEM), (7R)-7-(D-α-Amino-α-phenylacetamido)-3-methyl-3- cephem-4-carboxylic acid hydrate or (6R,7R)-7-{[(2R)-2-amino-2-phenylacetyl]amino}-3- methyl-8-oxo-5-thia-

1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid hydrate (Figure 1), is an antibiotic which has high oral absorption and lack of serum binding [14]. CEM has low water solubility (less than 0. 1 mg/g) has been using in the treatment of bacteria caused infections. Meanwhile, CEM has an important role for patients who have hypersensitivity to penicillin as being an alternative [15].

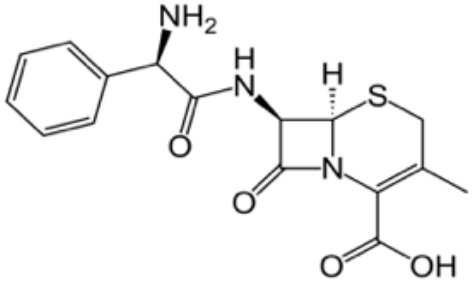


Figure 1. Structural formula of cephalexin

In addition to all mentioned above, for treatment of antibacterial infections CEM loaded microemulsions takes an important corner because of advantageous features such as avoidance of gastrointestinal side effects, increased solubility and improved targeting [16]. The aim of the present study was to prepare novel CEM loaded microemulsion formulations and to evaluate a better formulation of CEM for dermal delivery. For this aim, the physico-chemical characterization, *in-vitro* release, stability and microbiological tests were evaluated.

MATERIALS AND METHODS

Materials

CEM was purchased from DSM Sinochem, Spain. Isopyropyl myristate (IPM), Span 20 and phospate buffer tablets were purchased from Sigma, USA. Tween 80 (Polysorbate 80), and ethanol were purchased from Merck, Germany. Cremophor EL (Macrogolglycerol ricinoleate) kind gift from BASF, Germany. Oleic acid was purchased from Doga Ilac, Turkey. Dialysis membrane (Spectro/por Dialysis Mebrane, Spectra/por 4, diameter 16 mm, molecular weight of 12-14 kDa) were purchased from Spectrum. All other chemical reagents and solvents were of analytical grade and used as received.

Preparation of microemulsion formulations

In order to discover the existence range of microemulsions, pseudo-ternary phase diagrams were constructed by using titration method. A series of oil and surfactant/cosurfactant (S/Cos) mixtures with phosphate buffer at ambient temperature (25 ± 2 °C) were titrated. After being equilibrated, microemulsions

were determined by visually examining the mixtures[17]. The phase diagrams were constructed by using a software program[18]. All experiments were replicated at least four times.

Two different microemulsion formulations were prepared in accordance with the microemulsion areas in the phase diagrams. The microemulsion systems were prepared using IPM and oleic acid as oil phase, Span 20, Tween 80, Cremophor EL as surfactants, ethanol as co-surfactant and phosphate buffer as aqueous phase. After gently equilibrating selected microemulsions for 5 min with magnetic stirring, appropriate amount of CEM was dissolved in these microemulsions. The final concentration of CEM in formulations was 0.02% (w/w).

Characterization of microemulsion formulations

The characteristic features of microemulsions such as pH, viscosity, refractive index, electrical conductivity, droplet size, polydispersity index (PDI), and zeta potential was evaluated in order to discover the suitability of microemulsions for topical administration.

Dynamic Light Scattering method (Nano ZS, Malvern Instruments, U.K.) was used to measure the average droplet size and PDI. The particle size and PDI values (repeated five times at 25 °C) were obtained by averaging of five measurements at an angle of 173° by using disposable cells.

In order to measure the zeta potential of samples, disposable plain folded capillary zeta cells (Malvern Zetasizer Nano ZS) were used. The zeta potential was calculated from the electrophoretic mobility using the Helmholtz–Smoluchowski equation under an electrical field of 40 V/cm. Software involved system was used for the process. The measurements were repeated five times at 25±2 °C.

In order to measure the viscosities of formulations, AND Vibro Viscometer- SV-10 as viscosimeter was used. The pH values of the formulations were determined by a digital pH-meter (Mettler Toledo, Switzerland). The refractive index values of formulations were evaluated using a refractometer (Krüss DR301-95, Germany). Electrical conductivity of the formulations was studied using a conductometer (Milwaukee MW 801, USA) to determine the type of microemulsion. Experiments were performed at 25±2 °C five times for each sample, and the results are presented as mean ± SD.

Evaluation of *in vitro* Cephalexin release

A synthetic membrane (Spectro/por Dialysis Mebrane, Spectra/por 4, diameter 16 mm, molecular weight of 12-14 kDa) was filled with 3 ml CEM loaded microemulsion formulations. The receiver compartment (37 mL) consisted of ethanol

and PBS pH 7.4 (ratio of 20:80) in order to ensure sink condition. The receptor compartment was exposed to ambient temperature and covered with parafilm to prevent evaporation. The temperature of the receptor compartment was maintained at 37±1°C while the buffer solution was stirred at 600rpm continuously with a magnetic bar. Samples (1 mL) were withdrawn from the release medium at predetermined time**s** (0, 0.5, 1, 2, 3, 4, 5, 6, and 7h). The samples were analyzed by UV-Visible spectrophotometer (UV-1800, Shimadzu, Japan) at 261 nm. The analytical method was validated. Calibration curve was drawn.

Antibacterial activity studies of CEM loaded microemulsion formulations

Isolates of *Staphylococcus epidermidis* (1C1, 7N5, 7C5, 7N6, 7K9, 11C8, 11K8 and 12K2.1) which were isolated from vaginas of healthy women and strains of *S. epidermidis* (ATCC 12228), *S. aureus* (ATCC 6538, ATCC 2593) were used to evaluate antibacterial activity of microemulsion formulations by performing disk diffusion method. All microorganism samples were incubated 24 hours in brain-heart infusion broth medium at 37°C. The decision of the ethical committee of the study was taken from Istanbul Medipol University Ethics Committee of Non-Interventional Clinical Researches in Istanbul with the number 38 of Decision dated 11.04/2013.

Pure cultures of the microorganisms were prepared in 0.85% sterile saline solution and were adjusted to give an inoculum with an equivalent cell density to 0.5 McFarland turbidity standards. The entire surface of the Mueller-Hinton II Agar (MHA) plate (diameter, 90 mm) (Bio-Rad) was covered with the required inoculum, and the plate was air dried for 15 min before the disks were laid on the sterile discs were then placed onto agar plates, and 5µL of every formulation was applied to the discs. Discs were 1.5 cm away from the side of the petri dish and 2 cm away from each other. Plates were incubated at 37°C for 24h, and the zone diameters of each formulation for each isolate were measured [18–21].

The experimental groups were listed as:

- G1: M1 Formulation (unloaded) (F1)
- G2: M1 CEM (containing 0.02% CEM) (F1+CPH)
- G3: M2 formulation (unloaded) (F2)
- G4: M2 CEM (containing 0.02% CEM) (F2+CPH)
- G5: 0.02% CEM solution (CPH)
- G6: Solvent (S)

Results and discussion

Cephalosporins inhibit peptidoglycan cross-linkage by crossing the bacterial cell wall. They show bactericidal effect to microorganisms which contain autolysin enzymes and bacteriostatic to microorganisms that lack autolysins [22]. CEM is a first-generation cephalosporin antibiotic. The mainly usage of CEM in the treatment is the susceptible infections of the respiratory tract, urinary tract, and skin because of their safety profile [23]. CEM is mostly used because of its activity against both the gram-positive and gram negative microorganisms [11].

Nowadays, for the treatment of skin infections, the oral route is being chosen for CEM usage Even though CEM has high safety profile, systemic antibiotic usage has some disadvantages such as antibiotic resistance, which is a major threat to public health, and systemic toxicity and side effects and also low concentration of drug at the site of infection [24]. In order to improve drug concentration at the infection site and decrease the systemic toxicity and side effects, and avoid from the bacterial resistance topical administration of CEM can be advantageous [25].

In order to overcome the disadvantages of oral drug delivery for skin infections, development of novel topical drug delivery systems such as microemulsions, nanoparticles, liposomes etc. would be helpful [26]. In this context, microemulsions shows favorable characteristic features because of having the simple and economical preparation method, showing long term stability, biocompatibility and high solubility of poorly soluble drugs [1]. For instance, it is not possible to use CEM in the treatment of some specific skin infections such as acne due to the very decreased penetration of CEM which has hydrophilic structure into the microcomedones where bacteria are located [27]. On the other hand, because of structural features of microemulsions, they enhance the penetration of drug into the skin and improve the dermal bioavailability with a good topical tolerance [2].

In our present study, it was aimed to prepare of the microemulsions for the treating of bacterial skin infections.

Preparation of CEM loaded microemulsion formulations

In order to prepare microemulsions, IPM and oleic acid were selected as oil phase. IPM has a strong permeation enhancing effect and can increase the diffusion coefficient in skin [28]. Meanwhile, oleic acid which is one of the fatty acids is able to induce lipid fluidization as well as phase separation within the membrane and so enhance the permeation through skin [29]. In addition, to prepare microemulsions, high concentration of surfactants and co-surfactants are necessary to develop these diagrams and latter to determine the microemulsion region. This is the reason why determining the dermal tolerance of these systems is an

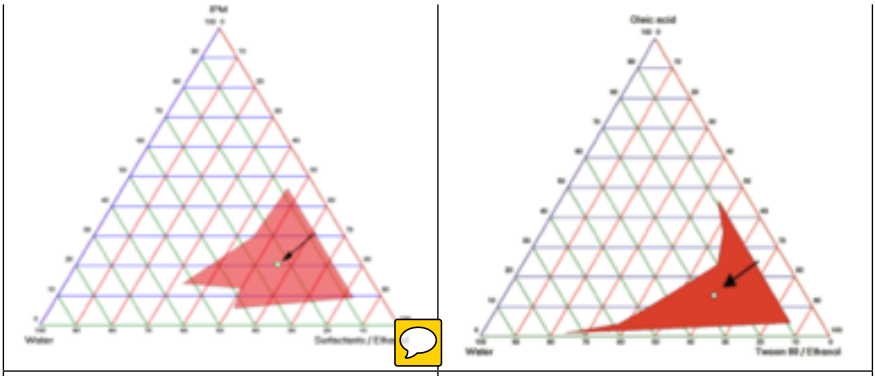
important procedure to eliminate the possibility of irritation. In order to prepare microemulsions which have ideal formulation characteristics, Span 20, Tween 80 and Cremophor EL as nonionic surfactants that are considered as less toxic compared to ionic ones were investigated for their suitability to form a microemulsion [30]. Meanwhile, ethanol which is commonly used in dermal microemulsions was used as a cosurfactant to prepare CEM loaded microemulsions.

In order to prepare ideal microemulsions, the optimum concentration range of components involved in microemulsion itself should be determined. The construction of phase diagrams makes it easy to find out required the concentration range of components. The construction of pseudo-ternary phase diagrams was used to obtain appropriate concentration ranges of components in the areas of forming microemulsions. Gravity center of phase diagrams provided the percentage of components in order to prepare the drug free microemulsion formulations. It was seen that all formulations formed clear and transparent. Figure 2 shows the pseudo-ternary phase diagrams of drug free microemulsions. Compositions of microemulsion formulation according to the pseudo-ternary phase diagrams and area values were presented in Table 1. The area of M1, M2 microemulsions was determined as 732 and 652 respectively. M1 microemulsion has higher area than the other microemulsion. Once CEM [0.02% (w/w)] was dissolved in the formulations, inversion of microemulsion, which is an unwanted conversion, was not seen.

Table 1. Composition of microemulsion formulations (M1 and M2)

Components (%)	M1	M2
IPM	21	-
OA	-	14
Span 20	12.7	-
Tween 80	-	40
Cremophor EL	24.3	-
Ethanol	18.7	20
Phosphate buffer (pH: 7.4)	23	26

* For both formulations, surfactant/co-surfactant ratio=2:1



M1 M2

Figure 2. The pseudo-ternary phase diagrams of microemulsion formulations (M1 and M2) composed of IPM, oleic acid, Span 20, Cremopor EL, Tween 80, ethanol and phosphate buffer (pH: 7.4).

Characterization of microemulsions

Droplet size, PDI, zeta potential, pH, viscosity, refractive index and electrical conductivity were measured in terms of determining the physicochemical properties of each microemulsion. Table 2 shows the physicochemical parameters and characteristic features of microemulsions during the presence and the absence of CEM.

The droplet size of CEM loaded microemulsion formulations were found different than each other and was determined in between 102.110±9.966nm and 152.750±5.321nm. The present droplet size of current formulations are in the usual microemulsion droplet size range 20. The incorporation of CEM into M1 microemulsion significantly increased the droplet size ($p5<0.05$). Different droplet size of the formulations can be interpreted in the manner of difference optimized oil and surfactants phases used. Optimization of different surfactant and oil phases may cause the minimal droplet size distribution with narrow PDI. Narrow PDI value shows the homogeneity of the size distribution of droplets in the developed microemulsions. This is the reason why the polydispersity index below 0.5 of all formulations developed can be formed an estimate of the indication of uniformity of the droplets. Zeta potential values of microemulsions were found almost neutral due to microemulsion components like nonionic surfactants.

The conductivity of CEM loaded microemulsion formulations was found between 342±4.472 μS/cm and 374±5.477 μS/cm. In terms of dermal application, oil-in-water type microemulsion formulations are mostly required. According to electrical conductivity studies performed in this study, it shows that developed formulations are also oil–in-water type and are suitable for dermal applications of CEM. The refractive indexes of all microemulsions were ranged between 1.415 and 1.418±0.0002 and thus signify that prepared microemulsions were clear and transparent.

Table 2. Characterization of the developed blank and CEM loaded microemulsion formulations (Mean±S.D., n = 5).

Formulation / parameters	pH	Droplet size (nm)	PDI	Zeta potential (mV)	Refractive index	Conductivity (µS/cm)	Viscosity (cP)
M1	4.796±0.053	65.45±1.497	0.127±0,091	0.325±0.047	1.415	374±5.477	104.4±0.548
$M1_{CEM}$	4.844±0.013	152.750±5.321	0.364±0.053	0.209±0.041	1.416±0,0002	362±4.472	104.2±0.447
M2	5.479±0.013	150.1 ±1.019	0.166 ±0.007	- 0.105 ±0.068	1.418±0,0002	352±4.472	139.2±0.447
$M2_{CEM}$	5.287±0.027	102.110±9.966	0.489± 0.062	0.141±0.024	1.418±0,0002	342±4.472	134±0

All formulations were found clear on visual inspection. In terms of patient compliance, pH values of prepared formulations should always be taken in consideration for preventing irritating sensations. The pH values were found in between 4.796±0.053 and 5.479±0.013. Ideally, dermal formulations should possess pH in the range of 4-7, for minimizing discomfort of patient or irritation on the skin due to acidic pH and microbial growth on the skin because of basic pH [20]. Table 2 shows that $M2_{CEM}$ has higher viscosity compared to $M1_{CEM}$. This result can be interpreted that $M2_{CEM}$ would be expected of controlled releasing drug content during *in vitro* studies. The results of characterization study indicate development of successful CEM loaded microemulsion formulations with optimum characteristics.

In vitro CEM release studies

The samples were analyzed by UV-Visible spectrophotometer (UV-1800, Shimadzu, Japan) at 261 nm. The analytical method was validated in terms of analyzing the samples. Calibration curve was created with eight-point calibration concentration with the range of 0.001-0.2mg/mL for standard solution of bulk CEM. Three independent determinations were performed at each concentration. Linear relationship between absorption and concentration of CEM was observed. The standard deviation of the slope and intercept were low. The determination coefficient R^2 for regression line is 0.99854 with slope of 20.624x and y + intercept of + 0.0149 for standard solution of CEM.

CEM loaded microemulsion formulations and CEM solution component were studied for *in vitro* release through synthetic membrane to assess and compare the performances of formulations. Figure 3 shows the in vitro release graphics. As it can be seen in Figure 3, $M1_{CEM}$ and CEM solution shows 100% release at the end of the 7^{th} hour.

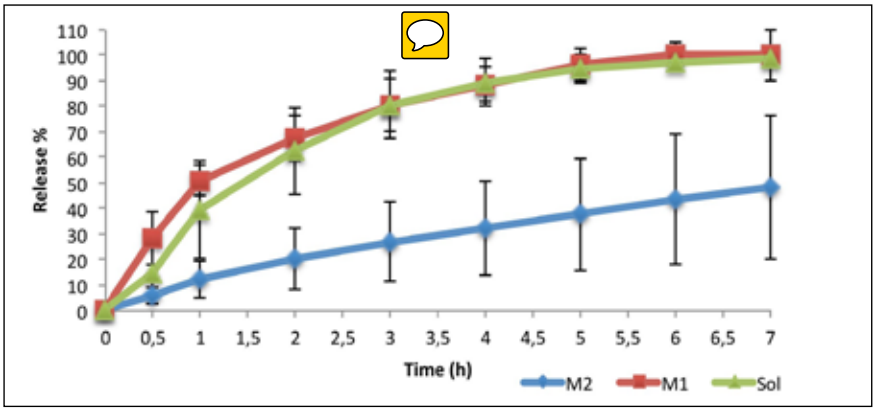


Figure 3. Percentage of release and time

Evaluation of antibacterial activity studies of CEM loaded microemulsion formulations

Table 3 shows the results of antibacterial activity tests of microemulsion formulations and their control groups which were performed on isolates of *Staphylococcus epidermidis* (1C1, 7N5, 7C5, 7N6, 7K9, 11C8, 11K8 and 12K2.1), strains of *S. epidermidis* (ATCC 12228), *S. aureus* (ATCC 6538, ATCC 2593). As a result, it was noted that there was no zone when blank microemulsion formulations were applied to the bacteria tested. On the other hand, it was found that when the CEM loaded formulations were applied on the test bacteria, the zone diameters varied between 12 and 32 mm.

When the CEM solution was applied on the bacteria alone, zone diameters were determined in between 16-32mm. Once the CEM loaded M1 and M2 microemulsion were applied on bacteria, zone diameter was measured between 12-30 mm, 18-28 mm, respectively. No zone formation was observed in the petri dishes in which the solvent was applied on.

As a result, blank M1 and M2 formulations were found to have no antibacterial activity when applied alone to the tested bacteria. In the meantime, CEM loaded M1 and M2 microemulsion was applied on the bacteria separately, it was seen that both of the formulations have close antibacterial activity compared to CEM solution. In addition, it was observed that the solvent used as a negative control in the study did not have any inhibitory effect on the bacteria. The study can be concluded that the M1 and M2 formulations could be used in combination with CEM antibiotic. Figure 4 shows the images of the zone diameters. At this figure, G1, G3, G5 refers to the above zones at each petri dish and G2, G4, G6 are for the below zones at each petri dish.

Table 3. Zone inhibition diameters

MICROORGANISMS	DIAMETER of ZONE INHIBITION RING (mm)					
	G1	G2	G3	G4	G5	G6
S. epidermidis 1C1	-	20	-	22	24	-
S. epidermidis 7N5	-	16	-	16	16	-
S. epidermidis 7C5	-	18	-	18	26	-
S. epidermidis 7N6	-	12	-	20	18	-
S. epidermidis 7K9	-	24	-	24	28	-
S. epidermidis 11C8	-	16	-	18	18	-
S. epidermidis 11K8	-	22	-	22	26	-
S. epidermidis 12K2.1	-	24	-	26	22	-
S. epidermidis ATCC 12228	-	30	-	26	30	-
S. aureus ATCC 6538	-	24	-	28	32	-
S. aureus ATCC 25923	-	18	-	26	26	-

Conclusion

In this study, blank and CEM loaded microemulsions were prepared, characterized, and evaluated for *in vitro* drug release and microbiological activity. The pseudo ternary phase diagram was used to optimize the microemulsion formulations. The present study showed that CEM microemulsions can successfully be prepared with titration method with narrow particle size and PDI range. According to the results of the characterization, and *in vitro* release studies, both of the formulations can be used for the treatment. $M1_{CEM}$ is a convenient formulation when prompt affect is required but on the other hand, if relatively prolonged release is necessary, $M2_{CEM}$ can be seen as a desirable formulation. The present study can open up a window for dermal application of microemulsions loaded with CEM; they would be a better alternative to conventional formulations in the treatment of various SSTIs with less systemic side-effects.

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DECLARATION OF INTEREST

The authors declare no conflict of interest.

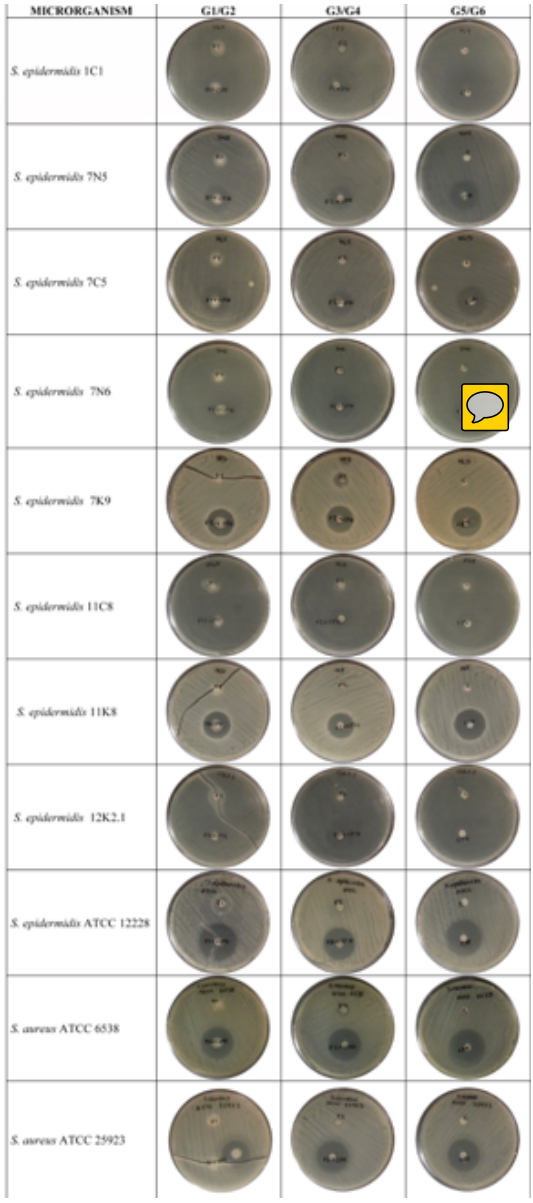

Figure 4. The images of zone diameters

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Synthesis and Characterization of Novel 1,3-oxazepin-5(1H)-one Derivatives via Reaction of Imine Compounds with Isobenzofuran-1(3H)-one

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ABSTRACT

The objective of this work is preparation of imine compounds from aromatic aldehyde reaction with aromatic primary amines to interfere with the preparation of disubstituted-oxazepine derivatives from the reaction of prepared imine compounds with isobenzofuran-1(3H)-one compound. Experimental part included synthesis of imine compounds (S_1-S_5) and synthesis of disubstituted-oxazepine derivatives (S_6-S_{10}). A number of new disubstituted-oxazepine derivatives were synthesized by acid-catalyzed cycloaddition- reaction of imine compounds with isobenzofuran-1(3H)-one in anhydrous THF under dry and reflux conditions with high yields. Imine compounds were synthesized by thermal condensation reaction of aromatic aldehydes, with aromatic primary amines. The products were identified by their melting point, FT-IR and 1H-NMR spectra. The formation of stable 7th – membered 1,3- oxazepine ring has been achieved by (5+2) cycloaddition reaction of isobenzofuran-1(3H)-one compound and imine group. The results of FT-IR and 1H-NMR showed that the target molecules were clearly formed due to the least obstructive effect in all preparation processes.

Keywords: Imine compound; isobenzofuran-1(3H)-one; disubstituted-oxazepine derivatives.

INTRODUCTION

Imine compounds

Imine compounds are class of compounds containing the imine group (-HC=N), usually prepared by the condensation of amino group in primary amines with an active carbonyl group of aldehydes and ketones, they are versatile precursors in the synthesis of industrial compounds via ring closure, and they exhibit a wide

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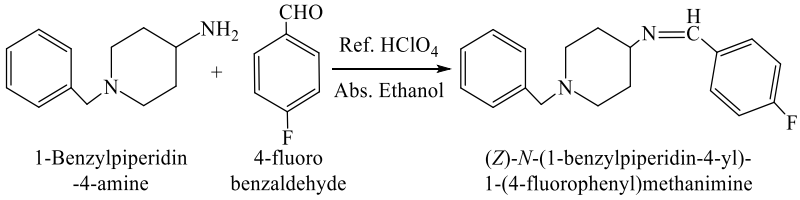
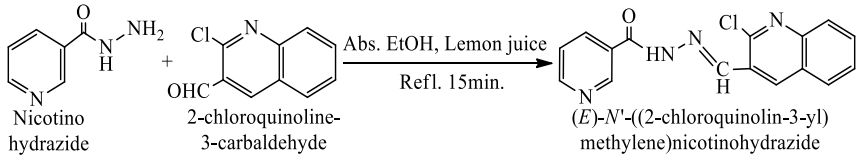
range of biological activities and pharmacological applications.[1-3] The reaction of 4-fluorobenzaldehyde with 1-benzylpiperidin-4-amine presence of per chloric acid efficiently gave the imine product (Scheme 1).[4]



Scheme 1. The effect of catalyst on imine compound formation

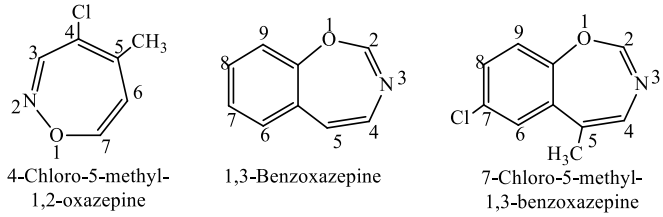
As well as the reaction of nicotinohydrazide with 2-chloro quinoline-3-carbaldehyde produce the imine compound in good yield (Scheme 2).[5]



Scheme 2. Uses of lemon juice to prepare imine compound

Oxazepine Derivatives

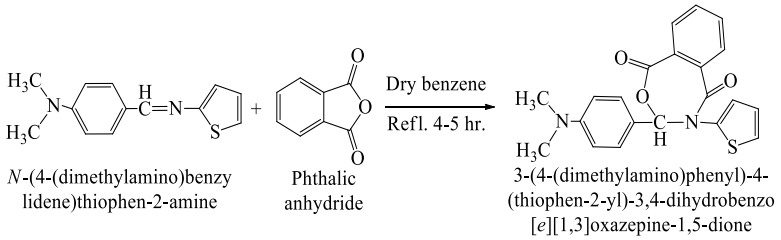
Oxazepines are class of heterocyclic compounds of seven- membered ring with two hetero- atoms (O and N), oxygen atom is located at position (1) and nitrogen atom in the (-2, -3 or-4) positions as shown in scheme 3.[6]



Scheme 3. Structures of oxazepines

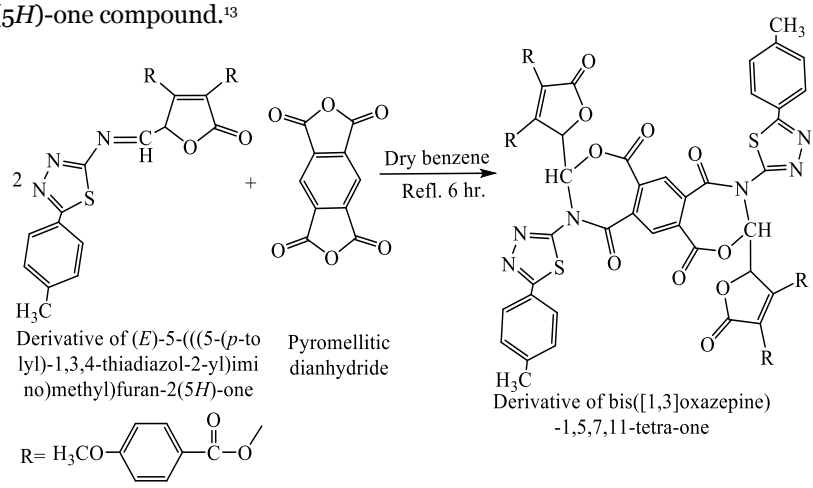
Oxazepines have been synthesized mainly by dipolar cycloaddition reaction of imine compounds with five atoms cyclic anhydride, such as phthalic, succinic, maleic pyromellitic and others.[7-14]

For example, the reaction of phthalic anhydride with N-(4-(dimethylamino) benzylidene) thiophen-2-amine in dry benzene gave an 1,3-oxazepine derivaties (Scheme 4).[15]



Scheme 4. Synthesized of oxazepine-1,5-dione derivatives

In scheme 5, the product of the reaction between pyromellitic anhydride and derivative of (E)-5-(((5-(*p*-to 1yl)-1,3,4-thiadiazol-2-yl)imino)methyl)furan-2(5*H*)-one compound.[13]



Scheme 5. Pyromellitic anhydride in bis([1,3]oxazepine)-1,5,7,11-tetraone synthesis

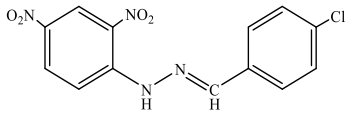
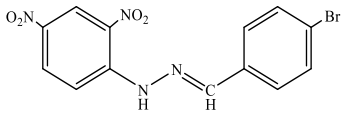
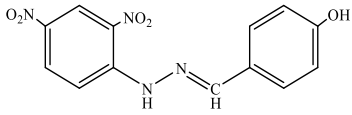
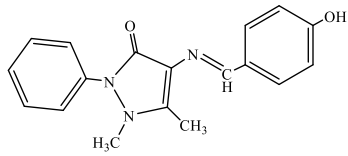
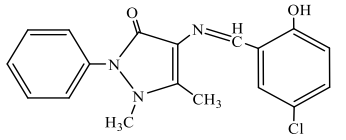
METHODOLOGY

Melting points were recorded on Electrothermal Melting Point Apparatus (uncorrected). FT-IR spectra were recorded at room temperature from (4000-400) cm^{-1} on Infrared Spectrophotometer Model Tensor 27 Bruker Co., Germany, and the ^{1}H-NMR spectra was recorded on Bruker Ac-300MHz spectrometer.

Synthesis of imine compounds (S_1-S_5)

Imine compounds were synthesized according to literature procedure.[9,16,17] An equimolar mixtures (0.02mol) of aldehydes and aromatic amines and trace of glacial acetic acid as catalyst in absolute ethanol (25ml) was placed in a (100ml) round-bottom flask equipped with condenser and stirring bar. The mixture was allowed to react at reflux temperature for 4hr, then to cool down to room temperature, whereby a crystalline solid separated out. The solid product was filtered off and recrystallized form ethanol. The structural formuli, nomenclature, melting points, colors, and percentage yields for the synthesized Imine compounds are given in Table 1.

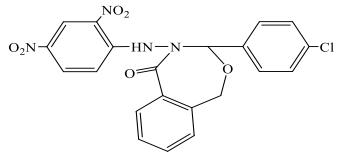
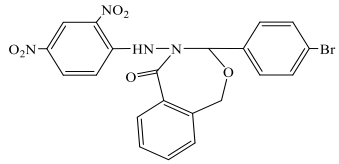
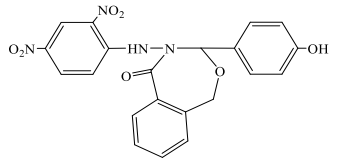
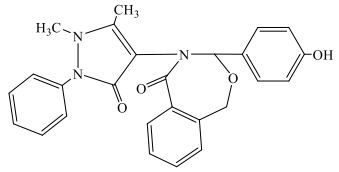
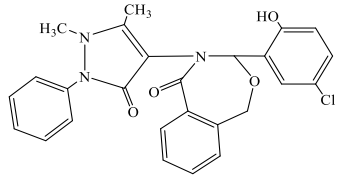
Table 1. Structural formuli, nomenclature, melting points, colors, and % yields of imines compound (S_1-S_5).

Comp. Code	Structural formuli	Nomenclature	Yield %	m.p. °C	Color
S_1		(E)-1-(4-chlorobenzylidene)-2-(2,4-dinitrophenyl) hydrazine	82%	236-238	Orange
S_2		(E)-1-(4-bromobenzylidene)-2-(2,4-dinitrophenyl) hydrazine	84%	232-234	Orange
S_3		(E)-4-((2-(2,4-dinitrophenyl) hydrazono)methyl) phenol	80%	240-242	Bright dark red
S_4		(E)-4-(4-hydroxybenzylidene amino)-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one	83%	218-220	Bright pale yellow
S_5		4-(5-chloro-2-hydroxybenzylid eneamino)-1,5-dimethyl-2-phe nyl -1H-pyrazol-3(2H)-one	89%	138-140	Bright pale yellow

Synthesis of disubstituted-oxazepine derivatives (S_6-S_{10})[11, 14]

In well dried 100-ml round-bottom flask equipped with condenser a mixture of Imine compound (0.01mol) and isobenzofuran-1(3*H*)-one (0.01mol) dissolved in (20ml) of tetrahydrofuran (THF) with trace of glacial acetic acid as catalyst was refluxed for 3hr and left to stand for 24hr at room temperature then solid product separated out. The solid product was filtered off and recrystallized form ethanol. The structural formuli, nomenclature, melting points, colors, and percentage yields for the synthesized disubstituted-1,3-oxazepine derivatives are given in Table 2.

Table 2. Structural formuli, nomenclature, melting points, colors, and % yields of disubstituted-oxazepine derivatives (S_6-S_{10}).

Comp. Code	Structural formuli	Nomenclature	Yield %	m.p. °C	Color
S_6		3-(4-chlorophenyl)-4-(2,4-dinit rophenylamino)-3,4-dihydrobe nzo[e][1,3] oxazepin-5(1H)-one	95%	194-196	Orange
S_7		3-(4-bromophenyl)-4-(2,4-di nitrophenylamino)-3,4-dihydro benzo[e][1,3] oxazepin-5(1H)-one	96%	198-200	Orange
S_8		4-(2,4-dinitrophenylamino)-3-(4-hydroxyphenyl)-3,4dihydro benzo [e][1,3] oxazepin-5(1H)-one	93%	184-186	Bright dark red
S_9		4-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-3-(4-hydroxyphenyl)-3,4-dihydro benzo [e][1,3]oxazepin-5(1H)-one	83%	239-240	Yellow
S_{10}		3-(5-chloro-2-hydroxyphenyl)-4-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-3,4-di hydrobenzo[e] [1,3] oxazepin-5(1H)-one	96%	138-140	Pale yellow

RESULTS AND DISCUSSION

Imine compounds were synthesized from commercially available aldehydes with primary amines and identified by their melting points, FT-IR, the FT-IR spectra, example figures 1 and 2, showed the appearance of the stretching absorption bands of the characteristic groups of the resulting imine (C=N) at (1573-1611) cm^{-1} beside the characteristic bands of the residual groups in the structure, Table 3, indicative of formation of the products.[18] The mechanism of imine compounds formation, Scheme 6, was thoroughly studied and established by many authorized literatures.[19]

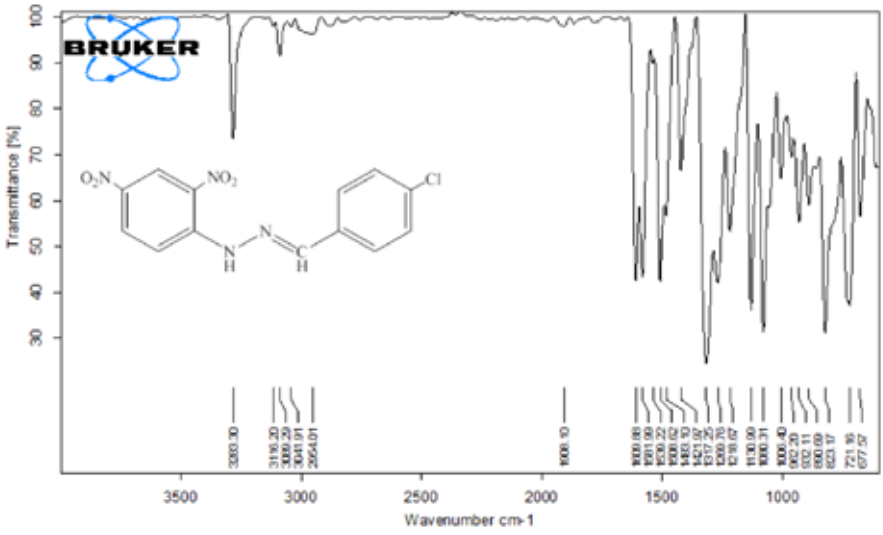


Figure 1. FT-IR spectra of S_1

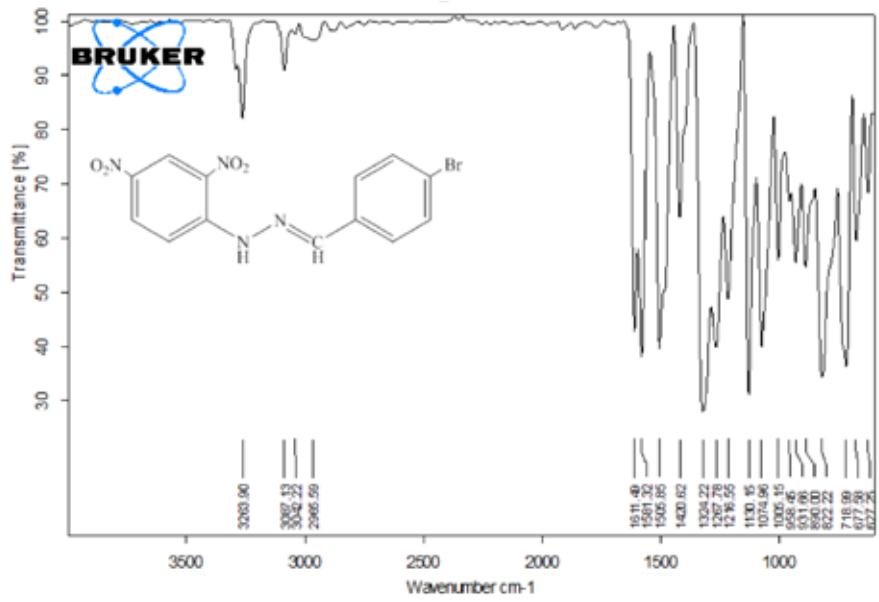
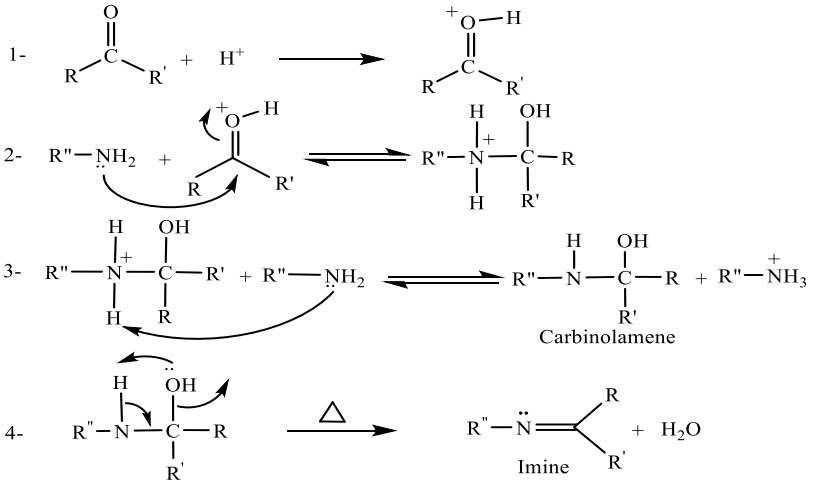


Figure 2. FT-IR spectra of S_2

Table 3. FTIR of imine compounds (S_1-S_5).

FT-IR, ν(cm^{-1})							
Comp. Code	C=N	C=C Aromatic	C-H Aromatic	C-H Alkene	C-H Ali.		Others
					Asymmetric	Symmetric	
S_1	1609	1581	3041	3089	--	--	NO_2 1508, 1317 N-H 3283 C-Cl 823
S_2	1611	1581	3042	3087	--	--	NO_2 1505, 1324 N-H 3263
S_3	1600	1584	3042	3112	--	--	NO_2 1508, 1305 O-H 3422 N-H 3257
S_4	1573	1507	3044	3114	3980	2892	C=O 1601 O-H 3582
S_5	1594	1559	3044	3075	2983	2874	C=O 1634 C-Cl 815 O-H 3450

The reaction of the aldehydes compounds and amine compounds to prepare imine compounds is given in the following equation (See scheme 6).



Scheme 6. Mechanism for the formation of imine compounds

In this work, the synthesis of new disubstituted-oxazepine derivatives by direct reaction of several imine compounds with Isobenzofuran-1(3*H*)-one in dry THF is reported. The synthesis of these compounds was achieved by the reaction of imine compounds and isobenzofuran-1(3*H*)-one in anhydrous THF at dry and

reflux conditions. The resulting products were identified by their melting points, FT-IR and ^{1}H-NMR spectra. The FT-IR spectra, figures (3) and (4), table (4) showed characteristic stretching absorption bands at (1613-1654) cm^{-1} indicative of C=O (lactam) bond formation beside the characteristic stretching absorption bands of the residual groups in the structure.[18]

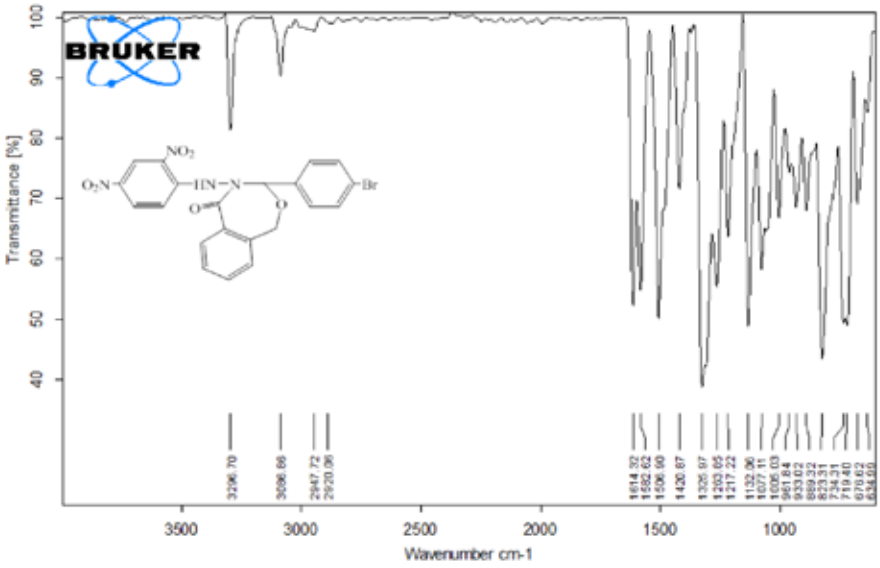


Figure 3. FT-IR spectra of S_7

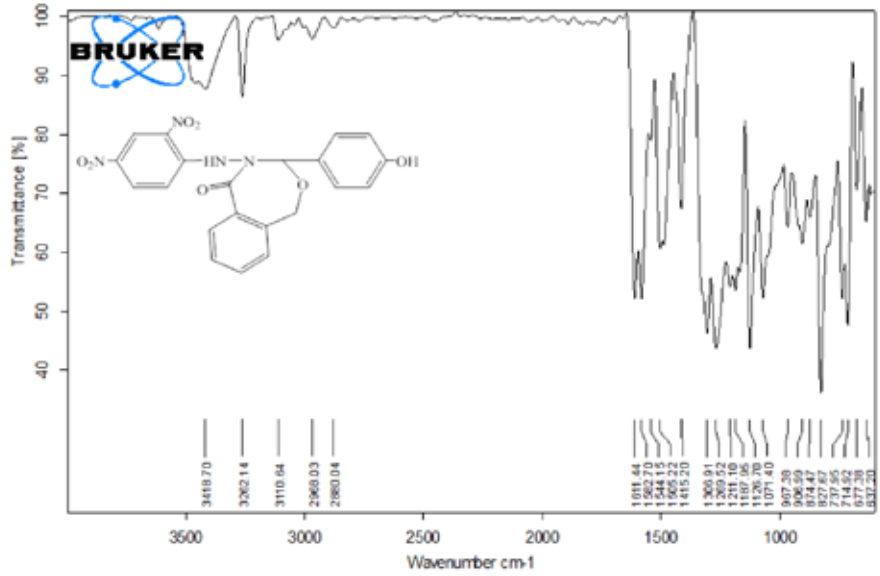


Figure 4. FT-IR spectra of S_8

Table 4. FT-IR of disubstituted-oxazepine derivatives (S_6-S_{10}).

FT-IR, ν(cm^{-1})								
Comp. Code	C=O Lactam	C-O Lactam	C-N Lactam	C=C Aromatic	C-H Aromatic	C-H Aliphatic		Others
						Asymmetric	Symmetric	
S_6	1613	1137	1223	1585	3091	2995	2890	NO_2 1515, 1327 N-H 3286 C-Cl 825
S_7	1614	1132	1263	1582	3086	2947	2920	NO_2 1513, 1330 N-H 3299
S_8	1611	1126	1269	1582	3110	2968	2880	NO_2 1505, 1306 O-H b3412 N-H 3262
S_9	1654	1158	1257	1582	3015	2988	2825	O-H 3450
S_{10}	1647	1134	1290	1580	3064	2962	2915	O-H b3462 C-Cl 819

The ^{1}H-NMR spectrum of compound S_9 in solvent DMSO, Figure (5) showed chemical shifts, δ(ppm), single in 1.23 (3H, N-CH_3), single in 2.44 (3H, =C-CH_3), single in 3.13 (2H, O-CH_2), single in 9.46 (1H, N-CH), single in 9.93 (1H, OH), multiplet 7.67-6.82 (13H, aromatic proton) and spectrum of compound S_{10}, Figure 6 showed chemical shifts, δ(ppm), singlet in 1.23 (3H, N-CH_3), singlet in 2.42 (3H, , =C-CH_3), singlet in 3.43 (2H, O-CH_2), singlet in 9.67 (1H, N-CH), singlet in 12.77 (1H, OH), multiplet 7.63-6.90 (13H, aromatic proton),[20] other chemical shifts, δ(ppm) of compounds (S_6-S_8), are given in Table 5.

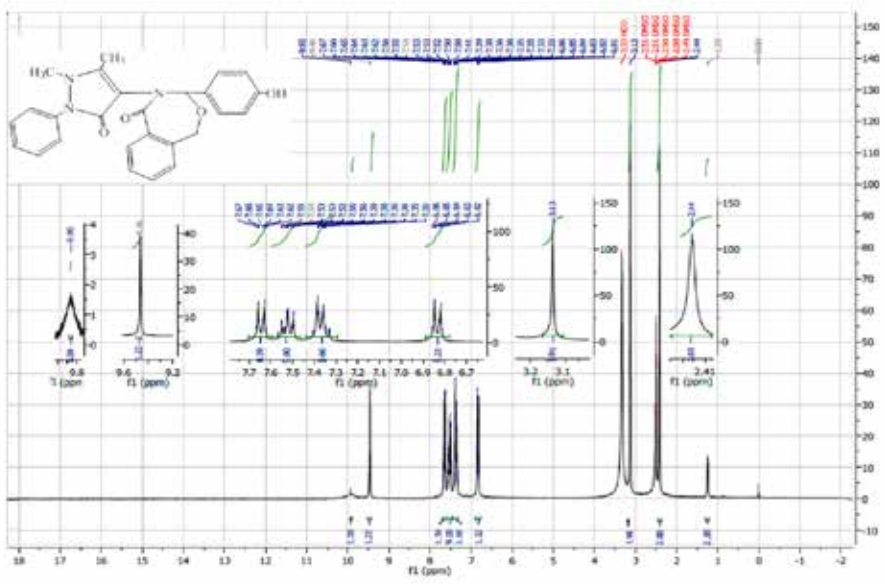


Figure 5. ^{1}H-NMR spectra of S_9

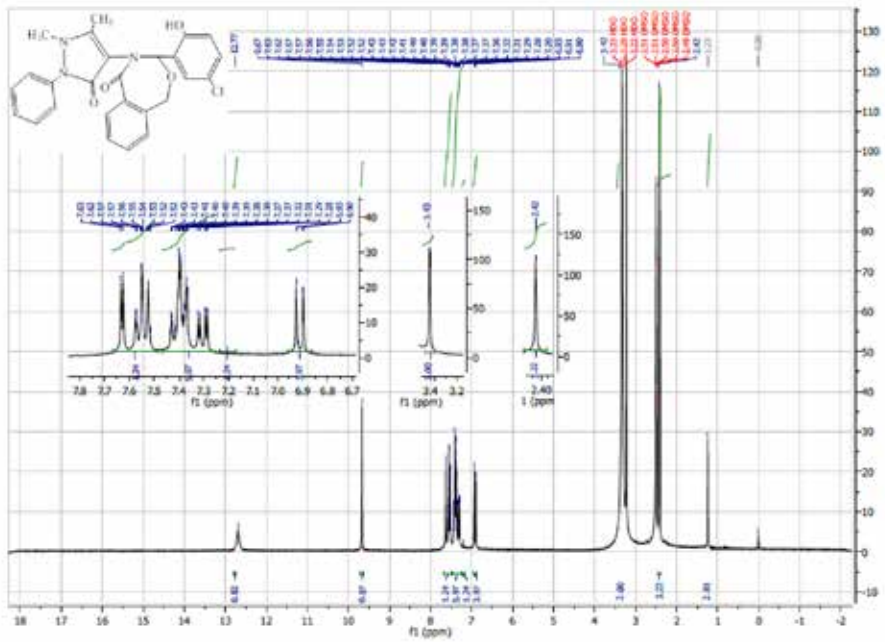


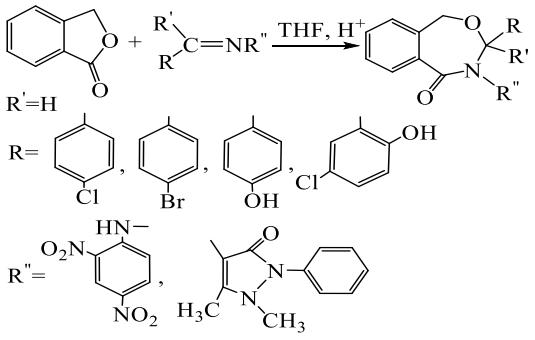
Figure 6. ^{1}H-NMR spectra of S_{10}

Table 5. The ^{1}H-NMR spectra of disubstituted-oxazepine derivatives (S_6-S_{10}) in DMSO.

Comp. Code	Chemical Shift δ ppm
S_6	Singlet in 2.26 (2H, O-C<u>H</u>$_2$), singlet in 4.71 (1H, -N<u>H</u>) singlet in 11.71 (1H, N-<u>C</u>H), multiplet in 7.56-8.88 (11H, aromatic proton).
S_7	Singlet in 3.26 (2H, O-C<u>H</u>$_2$), singlet in 4.70 (1H, -N<u>H</u>), singlet in 11.71 (1H, N-C<u>H</u>), multiplet in 7.69-8.89 (11H, aromatic proton).
S_8	Singlet in 3.35 (2H, O-C<u>H</u>$_2$), singlet in 4.37 (1H, -N<u>H</u>), singlet in 10.07 (1H, N-C<u>H</u>), singlet in 11.57 (1H, O<u>H</u>), multiplet 6.86-8.87 (11H, aromatic proton).
S_9	Singlet in 1.23 (3H, N-C<u>H</u>$_3$), singlet in 2.44 (3H, =C-C<u>H</u>$_3$), singlet in 3.13 (2H, O-C<u>H</u>$_2$), singlet in 9.46 (1H, N-C<u>H</u>), singlet in 9.93 (1H, O<u>H</u>), multiplet 7.67-6.82 (13H, aromatic proton).
S_{10}	Singlet in 1.23 (3H, N-C<u>H</u>$_3$), singlet in 2.42 (3H, , =C-C<u>H</u>$_3$), singlet in 3.43 (2H, O-C<u>H</u>$_2$), singlet in 9.67 (1H, N-<u>C</u>H), singlet in 12.77 (1H, OH), multiplet 7.63-6.90 (13H, aromatic Proton).

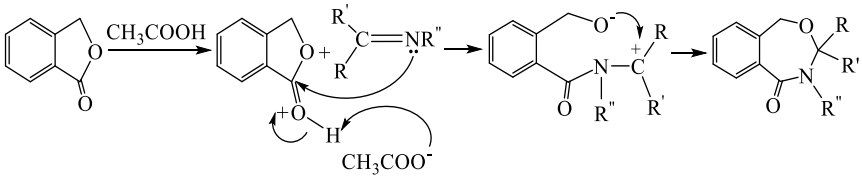
It may be concluded that the reaction takes place via concerted (5+2) dipolar cycloaddition mechanism in which the mild nucleophile (imine) attacked the electrophilic carbon atom of the carbonyl group to give a dipolar intermediate, which collapses to give the target molecule, the roll of the acid-catalyst is to enhance the electro positivity of the carbon nucleus.

The reaction of the prepared imine compounds with Isobenzofuran-1(3*H*)-one is given in the following equation (See scheme 7).



Scheme 7. Synthesized of disubstituted oxazepine derivatives

The reaction course and the suggested mechanism is given by Scheme 8.



Scheme 8. Mechanism for the formation of disubstituted oxazepine derivatives

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ABDiİBRAHİM

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Gastro-protective Effects of the Methanolic Extract of the Rind of Citrullus Lanatus on Indomethacin Induced Gastric Ulceration in Male Wistar Rats

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ABSTRACT

Objectives: Study investigated methanolic extract of rind of *Citrullus lanatus* for possible benefit in ameliorating indomethacin induced gastric ulceration.

Methodology: Adult male Wistar rats were assigned 5 groups of 6 rats each. Group 1 received extract vehicle; Groups 2, 3 and 4 received 100mg/kg, 200mg/kg and 500mg/kg bw of extract; Group 5 received 200mg/kg bw of cimetidine. Treatment was orally for 21days. Gastric ulcer was subsequently induced by oral indomethacin. Gastric juice volume, acid concentration, ulcer index, percentage ulcer inhibition was determined. Photomicrograph sections of the gastric mucosa of the various groups were obtained.

Results: Compared to cimetidine, extract protected gastric mucosa against indomethacin induced gastric ulceration with significant reduction in gastric juice volume, acid concentration and ulcer index and increase in percent ulcer inhibition in a dose dependent manner ($p<0.05$); consistent with gastric histological changes.

Conclusion: Extract of the rind of *Citrullus lanatus* exerts potential gastro-protective effects against indomethacin induced gastric ulceration in male Wistar rats.

Keywords: Citrullus lanatus, gastric protection, gastric ulcer, indomethacine

INTRODUCTION

Although many useful drugs have been identified in the treatment of ulcers, these drugs are often accompanied with undesirable side effects such as drowsiness, muscular pain, diarrhea, fatigue and headache.[1] Watermelon (*Citrullus lanatus)*, is a fruit with about 93% water which gives it the name "watermelon". The *Citrullus* part of watermelon is derived from the Greek word 'citrus;' while *lana-*

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tus, a Latin word meaning wooly, describes the tiny hairs present on the stems and leaves of the plant.[2] Watermelon has been cultivated in Africa for over 4,000 years.[3] It is a very rich natural source of lycopene; an antioxidant with potential health benefits.[4, 5] It also belongs to the family of Cucurbitacea plants which are known to possess bioactive compounds like alkaloids, triterpenes, sterols and cucurbitacin.[6] The various parts of watermelon have beneficial values and these include the rind and seeds. The rind which is the thick smooth exterior part of the fruit has been reported to be prescribed in cases of diabetes and alcoholic poisoning.[7] The rind contains alkaloids, saponin, cardiac glycosides, flavonoids, phenol, moisture, lipid, protein, fiber, and carbohydrates.[8] In a recent report from our center, we described the possible ameliorative effects of the methanolic extract of the rind of *Citrullus lanatus* on semen parameters and reproductive hormones following lead acetate induced toxicity in male wistar rats.[9]

Plant based medicines are now considered as better alternatives to control and treatment of various diseases; because of safety considerations since they have minimal side effects likely due to the presence of naturally occurring ingredients.[10] On account of these, the present study attempted to evaluate the methanolic extract of the rind of *Citrullus lanatus* for possible benefit in indomethacin induced gastric ulceration using male Wistar rats as models.

METHODOLOGY

Plant material and preparation of extracts

Fresh plant and fruits of watermelon were obtained from a local market in Rivers State, Nigeria. The fruits were identified and authenticated by Dr. C Ekeke of the Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria. Herbarium number: UPH/V/1214 was assigned and voucher specimens deposited. The rinds were peeled off from the whole fruit washed thoroughly, sun-dried and milled into a fine powder. The method of extraction employed is percolation as described by Adesanya *et. al.,* 2011.[11] 24g of the powdered sample was soaked in a beaker containing 100ml of 98% methanol for a period of 48 hours and then filtered with a Whatman No. 1 filter paper size. The volume of filtrate obtained was 150ml before concentration; the filtrate was subsequently concentrated using a rotary evaporator. The weight of residue obtained was 8.5g. The study protocol was approved by the College Research Ethics Committee, College of Health Sciences, University of Port Harcourt, Nigeria with the reference number: UPH/CHS/CREC/2011/A035 vide a communication dated 24th April 2011. Furthermore, the study was conducted in accordance with the guidelines for the care and use of laboratory animals issued by the United States Institute for Laboratory and Animal Research (1996).[12]

Determination of Median Lethal Dose (LD_{50})

Acute toxicity study (LD_{50}) was determined using the method described by Lorke 1989.[13] The (LD_{50}) of the extract was found to be greater than 2000mg/kg body weight.

Experimental design

Thirty male Wistar rats were used for this study. The rats were aged 8 to10 weeks and weighed between 170 and 200g. They were randomly divided into five groups: Groups 1 to 5 consisting of 6 rats each. Rats in each group were numbered 1 to 6 and placed in separate cages in the Animal House of Madonna University, Nigeria under natural day and night cycles. The rats had free access to normal rat chow and tap water *ad libitum*. They were allowed two weeks of acclimatization to their environment and subsequently treated as follows:

Group 1: Control group; rats in this group were given 2ml/kg body weight of extract vehicle.

Group 2: Low dose extract group; rats in this group were treated with 100mg/kg body weight of the extract of the rind of *Citrullus lanatus*.

Group 3: Medium dose extract group; rats in this group were treated with 200mg/kg body weight of the extract of the rind of *Citrullus lanatus*.

Group 4: High dose extract group; rats in this group were treated with 500mg/kg body weight of the extract of the rind of *Citrullus lanatus*.

Group 5: Positive control group; rats in this group were given 200mg/kg body weight of Cimetidine.

The extract of the rind of *Citrullus lanatus*, cimetidine and extract vehicles were administered to the rats daily, using an oral cannula. All the rats were treated for a total of 21 days.

Induction of gastric ulceration

After 21 days of treatment, the rats were fasted for 24 hours following which gastric ulceration was induced by oral administration of 40mg/kg body weight of indomethacin.

Determination of gastric juice volume, gastric acid secretion, and ulcer index

This was performed as earlier described by Heeba *et. al.,* 2009.[14] Four hours after the induction of gastric ulcer, the rats were killed by cervical dislocation, the abdomen was opened to remove the stomach, and gastric contents was collected to determine the gastric juice volume. Five milliliters of distilled water was add-

ed to the gastric juice and the resultant solution was centrifuged at 3,000 rpm for 10 minutes. Gastric juice acid concentration in mEq/L was determined in the supernatant volume by titration to pH 7 with 0.0025 N of sodium hydroxide.

After removal of gastric content from the stomach, the stomach was pinned onto a soft board. Scoring of ulcer was subsequently done as follows: 1 = erosions of 1mm or less in diameter; 2 = erosions of between 1 to 2mm in diameter; 3 =erosions greater than 2mm in diameter. The overall scores were divided by a factor of 10 and the result obtained designated as the ulcer index.[15] The percentage of ulcer inhibition was also calculated as follows:

$$\text{Percent ulcer inhibition} = \frac{\text{(Mean ulcer index of control-Mean ulcer index of test)}}{\text{Mean ulcer index of control}} \times 100$$

Gastric histology

Portions of the stomach of all rats were carefully obtained and fixed in 10% formalin, dehydrated stepwise in graded alcohol, cleared in xylene and then embedded in liquid paraffin. A 5µ thickness paraffin section was cut and stained in hematoxylin and eosin, followed by examination under a light microscope at x200 magnification. The slides obtained were analyzed and then re-analyzed by two different but widely experienced pathologists. Appropriate photomicrographs were subsequently obtained. Typical results for each respective Group are as presented in Plates A to E.

Statistical analysis

The results of this study were expressed as mean and standard error of mean (Mean ± SEM). Significant differences between the groups was assessed using the one-way analysis of variance (ANOVA); followed by the LSD post-hoc test. A p value less than 0.05 was considered statistically significant. Results are as presented in Table 1 and Plates A to E.

Table 1. Effects of the methanolic extract of the rind of *Citrullus lanatus* on gastric juice volume, gastric juice acid concentration, gastric ulcer index, and percentage ulcer inhibition in male Wistar rats.

Groups	Group 1: Control group [2ml/kg bw of extract vehicle]	Group 2: Low dose extract group [100mg/ kg bw of extract]	Group 3: Medium dose extract group [200mg/ kg bw of extract]	Group 4: High dose extract group [500mg/ kg bw of extract]	Group 5: Cimetidine group (Positive control) [200mg/ kg bw of Cimetidine]
Gastric juice volume [ml/4hr]	5.26 ± 0.08	5.22 ± 0.08	5.16±0.05*	5.14 ± 0.05*	5.02 ± 0.04*
Gastric juice acid concentration [mEq/L]	0.81 ± 0.13	0.57 ± 0.03*	0.66 ± 0.03*	0.50 ± 0.03*	0.53 ± 0.08*
Gastric ulcer index	2.76 ± 0.06	2.36 ± 0.15*	1.21 ± 0.08*	0.50 ± 0.08*	1.20 ± 0.14*
Percentage ulcer inhibition [%]	-	14.5*	56.2*	81.9*	56.5*

*Values expressed as Mean ± SEM; * significantly different as compared to control*

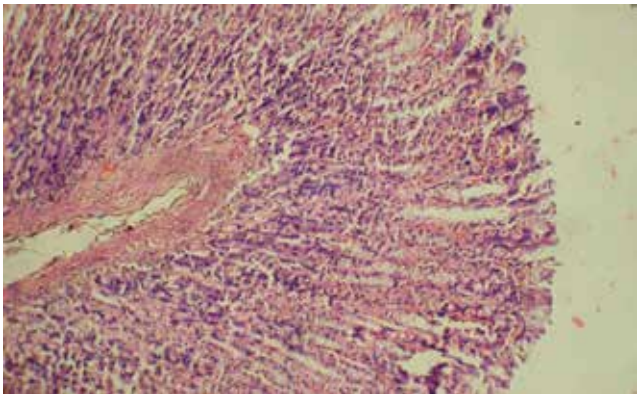

Plate A. Cross section of gastric epithelium of Group 1 control rats (Ulcerated untreated group)

Plate A is a cross section of the gastric epithelium obtained from Group 1 ulcerated and untreated control rats showing blood stained hemorrhagic ulcers, a disrupted epithelium and blood-stained mucosa.

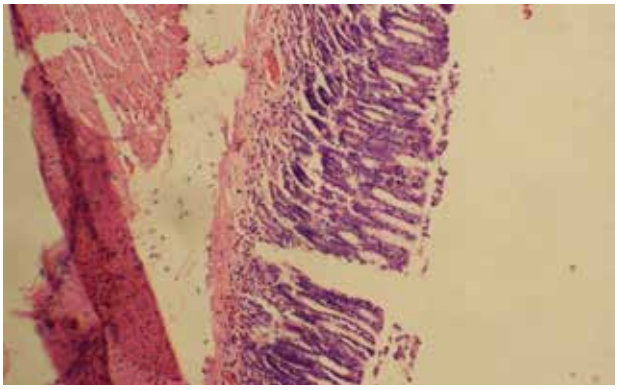

Plate B. Cross section of gastric epithelium of Group 2 rats (Low dose extract group)

Plate B is a cross section of the gastric epithelium obtained from Group 2 low dose extract treated rats showing severe disintegration of the epithelial cells.

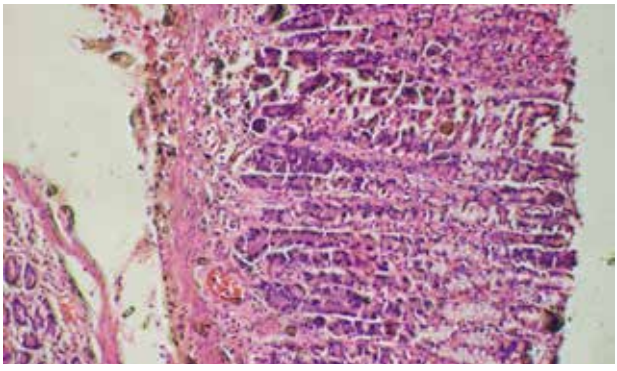

Plate C. Cross section of gastric epithelium of Group 3 rats (Medium dose extract group)

Plate C is a cross section of the gastric epithelium obtained from Group 3 medium dose extract treated rats showing severe erosion of the mucosa layers and evidence of mild ulceration.

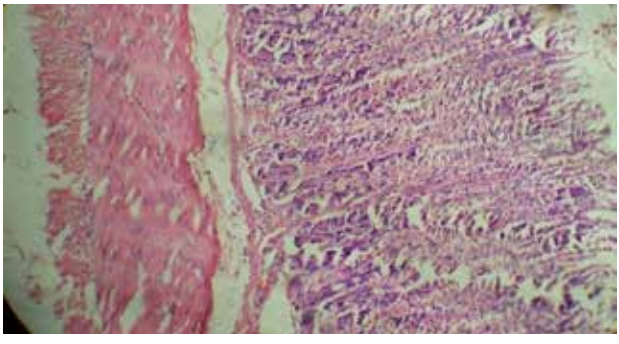

Plate D. Cross-section of gastric epithelium of Group 4 rats (High dose extract group)

Plate D is a cross-section of gastric epithelium obtained from Group 4 high dose extract treated rats showing a fairly intact and tightly arranged epithelial cells with slight disruption of the uppermost epithelium of the mucosa.

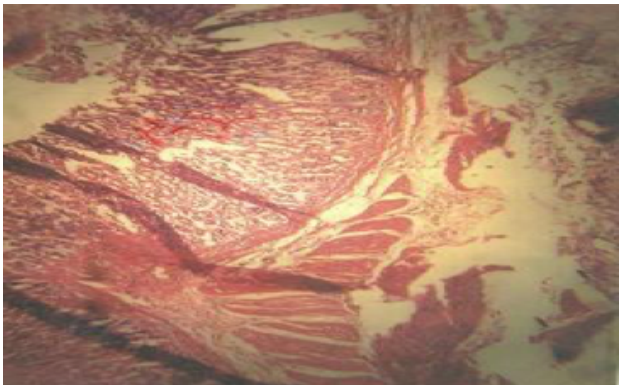

Plate E. Cross-section of gastric epithelium of Group 5 rats (Cimetidine/positive control group)

Plate E is a cross-section of the gastric epithelium obtained from Group 5 cimetidine treated positive control rats showing minimal disruption of the gastric epithelium and blood stained mucosa.

RESULTS AND DISCUSSION

Effects of the extract of *Citrullus lanatus* on gastric juice volume, gastric acidity, ulcer index and percentage ulcer inhibition

Table 1 shows the effects of the extract the rind of *Citrullus lanatus* on gastric juice volume, gastric juice acid concentration, mean ulcer index and percentage ulcer inhibition in male Wistar rats in the present study. At doses of 200 and 500 mg/kg body weight the extract significantly reduced gastric juice volume in a dose dependent manner ($p<0.05$), compared to rats in the control group (Group 1). This effect is similar to that of the administration of cimetidine at a dose of 200mg/kg body weight seen amongst rats in Group 5. Similarly, at all doses administered, the extract significantly reduced gastric juice acid concentration amongst Groups 2, 3 and 4 rats ($p<0.05$), an effect comparable to that of cimetidine at a dose of 200mg/kg body weight. Furthermore, at all doses administered, the extract significantly reduced the mean ulcer index in a dose dependent manner ($p<0.05$) compared to rats in the control group (Group 1). This effect of the extract was observed to be also similar to those induced by the administration of cimetidine at a dose of 200mg/kg body weight amongst Group 5 rats. At all doses, administration of the extract caused a significant increase in percentage ulcer inhibition in a dose dependent manner ($p<0.05$), similar to the effect of cimetidine and comparable to rats in the control group (Group 1).

Histologic changes in the gastric epithelium

Plate A is a cross section of the gastric epithelium obtained from Group 1 ulcerated and untreated (control) rats showing blood stained hemorrhagic ulcers, a disrupted epithelium and blood stained mucosa. Plate B is a cross section of the gastric epithelium obtained from Group 2 low dose extract treated rats showing severe disintegration of the epithelial cells. Plate C is a cross section of the gastric epithelium obtained from Group 3 medium dose extract treated rats showing severe erosion of the mucosa layers and evidence of mild ulceration. Plate D is a cross-section of gastric epithelium obtained from Group 4 high dose extract treated rats showing a fairly intact and tightly arranged epithelial cells with slight disruption of the uppermost epithelium of the mucosa. Plate E is a cross-section of the gastric epithelium obtained from Group 5 cimetidine treated positive control rats showing minimal disruption of the gastric epithelium and blood stained mucosa.

The present study attempted to determine the possible beneficial effects of the extract of the rind of *Citrullus lanatus* against indomethacin induced ulceration of the gastric mucosa in male Wistar rats. The results obtained indicating a reduction in gastric juice volume, gastric juice acid concentration and mean ulcer index and an increase in percent ulcer inhibition, clearly suggest that the extract of *Citrullus lanatus* apparently protected the gastric mucosa against indomethacin induced ulceration in a dose dependent manner; comparable to the effect of cimetidine a known histamine (H_2) receptor antagonist used commonly in the management of peptic ulcer disease. The possible active ingredients in the rind of *Citrullus lanatus* responsible for its protective effects are at present uncertain. However, phytochemical screening has revealed the presence of tannins and flavonoids amongst other constituents.[8] These compounds have been shown to have a contributory role in ameliorating the development of peptic ulcers: tannins are known to 'tar' the outermost layer of the gastric mucosa rendering it less permeable and more resistant to chemical and mechanical injury or irritation.[16] It is possible that flavonoids present in the rind of *Citrullus lanatus* may also play a role in this regard: flavonoids possess antioxidant properties in addition to strengthening the mucosal defense system through stimulation of gastric mucus secretion.[17]

The findings of this study also suggest that watermelon rind causes a significant reduction in gastric acid secretion. When compared with the control group, all treatment groups exhibited a decrease in gastric acid output. The results also suggest that watermelon rind inhibits gastric acid secretion in a dose dependent manner. It has been earlier reported that nitric oxide inhibits gastric acid secretion in rats.[18] Watermelon rind is an edible source of citrulline; a compound vital

for the production of nitric oxide. Watermelon consumption increases the level of citrulline significantly.[19] It is therefore safe to propose that one of the possible mechanisms by which watermelon rind causes a decrease in gastric acid secretion is by increasing citrulline levels thereby stimulating an increase in nitric oxide production. This will lead to a decrease in gastric acid secretion as confirmed by previous reports [20, 21] which in turn accounts, at least in part, for the observed gastro-protective effects of watermelon rind in indomethacin-induced gastric ulceration described in the present study. It is also pertinent to state that other mechanisms of gastric mucosal protection such as increase in mucus secretion, stimulation of prostaglandins release, increase in mucosal blood flow etc., could also be potentially involved in the anti-ulcerative actions of watermelon rind. However, these effects were not investigated in the present study. The histological changes described in the present study are fairly consistent with findings on gastric juice volume, gastric juice acid concentration, gastric ulcer index, and percentage ulcer inhibition as presented in Table 1.

In conclusion, the results of the present study show that the methanolic extract of the rind of *Citrullus lanatus* reduces gastric juice volume, gastric juice acid concentration, mean ulcer index and increases percentage ulcer inhibition following indomethacin induced gastric ulceration in male Wistar rats; these findings along with the histological changes described suggests a possible beneficial and potential gastric protective and anti-ulcer effects of the extract. The results indicate a possible beneficial effect of the rind of *Citrullus lanatus* and perhaps a therapeutic potential in the management of peptic ulcer disease. We therefore recommend further studies in this regard.

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Characterization of Cucumis sativus (Linnaeus) Mucilage and its Excipient Potentials in Metronidazole Tablet Formulation

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ABSTRACT

The objective was to characterize *Cucumis sativus* mucilage (CSM) and evaluate its binding potentials in metronidazole tablet formulation.

Characterization was done using proximate, elemental, material and rheological properties including FTIR. Tablets were produced by wet granulation with CSM, corn starch or acacia as binder (1-4%$^{w}/_{w}$) and evaluated using mechanical and release properties.

Generally, the properties of CSM showed that it can be used for oral formulations and it has significantly higher swelling index. The mechanical properties of metronidazole tablets as described by crushing strength–friability ratio ranked acacia > corn starch > CSM. An increase in the concentration of CSM produced faster disintegration for all tablets as opposed to corn starch and acacia which led to slower disintegration. The dissolution profiles of the tablets from CSM (4%$^{w}/_{w}$) showed highest similarity (f2=61.60) to those of acacia at 2%$^{w}/_{w}$.

CSM has excipient potentials that can be further developed for tablet production.

Keywords: *Cucumis sativus* mucilage, binding potential, metronidazole tablet.

INTRODUCTION

Excipient development is a research area that leads to discovery of new materials that may have assets similar to existing ones or sometimes offer improved properties to a dosage form. More so, development from locally available raw materials lowers manufacturing costs and boosts national status by creating jobs in different areas like planting, harvesting and storage systems[1]. In addition, the need to search for newer materials cannot be overemphasized especial-

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ly in oral drug delivery systems. Despite diverse advances in oral drug delivery systems, tablets remain tangible due to its being compact, simple and easy to use; offers opportunity for mass production, variability of designs can also be prepared compared to liquid dosage forms. Tablets also accounts for over 60% of solid dosage forms because of its high patient acceptance, compliance and adherence to medication.

Excipients used in tablet formulation include diluents, lubricants, glidants, sweetners, disintegrants and binders. Binders are agents which may be added in wet or dry form to assist the cohesiveness of powders thus ensuring the formation of intact tablets[2]. It has been estimated that less than 20 percent of active pharmaceutical materials can be compressed directly into tablets, the rest requires the use of binders either for direct compression or wet granulation. The use of natural polymers such as starches, gums and mucilages as pharmaceutical excipients grants several benefits such as biological compatibility, ready availability, non-toxicity, low cost and ease of chemical modification to suit diverse formulation requirements in comparison to synthetic ones[3].

Cucumis sativus Linnaeus (Family Cucurbitaceae) is commonly known as cucumber and was originally cultivated in Southern Asia, but now grows in most continents thriving both in temperate and tropical regions. Cucumber is a frost-sensitive annual plant whose heat requirement is greater than that for most common vegetables. It has a hairy climbing, trailing, or creeping stem, and is often grown on frames or trellises. The leaves are hairy and have 3–5 lobes; branched tendrils at leaf axes which support climbing or creeping. The plant bears cylindrical edible fruit when ripe with skins which can be smooth and thin, or thick and rough.

Jyoti and colleagues[4] studied the proximate and antimicrobial properties of cucumber extract and concluded that it has nutritional ingredients with considerable antimicrobial properties. Cucumber fruit has high water content, low calories, potential antidiabetic, lipid lowering and antioxidant activity[5]. Furthermore, several bioactive compounds have been isolated from cucumber including cucurbitacins, cucumegastigmanes I and II, cucumerin A and B, vitexin and orientin[5]. The medicinal properties of this valuable crop have been widely studied, and different parts of it have been acclaimed useful.

Cucumis sativus fruit has mucilage inside which helps the seeds to attach to the pulp and the excipient potentials of this mucilage is largely unharnessed. In our first study of this novel mucilage, the emulsifying properties in olive oil and liquid paraffin emulsions was assessed and the outcome showed that *Cucumis sativus* mucilage (CSM) may be used as a primary emulsifying agent for o/w emul-

sions[6]. To further increase the application of CSM, there is need to characterize it and evaluate its excipient properties in tablet formulation. In the present study therefore, an attempt was made to characterize the mucilage and its excipient potentials in metronidazole tablet formulation were evaluated in comparison with acacia and corn starch. The main finding showed that *Cucumis sativus* mucilage could be further developed for excipient use in tablet formulations.

METHODOLOGY

Materials

The materials used include Metronidazole, corn starch, lactose and xylene all obtained from BDH Laboratories (London, UK). Ethanol and diethyl ether were procured from Sigma (St Louis, MO, USA). Fruits of *Cucumis sativus* were purchased from Eleyele market, Ibadan town, South-west, Nigeria. All other chemicals and reagents were of analytical grade.

Methods

Extraction and purification of *Cucumis sativus* mucilage

The fruits of cucumber were cut open and the internal part was scooped out and hydrated for 72 h in chloroform-water DS (double strength) with intermittent stirring. The extraneous materials were removed by straining through a muslin cloth. To the filtrate, absolute ethanol (96%$^{v}/_{v}$) was added to precipitate the polymeric material. The precipitated mucilage was filtered, washed with diethyl ether, dried in a hot air oven (Laboratory oven TT-9083; Techmel and Techmel, TX, USA) at 40 ^{o}C, milled and sieved with 250 μm sieve and stored in airtight containers[7].

Proximate and elemental composition of *Cucumis sativus* mucilage

The ash, crude fat and crude fibre contents were determined using the Association of Official Analytical Chemists (AOAC) methods[8]**.** The protein content was calculated from the nitrogen content determined by elemental analysis using Atomic Absorption Spectrophotometer (AAS, Model 2500 Torontech Inc., Toronto, ON, Canada) using a conversion factor of 6.25. All determinations were done in triplicate and results were presented as mean and standard deviation.

Furthermore, elemental composition for cucumber mucilage was obtained by digesting an accurately weighed amount (2 g) of the sample to obtain a solution. The solution thus obtained was analysed for heavy metals using Atomic Absorption Spectrophotometer (AAS, Model 2500 Torontech Inc., Toronto, ON, Canada). The instrument was calibrated using manually prepared standard solutions of respective metals.

Density measurements

The loose bulk volume of the CSM or the granules was determined by pouring 30 g of powder at an angle of 45 ^{o} through a funnel into a 50 mL glass measuring cylinder and the height was measured. The density was calculated from the ratio of the mass to the volume. The tapped volume was measured by applying 100 taps to 30 g of CSM or granules in a graduated glass cylinder at a standardized rate of 38 taps per min[9]. The particle density was measured using a 50 mL liquid pycnometer bottle with xylene as the displacement fluid[10].

Hausner's ratio and Carr's index determination

The Hausner's ratio was determined as the ratio of the initial bulk volume to the tapped volume.

The Carr's index was calculated from the results obtained from the bulk and tapped densities by using the equation below:

$$\text{Carr's index (\%)} = \frac{\text{Tapped density-Bulk density}}{\text{Tapped density}} \text{ x 100} \quad (1)$$

Swelling index

The swelling index of the excipients was determined using a standard method[11]. Excipient (2.5 g) sample was poured into a 100 mL measuring cylinder (v_1) and distilled water (40 mL) was added. The dispersion was well shaken for 5 min, made up to 50 mL with distilled water and allowed to stand for 24 h before sedimentation volume was obtained (v_2). The swelling power was then calculated as follows:

$$\text{Swelling index} = {}^{V_2}/_{V_1} \quad (2)$$

Solubility

The solubility of the CSM was determined using the method of Leach *et al* [12]. CSM (1 g) sample (w) was weighed into a conical flask; distilled water (15 mL) was then added and shaken slowly for 5 min. This was then transferred into a pre-weighed centrifuge tube; distilled water (7.5 mL) was added and centrifuged at 2200 rpm for 20 min. The supernatant was carefully decanted into a pre-weighed dish (W_2), dried at 100 ^{o}C to constant weight (W_3) and cooled for 30 min. The solubility was determined using the equation:

$$\text{Solubility (\%)} = \{[W_2\text{-}W_3]\}/w \text{ x } 100 \quad (3)$$

Water absorption capacity

The water absorption capacity (WAC) was determined using the method of Solsulski[13]. CSM (2.5 g) was added into a pre-weighed 50 mL centrifuge tube, distilled water (15 mL) was added and agitated on a vortex mixer for 2 min. The

mixture was centrifuged at 400 rpm for 20 min and the supernatant was decanted. The residue was weighed (W_1) and the absorbed water was removed by drying the residue at 60 °C to constant weight (w_2) in an oven. WAC was then expressed as:

$$WAC = \{(W_1 - W_2)/2.5\} \times 100 \quad (4)$$

Determination of Rheological profiles

The rheological profiles of the excipients were obtained using a heating and cooling viscometer coupled with Thermocline Windows Software (Rapid Viscosity Analyzer series 3 RVA, Newport Scientific Pty Ltd. Warriewood, Australia). Excipient (3 g), was weighed into the canister, distilled water (25 mL) was added and the slurry was heated under constant rate of shear. The increase in viscosity of the material was measured as torque on the spindle and the viscoamylographs obtained.

Microscopy of particles

The particle size and shape of the excipients were determined by optical microscopy on approximately 100 particles for each. The particles of each excipient sample was thinly spread over glass slides and observed under a light microscope (Olympus BX40 Research Microscope, New York Microscope Company, NewYork, USA) and photomicrographs were taken using an attached Digital Camera (Cannon EOS SL1, Cannon Inc, Tokyo, Japan).

Fourier Transform Infrared (FTIR) Spectroscopy

Sample (2 mg) was mixed with 100 mg of KBr and compressed into pellets in a hydraulic press. The pellet was placed in the light path and the spectrum was recorded in the wavelength region of 4000- 350cm^{-1} using FTIR Spectrophotometer (BXV5.3.1, PerkinElmer Inc., Massachusets□, U.S.A). The details of functional groups and assignment obtained from the FTIR spectroscopy were done using a Table-driven Infrared application (FTIR-Interpret software IR Pal, Version 2.0).

Preparation of granules

Different batches (100 g) of metronidazole granules with a basic formula containing Metronidazole (60%$^{w}/_{w}$), Corn starch (10%$^{w}/_{w}$) and Lactose (30%$^{w}/_{w}$) were prepared using the wet granulation method. The required quantities were weighed out and mixed in a mortar. Mucilage of 1, 2 and 4%$^{w}/_{w}$ concentration of the binding agent (CSM, acacia gum or corn starch) were prepared in distilled water and used to moisten the powder mixture. The wet masses obtained were then granulated by passing it through a 1400 μm mesh size and dried in a hot

air oven (Laboratory oven TT-9083: Techmel and Techmel, USA) at 50 °C. Dry-screening was done through a 1000 μm mesh sieve before being stored in an air tight container.

Tablet compression

The granules produced from the various batches of the formulation were compressed into tablets (400 mg) using a single punch Carver hydraulic hand press (Model C, Carver Inc., Menomonee Falls, Wisconsin, USA) at pre-determined pressures. Tablet compression was done for 30 sec using a die having diameter of 10.5 mm. A dispersion of magnesium stearate in acetone ($1\%^{w}/_{v}$) was used to lubricate the die and punch surfaces before compression. After compression of the tablets, they were stored over silica gel for 24 h.

Mechanical properties of the tablets

The crushing strength (Cs) of the tablets was determined using the tablet hardness tester (MHT-100, Model P&M 01, Pharma Alliance Group, California, USA). The tablet was placed between the anvil and the spindle of the tester. The force at which the tablet broke into two halves was then recorded.

Tablet friability (Fr) was determined using a DBK Friabilator (Model 40FTA01, DBK Instruments, Mumbai, India) at a speed of 25 rpm for 4 min. Five tablets were selected at random, weighed with an electronic balance and transferred into the drum of the Friabilator before it was switched on to begin its rotation. The tablets were then dusted and re-weighed from which the percentage loss was calculated.

The crushing strength-friability ratios (Cs/Fr) of the tablets were then calculated from the values of crushing strength and friability.

Release properties (Disintegration and dissolution) of the tablets

The disintegration time (Dt) of the tablets was determined in distilled water at 37 ± 0.5 °C using the DBK disintegration testing apparatus (Type 40TDA01, DBK Instruments, Mumbai, India). The time taken for the tablets to disintegrate and pass through the mesh was recorded. Determinations were made in triplicates.

The disintegration efficiency ratio (DER) for the tablets was calculated as a ratio of Cs, Fr and Dt. The DER, a measure of the balance between mechanical and disintegration property of tablets, was obtained using equation 5 where Cs is crushing strength, Fr is friability, and Dt is disintegration time.

$$DER = \frac{Cs/Fr}{Dt} \quad (5)$$

The dissolution profiles of the tablets were determined using the DBK dissolu-

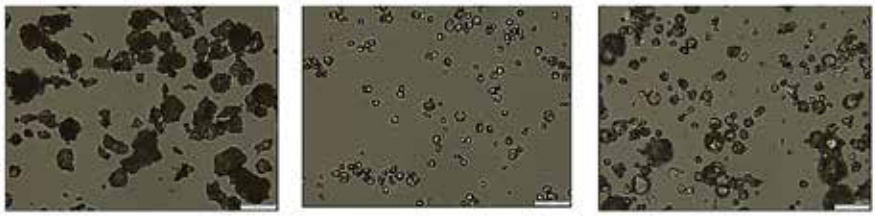
tion test apparatus (Type 40DRT01, DBK Instruments, Mumbai, India). Each tablet was placed in a cylindrical basket of stainless steel wire mesh which is attached to a rotor that can be regulated to varying speed and suspended in a glass vessel containing 900 mL of 0.1N HCl. The glass vessel was immersed in a water bath controlled at a temperature of 37 ± 0.5 °C. The apparatus was set to rotate at 50 rpm and 5 mL of the dissolution medium was removed from the glass vessel at specific time intervals and replaced simultaneously with an equal volume of fresh dissolution medium. The absorbance of the removed samples was measured at a wavelength of 277 nm and the drug concentration determined mathematically.

Data presentation and analysis

All experiments were performed using appropriate replicates and data were presented as mean ± standard deviation (SD) except for ratios. DD solver software was used to obtain dissolution times and compare the dissolution profiles by determining the similarity factor (f_2).

RESULTS AND DISCUSSION

The mucilage extracted from *Cucumis sativus* fruit was observed to be cream in colour, with a pleasant odour and a rough texture which is similar to *Chrysophyllum albidum* mucilage[14] and *Hibiscus esculenta* gum[15]. The photomicrographs of the excipients in Figure 1 showed that CSM particles are irregularly shaped and the surface is rough contrary to corn starch particles which are spherical. Acacia particles also have irregular shapes. The shape of the particles of a material is related to the botanical source of such materials and it affects how well such materials will pack in addition to other factors.



Cucumis sativus mucilage *Corn starch* *Acacia gum*

Figure 1. Photomicrographs of the particles of excipients (X 400)

The results obtained from the proximate analysis of CSM are presented in Table 1. It showed that CSM contains protein, fibre, ash, fat and carbohydrate in varying degrees. As expected, carbohydrate has the highest percentage (57.65%) indicating that CSM is a polysaccharide, protein content is much less than carbohydrate but, the content is important as well because gums and mucilages have

nitrogenous compounds. Generally, protein, fat and ash are part of the minor constituents of gums and mucilages and their presence influences the functional performance such as pasting and gelling behaviour[16]. The moisture content of *Cucumis sativus* mucilage was 11.23%$^{w}/_{w}$. Generally, gums and mucilages absorb moisture from the surrounding air and this usually depends on the properties of the material and the environmental humidity. According to Williams and Phillips[17], the moisture in a material should be moderate, otherwise enzymatic activation could set in motion the process of degradation.

Table 1. Proximate and elemental composition of *Cucumis sativus* mucilage

Proximate composition	Crude protein (%)		Crude Fibre (%)		Crude Ash (%)		Crude Fat (%)		Carbohydrate (%)		Moisture Content (%)	
	20.13		40.19		3.59		2.12		57.65		11.23	
Elements	Mg	K	Ca	Na	Fe	Cu	Co	Cd	Pb	Ni	Mn	Zn
(%)	0.11	1.18	0.14	38.11	9.01	2. 11	0.00	0.00	0.00	0.00	1.50	3.053

The results obtained from the elemental analysis is presented also in Table 1 and it showed that *Cucumis sativus* mucilage contained Calcium, Magnesium, Potassium, Sodium, Manganese, Iron, Copper, and Zinc which are not harmful to the body system while heavy metals such as Lead, Cadmium, Cobalt, Chromium and Nickel were found to be absent. This gives CSM an acceptable biological profile hence it may be useful as a food or drug additive.

Material properties

The material properties of CSM and the reference standards (corn starch and acacia gum) are presented in Table 2. The bulk and tapped densities of the excipients were in the order CSM <corn starch <acacia while particle density ranked- corn starch <CSM <acacia. In addition, particle size was in the order of corn starch <acacia <CSM. The particle arrangement, packing and the entire compaction profile of a material can be perceived by the bulk and tapped densities[18]. CSM showed lower values of these parameters thus indicating that acacia and corn starch have higher capacity in reducing die fill-volume during tablet compression. Furthermore, a high bulk density, that is a low porosity, will result in a low deformation potential, due to a lack of space for deformation during compression causing less intimate contact between the particles within the tablets ultimately resulting in weaker tablets[19,20]. There were no significant differences ($p>0.05$) between the particle density of acacia and CSM while that of corn starch was significantly ($p<0.05$) lower than for both. Generally, materials with higher particle density may require greater compression forces but usually produce tablets with improved mechanical strength[21]. The particle size of CSM was

significantly higher ($p<0.05$) than for corn starch and acacia. The dispersion of gums and mucilages has been reported to improve with increase in particle size though materials with lower particle sizes shows faster dissolution[22]. It implies therefore that where fast dissolution rate is not crucial, higher particle size may be of immense benefit.

Table 2. Material properties

Parameters	Cucumis sativus	Corn starch	Acacia
Bulk density (g/cm^3)	0.411 ± 0.023	0.468 ± 0.022	0.581 ± 0.335
Tapped density (g/cm^3)	0.563 ± 0.112	0.625 ± 0.113	0.778 ± 0.447
Particle density(g/cm^3)	1.349 ± 0.025	1.233 ± 0.039	1.374 ± 0.37
Particle size	64.871 ± 27.959	13.257 ± 3.298	32.058 ± 10.193
Carr's index (%)	26.998 ± 5.223	25.121 ± 6.004	25.310 ± 3.448
Hausner's ratio	1.420 ± 0.552	1.360 ± 0.235	1.339 ± 0.110
Angle of repose (^{0})	27.860 ± 2.443	30.591 ± 3.211	16.802 ± 4.009
Compressibility index (%)	26.998± 6.443	26.560 ± 4.335	25.311 ± 3.898
Swelling index (%)	310.091 ± 5.678	3.749 ± 0.113	128.314 ± 6.887
Solubility (%)	22.013 ± 8.321	6.029 ± 2.543	44.435 ± 6.237
Water absorption capacity (%)	80.002 ± 8.667	68.012 ± 7.532	58.956 ± 6.745

The results of Carr's index for the excipients ranked CSM > acacia > Corn starch without significant differences ($p>0.05$); Hausner's ratio also ranked CSM > Corn starch > acacia while angle of repose was in the order of corn starch > CSM >> acacia. There were no significant differences ($p>0.05$) between the Hausner's ratio and acacia yielded significantly lower ($p<0.05$) angle of repose in comparison with CSM and corn starch. The high values of Carr's index, Hausner's ratio and angle of repose for the excipients showed that they do not possess good flow properties[21]. The Carr's index is an expression of compressibility of a powdered material while Hausner's ratio describes the degree of densification that can occur due to feed hopper vibration during tableting procedures.

The swelling indices of the materials were in the order of CSM > acacia >> corn starch with significant ($p<0.001$) differences. The swelling index of an excipient is crucial in tablet formulation as it impacts on disintegration properties of the tablet. Materials with high swelling index may confer faster disintegration compared to those with lower values[23]. Furthermore, the solubility ranked acacia>CSM>corn starch while water absorption capacities were in the order of CSM>corn starch>acacia. The differences thus observed in these properties may be attributed to divergent intensities of molecular association forces inside the particles. Higher water absorption capacity has been ascribed to loose structure

of the polymer particles while low values imply firmness. Generally, the materials are from different botanical sources hence there is variation in their material properties.

The parameters obtained from the viscoamylographs of the excipients are presented in Table 3. The peak viscosity ranked corn starch>CSM>>acacia. The peak viscosity of corn starch was significantly higher than for CSM and acacia because the materials were subjected to heating and starches gelatinize when subjected to heating. Generally, viscosity properties of excipients become useful when agglomeration of particles is required during tableting procedures. Materials with moderate to high viscosity may demonstrate superior binding properties during granulation compared to those with low values. The high peak temperature shows that the materials are not going to be adversely affected by the heat generated during tablet compression. Materials with low peak temperature are sensitive to heat and may thus form gel or paste which is not desired during tablet compression[24].

Table 3. Parameters obtained from viscoamylography of the excipients

Parameter	Cucumis sativus mucilage	Corn starch	Acacia gum
Peak viscosity (cp)	135.50	200.57	14.50
Trough viscosity	110.00	151.75	11.50
Breakdown viscosity (cP)	25.50	51.88	44.00
Final viscosity (cP)	201.00	192.67	18.00
Setback from trough (cp)	91.00	41.75	7.00
Peak temperature (^{0}C)	97.00	90.97	60.00
Peak time (min)	7.00	6.03	1.60

Fourier Transform Infrared Spectroscopy

The Fourier transform infrared spectroscopy (FTIR) of the excipients is shown in Figure 2. The FTIR interpret software used to analyze the spectra showed different functional groups depending on the material involved. For *Cucumis sativus* and acacia, the functional groups shown are alcohols, alkane, alkenes, amides, carboxylic acids, amines and alkyl halides. The FTIR of corn starch also revealed the presence of carboxylic acid, alkanes, alkenes, amides, aromatic compounds and alkyl halides. In general, the functional groups thus identified showed that these materials are polymeric in nature.

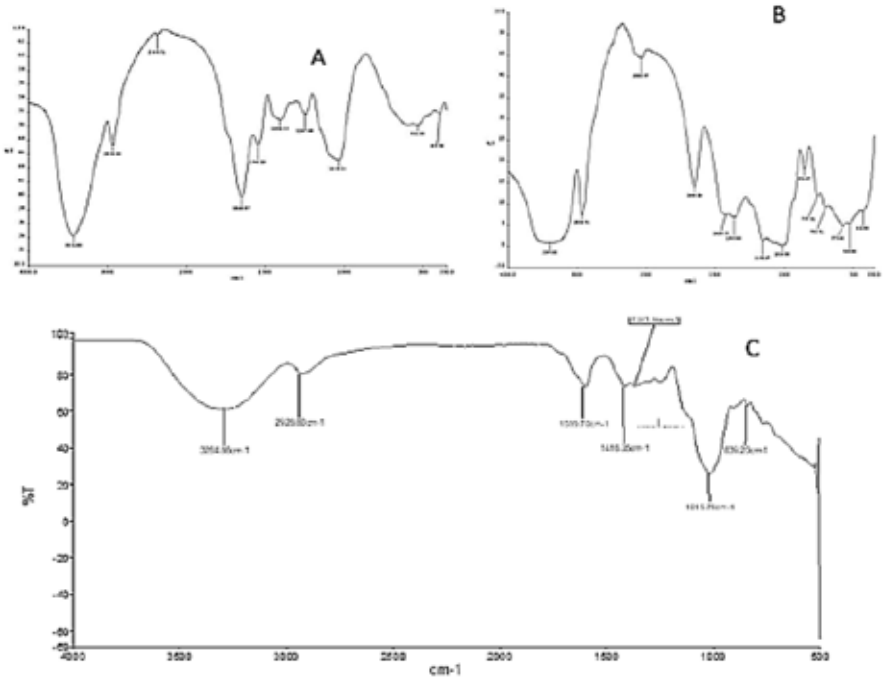


Figure 2. FTIR spectroscopy of the excipients (A-CSM, B-Corn starch, C-Acacia)

Mechanical properties of tablet

Tables 4, 5 and 6 shows the mechanical (Cs, Fr & Cs/Fr) properties of tablets prepared using the excipients (CSM, corn starch and acacia) as binding agents. The Cs of tablets prepared using the excipients generally increased with increase in binder concentration and compression pressure. The Cs were significantly ($p<0.05$) lower at every pressure for tablets prepared without a binder although the Cs for all the tablets were observed to be somehow small for tablets containing CSM. Tablets prepared using corn starch mucilage as binder had significantly higher Cs in comparison to those prepared using CSM. Generally, Cs values ranked CSM<corn starch<acacia.

Crushing strength is a measure of the bond strength and ability of tablets to withstand the stress of packaging, transportation and handling. It is dependent on the amount of binder solution used, compression pressure and also the tablet dimensions. It is also a function of the weight, density and porosity of materials used and the space between the upper and the lower punches at the moment of compression. Generally, an increase in the concentration of a binding agent has been shown to cause an increased particle–particle contact points resulting in the creation of more solid bonds; resulting in tablets with more resistance to fracture and abrasion[25]. It is not surprising therefore that the Cs of tablets containing

higher concentrations of the binders was improved. The effect of compression pressure on the Cs of the tablets was also expected because as forces are increased during compression, an increase occurs in the packing fraction of the granulation leading to a decrease in intra and inter-granular voids thus creating more contact points hence an increase in the degree of bonding between the particles[26].

The friability of all tablets is shown in Tables 4, 5 and 6. The results revealed that tablets prepared without using a binding agent were extremely friable with significant differences ($p<0.01$) in comparison to those with binders. Generally, the friability of all tablets reduced with increased compression pressure and concentration of binder. The ranking of friability among tablets produced with the different binders was CSM>acacia>corn starch showing that corn starch and acacia produced stronger tablets than CSM. Friability is a mechanical property of tablets with compendial specification of not more than 1%[27]. Most tablets in this study did not comply with official specification for friability and it could be that higher binder concentrations would be preferable. Generally, binders could be used up to 10% but lower concentrations have been used in a previous work[15] after which this study was patterned. While crushing strength is considered as a bulk deformation of tablets, friability is related to surface deformation which may be enhanced by tablet morphology[28]. Tablets with rough surfaces are usually more friable than smooth ones. Ideally, friability should decrease with increase in compression pressure and binder concentration[28]. This is the pattern followed by the tablets produced in this study, if higher binder concentrations were used however, the tablet friability would likely reduce significantly.

Table 4. Mechanical (Cs, Fr & Cs/Fr) and release (Dt & DER) properties of metronidazole tablets prepared using *Cucumis sativus* mucilage as excipient

Binder concentration ($\%^{w}/_{w}$)	Compression pressure (MN/m^2)	Cs (N)	Fr (%)	Cs/Fr	Dt (min)	DER
0.0 (No binder)	56.660	1.467 ± 0.462	93.550	0.016	0.142 ± 0.019	0.113
	84.840	5.067 ± 0.231	62.231	0.081	0.754 ± 0.120	0.107
	113.130	9.567 ± 0.611	27.320	0.552	0.787 ± 0.093	0.701
1.00	56.660	8.823 ± 0.042	31.232	0.283	0.471 ± 0.468	0.601
	84.840	10.931 ± 0.611	25.743	0.425	0.547 ± 0.024	0.777
	113.130	13.684 ±1.058	20.662	0.662	0.693 ± 0.019	0.955
2.00	56.660	10.870 ± 0.231	38.811	0.280	0.241 ± 0.072	1.162
	84.840	13.233 ± 0.401	26.090	0.507	0.614 ± 0.521	0.826
	113.130	18.623 ±1.058	18.440	1.010	0.680 ± 0.431	1.485
4.00	56.660	19.353 ± 1.007	15.501	1.249	0.193 ± 0.051	6.472
	84.840	19.977 ± 1.888	11.820	1.690	0.271 ± 0.093	6.236
	113.130	21.763 ± 3.274	4.000	5.441	0.390 ± 0.037	13.951

Table 5. Mechanical (Cs, Fr & Cs/Fr) and release (Dt, DER) properties for metronidazole tablets prepared using Corn starch as excipient

Binder concentration (%w/w)	Compression pressure (MN/m²)	Cs (N)	Fr (%)	Cs/Fr	Dt (min)	DER
0.0 (No binder)	56.660	1.467 ± 0.462	93.550	0.016	0.142 ± 0.019	0.113
	84.840	5.067 ± 0.231	62.231	0.081	0.754 ± 0.120	0.107
	113.130	9.567 ± 0.611	27.320	0.552	0.787 ± 0.093	0.701
1.00	56.660	15.901 ± 1.670	2.101	7.572	1.226 ± 0.072	6.176
	84.840	17.402 ± 1.210	1.569	11.091	1.472 ± 0.017	7.535
	113.130	20.900 ± 3.02	1.927	10.846	2.203 ± 0.300	4.923
2.00	56.660	17.10 ± 0.523	1.677	10.197	1.222 ± 0.210	8.345
	84.840	18.980 ± 2.433	1.469	12.920	1.567 ± 0.170	8.245
	113.130	24.903 ± 5.410	1.227	20.296	2.623 ± 0.038	7.738
4.00	56.660	23.374 ± 1.661	1.366	17.111	2.244 ± 0.032	7.625
	84.840	29.030 ± 0.231	1.213	23.932	3.293 ± 0.017	7.268
	113.130	32.431 ± 4.452	1.070	30.309	4.334 ± 0.108	6.993

Table 6. Mechanical (Cs, Fr & Cs/Fr) and release (Dt, DER) properties for metronidazole tablets prepared using acacia gum as excipient

Binder concentration (%w/w)	Compression pressure (MN/m²)	Cs (N)	Fr (%)	Cs/Fr	Dt (min)	DER
0.0 (No binder)	56.660	1.467 ± 0.462	93.550	0.016	0.142 ± 0.019	0.113
	84.840	5.067 ± 0.231	62.231	0.081	0.754 ± 0.120	0.107
	113.130	9.567 ± 0.611	27.320	0.552	0.787 ± 0.093	0.701
1.00	56.660	19.800± 0.67	3.298	6.004	0.588 ± 0.032	10.211
	84.840	24.740± 2.021	2.683	9.221	1.347 ± 0.034	6.846
	113.130	29.430± 2.402	2.212	13.305	1.453 ± 0.076	9.157
2.00	56.660	26.410 ± 0.952	2.434	10.850	1.082 ± 0.023	10.028
	84.840	29.950 ± 3.143	2.353	12.728	1.256 ± 0.062	10.134
	113.130	34.510 ± 3.241	1.542	22.380	1.372 ± 0.103	16.312
4.00	56.660	27.537 ± 3.006	1.793	15.358	1.734 ± 0.204	8.857
	84.840	35.283 ± 4.423	1.451	24.316	2.309 ± 0.107	10.531
	113.130	56.453 ± 5.045	1.184	47.680	2.937 ± 0.440	16.234

The mechanical strength of tablets can also be measured by the crushing strength – friability ratio (Cs/Fr). The Cs/Fr is a stronger parameter for determining the mechanical strength of tablets and the higher the Cs/Fr, the stronger the tablet since it provides a balance between tablet weakness and strength[25].

The Cs/Fr for the tablets was also presented in Tables 4, 5 and 6. Generally, the results showed that Cs/Fr increased with increase in compression pressure and

binder concentration with tablets prepared from acacia having the highest values. The ranking of Cs/Fr was acacia>corn starch> CSM. Tablets produced without using binding agents had significantly ($p<0.05$) low Cs/Fr compared to the others showing the usefulness of the binders for optimum mechanical properties.

Release properties of tablets

The release properties-Disintegration time (Dt) and Disintegration efficiency ratio (DER) of the tablet formulations prepared using the different binding agents are also shown in Tables 4, 5 and 6. Generally, all tablets prepared with the excipients passed the disintegration time test with values much less than the 15 min stipulated in the official compendia. In addition, the disintegration times increased with increase in compression pressure. It was observed also, that an increase in the concentration of CSM produced faster disintegration for all tablets compressed at the same pressure while an increase in the concentration of corn starch and acacia led to increased Dt. The disintegration for all the tablets could have been fast because of the high friability values, while the reduced disintegration time as CSM concentration increased could be a pointer to potential disintegration property of the excipient. Swelling has been reported as the most accepted mechanism for tablet disintegration[29]. In swelling, there is dimensional increase in size of particles in an omni-directional pattern and this pushes apart the adjoining components, thereby instigating break-up of the tablet matrix[30]. It is possible therefore since CSM has a high swelling index, this property may cause it to potentiate the effect of the disintegrant in the formulation.

Disintegration efficiency ratio (DER) otherwise known as Cs/Fr/Dt has been reported as a superior index for assessing tablet quality[31]. This parameter simultaneously evaluates tablet strength (crushing strength) and weakness (friability). In addition, DER also evaluates the negative effects of friability on Dt and the overall usefulness of a binder in a formulation[31]. In general, a higher value of DER suggests an enhanced balance between binding and disintegration properties[32]. The DER of tablets produced with CSM increased with increase in compression pressure and excipient concentration showing that tablets produced with 4% binder concentration and a compression pressure of 113.13MN/m2 were optimal. Generally, tablets without any binder had the least DER with significant differences ($p<0.001$) in comparison to all the tablets with binding agent showing a poor balance of mechanical and disintegration properties.

Dissolution is a significant parameter in tablet evaluation as tablets must dissolve before absorption can occur and may also be a rate-limiting step in drug bioavailability. Since drug absorption depends on amount dissolved, it is therefore imperative that tablets demonstrate acceptable dissolution characteristics.

Drugs having poor dissolution profile may fail to elicit its therapeutic action since availability of such drug in the systemic circulation cannot be guaranteed. Representative drug dissolution profiles for tablets produced with 2 and 4%$^{w}/_{w}$ of the excipients and compression pressure of 113.13 MN/m^2 are shown in Figure 3. The choice was based on the general outlook of the disintegration efficiency ratio. The higher the DER, the optimal the balance of mechanical and release properties of tablets. The amount of drug dissolved was observed to increase with time and formulations containing lower amount of binding excipient also released faster than those with higher concentration. From the plots, dissolution times - t_{50} and t_{80} (the time required for 50 and 80% of the drug to be released) were determined and the values are presented in Table 7. The dissolution times increased with increase in the concentration of the binding agent in the order- Corn starch <CSM<acacia implying that corn starch showed faster release than the other excipients.

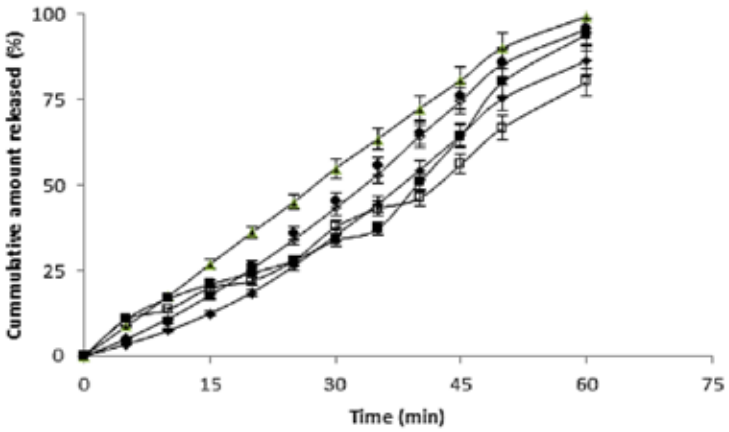


Figure 3. Dissolution profiles of the tablet formulations (2% Cucumis sativus, 4% Cucumis sativus, 2% Corn starch, 4% Corn starch l, 2% acacia n, 4% acacia o).

Table 7. Dissolution times for tablets prepared with the excipients

Excipient	t_{50} (min)	t_{80} (min)
CSM (2%)	30.389	48.623
CSM (4%)	34.727	55.623
Corn starch (2%)	27.705	44.328
Corn starch (4%)	29.994	47.991
Acacia (2%)	35.524	56.838
Acacia (4%)	39.412	63.060

Since the dissolution profiles from the formulations visually looked similar, there was a need to conduct further verification. A model independent mathematical

approach (similarity factor, f2) proposed by Moore and Flanner[33] was employed to compare the dissolution profiles using pairwise procedures on DDSolver. The f_2 is a mathematical treatment that uses the mean dissolution values from the curves at each time point. When the f_2 value is 100 then the profiles are identical, when it is 50, it indicates that there is an average difference of 10% at all measured points. Generally, an f_2 value between 50 and 100 designates sameness or equivalence of the dissolution profiles[34]. The use of f_2 is widely emphasized by the Food and Drug Administration in various documents except when the products are rapidly dissolving (more than 85% in 15 min). The similarity factor for the dissolution profiles are presented in Table 8. None of the formulations showed identical profile i.e f_2=100. However, there were similarities between formulations containing 2% of CSM and that of corn starch or acacia ($f_2 > 50$), in addition, 4% CSM and corn starch or acacia also yielded $f_2 > 50$. The highest f_2 in the groups was between 4% CSM and 2% acacia ($f_2 = 61.60$) showing superior similarity. This may imply that where 2% acacia is needed for acceptable release, as much as 4% of CSM will be required. Other formulations as shown on the table were dissimilar in dissolution profiles. In this study, the dissolution profile similarity between acacia and CSM was stronger (higher f_2 values) than for corn starch. This is to be expected as gums and mucilages have relative resemblance than starches. These results indicate that *Cucumis sativus* mucilage have excipient potential worthy of further development.

Table 8. Similarity factors for the dissolution profiles

Dissolution profiles compared	Similarity factor (f_2)
2% CSM and 2% corn starch	54.84
2% CSM and 2% acacia	54.51
4% CSM and 4% corn starch	53.28
4% CSM and 4% acacia	61.04
4% CSM and 2% acacia	61.60
4% CSM and 2% corn starch	41.09
2% corn starch and 2% acacia	42.08
2% corn starch and 4% acacia	38.36

CONCLUSION

Cucumis sativus mucilage was successfully extracted, characterized and its binding properties evaluated in a poorly compressible drug-metronidazole. Generally, CSM properties showed that it can be used as an excipient for oral formulations; its swelling index was significantly higher compared to corn starch and acacia.

Tablets obtained using the new excipient had low crushing strength showing that it may be more useful when soft tablets are required or a higher concentra-

tion may be needed to achieve stronger tablets. The mechanical properties of metronidazole tablets as described by Cs/Fr ranked acacia>corn starch> CSM. It was observed also, that an increase in the concentration of CSM produced faster disintegration for all tablets compressed at the same pressure as opposed to corn starch and acacia which led to slower disintegration. This may be a pointer to potential disintegrant properties of CSM due to its high swelling index and the less than 1 min disintegration time for all tablets produced using it. The dissolution profiles of the tablets from CSM (4%$^{w}/_{w}$) showed highest similarity (f2=61.60) to those of acacia at 2%$^{w}/_{w}$. These results showed that CSM could be further developed for excipient use in tablet formulations.

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Evaluation of Knowledge Level About Asbestos Exposure in Urban Transformation Construction Areas

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ABSTRACT

Since Turkey is in an earthquake zone, historically there has been a lot of demolition in various cities, including Istanbul which contains many historical places. Residents of Istanbul are subject to calamity regulations and plans that fall under these regulations. Older buildings, and those in a state of disrepair which are not in compliance with regulations, are demolished to make the city safer. In this context, urban transformations are based in Istanbul.

In many cases Istanbul's demolitions reveal carcinogenic asbestos fibers, which are known to have carcinogenic effects. Building employees are directly exposed to asbestos dust, and the local community is indirectly exposed. Protecting against the negative health affects of asbestos on building employees who are working in buildings unknown to have asbestos is under discussion.

The aim of this study is to create awareness of asbestos and to protect against its negative affects on building employees working in urban transformations areas. Surveys show that the employes are uninformed about asbestos and its effects. The projects must continue to create awareness.

Keywords: Asbestos, exposure to asbestos, urban transformation, occupational illness.

INTRODUCTION

Along with the development of industry, the notion of occupational illness has also developed. Many employees are exposed to occupational illnesses because of their workplaces and working conditions. Asbestos is a dust that causes occupational illnesses and deaths. Asbestos was used widely in industry because it was a durable, fire-retardant insulator at a time when few alternatives were available. The World Health Organization (WHO) estimates that globally about

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125 million people are exposed to asbestos in their workplace and each year more than 107.000 deaths are attributable to occupational exposure to asbestos.[1]

Asbestos exposure in industry was first realised in 1898 by factory supervisors in England, a world industrial leader. The harms of asbestos were discussed and safety precautions and limitations to asbestos usage were introduced at this time. Since occupational diseases in asbestos exposed workers increased, it was determined that restrictions on asbestos usage were insufficient, according to studies.[2,3] Asbestos was proven to be a human carcinogen in 1977 by the International Agency for Research on Cancer (IARC), in 1980 by the World Health Organization (WHO) and National Toxicology Program (NTP) and in 1986 by the United States Environmental Protection Agency (U.S. EPA).[4]

It is known that the use of asbestos in Europe was limited, and since the 1980's, asbestos has been prohibited.[5] With a shared directive in 1999 (1999/77/EC), European Union countries discussed the restriction of the use and marketing of asbestos, and on 1 January 2015, a complete ban on asbestos has been implemented in European Union countries.

With a 2003 directive related to the protection of workers against the risks of asbestos exposure (2003/18/EC), the prohitibion of any activity that exposes workers to asbestos fiber at the stage of asbestos removal and production was discussed in the council. As a result, this prohibition was applied in European Union countries from 2006 and special precautions were taken to protect the community from asbestos at the stage of repairing and/or demolishing buildings containing asbestos.[2]

One of the sectors where asbestos is prominent is the construction sector. In our country, according to Law 6306 on Renovating Buildings That Carry Disaster Risk, urban transformation has begun on older buildings. Workers involved in this process and residents of demolition areas could easily have been exposed to asbestos. Although formal limitations are being followed, there is a consensus among scientists that exposure to asbestos is not yet at safe levels.

The aim of this study is to determine the level of awareness among urban transformation site workers about carcinogenic asbestos dust, and to raise their awareness about the health effects of asbestos.

METHODOLOGY

This study was carried out with randomly selected volunteers aged 19 to 63 working in urban transformation sites in the Kartal, Maltepe and Kadıköy districts of Istanbul's Anatolian Side. Data were collected between April 2017 and June 2017 by face-to-face interview with surveys. Ethical approval of this study was

obtained from Istanbul Medipol University Social Sciences Scientific Researchs Ethical Committee on 13.04.2017 (Approval no 43037191-604.01.01.-E.9596).

In surveys, we asked questions to workers who are likely to be exposed to asbestos in order determine the following information:

-Rate of smoking

-Rate of staff who sought x-ray examinations

-Rates of hand and machine washing if work clothes are cleaned at home, and the rate of who washed the clothes

-Rate of asbestos-related disease in family

-Rate of awareness level of asbestos by age and occupation (engineers and others...)

-How many people think that protection precautions are taken at the demolition stage?

-Which personal protectors are used most?

-Rate of working on urban transformation projects

-Are health scans done?

-To which dust are workers most exposed?

-How are health problems shared with workplace physician?

In this study, we used the 'Simple Random Sample Size Estimation' method.

$$n = \frac{Nt^2pq}{d^2(N-1)+t^2pq}$$

N: Number of individuals in the environment
n: Number of individuals to be sampled
p: Occurrence frequency of the reviewed event
q: Non-occurrence frequency of the reviewed event
t: Theoretical value in the T table at a certain degree of independence and at a determined level of error
d: ± deviation to be made according to the frequency of occurrence of the event

$$n = \frac{(100)(1.96)^2(0.90)(0.10)}{(0.05)^2(1000-1)+(1.96)^2(0.90)(0.10)}$$

n=122 minimum total event quantity

We planned total event quantity as 125-130 when considering the losses and completed the study with 125 significant data.

The NCSS 2007 Statistical Software (Number Cruncher Statistical System) (NCSS LLC, Kaysville, Utah, USA) and IBM SPSS 23.0 programmes were used to evaluate the data. The reliability analysis of the questions which were developed for

determination of asbestos knowledge, was done with Kuder-Richardson-20 test. Along with descriptive statistical methods (Average, standard deviation, median, frequency, ratio, minimum and maximum), the Mann Whitney U test was used to compare quantitative data that don't show normal distribution between groups. For evaluation of relation between variants, non-parametric Spearman's Correlation Analysis was used and meaningfulness was evaluated on $p<0.05$ level.

RESULTS

This study was carried out with total of 125 construction workers of which 28.0% were white collar workers (n=35) and 72.0% were (n=90) blue collar workers. The age of the participants varied between 19-63 and the average age is 34.54±10.48. In a study that was conducted by Köksal et al., 488 subjects participated in that study and the mean age of female subjects were 49.8 ± 12.7 while male subjects were 52.1 ± 13.2.[6] Şahin et al. also conducted a study that consisted of 132 villagers in Karağı village, Isparta. Mean age of participants was 53.21 ± 13.57 years and age range was 30-79.[7]

Table 1. Distribution of descriptive properties.

	n	%
Exposed construction site dust[a]		
Asbestos	32	25.6
Iron dust	44	35.2
Wood dust	39	31.2
Lime dust	43	34.4
Other dusts	52	41.6
Occupation		
Blue collar	90	72.0
White collar	35	28.0
Time period of work		
< 1 month	8	6.4
1-3 months	27	21.6
3-6 months	18	14.4
6-12 months	20	16.0
> 12 months	52	41.6
Previous job (n=87)		
Blue collar	65	74.7
White collar	22	25.3
Smoking		
Yes	53	42.4
No	62	49.6
Quit smoking	10	8.0
Age (years)		
Minimum-Maximum (Median)	19-63 (32)	
Average ± SD	34.54±10.48	

[a]*Seen more than once*

In Table 1, 25.6% of the workers (n=32) were exposed to asbestos dust while 35.2% (n=44) were exposed to iron dust, 31.2% (n=39) to wood dust, 34.4% (n=43) to lime dust and 41.6% (n=52) to other dusts.

When evaluating the time periods of work; 6.4% of the workers (n=8) worked less than one month, 21.6% (n=27) worked between 1-3 months, 14.4% (n=18) between 3-6 months, 16.0% (n=20) between 6-12 months and 41.6 (n=52) worked more than 12 months. 74.7% of the workers (n=65) were a blue collar position in their previous employment while 25.3% of the others (n=22) had white collar jobs.

42.4% of the participants (n=53) were smokers, 49.6% of them (n=62) were non-smokers, and 8.0% of (n=10) the workers had quit smoking. In a study that was conducted by Şahin et al., 55 (41.7%) of 132 participants were smokers and 77 (58.3%) of others were non-smokers.[7] According to another study, 41.2% (n=54) of villagers in Eskisehir who were exposed to asbestos were smokers and 58.8% (n=77) of others were non-smokers.[8]

In Table 2, there are no genetic diseases in 83.2% of participants (n=104) and/or their families while 16.8% of others (n=21) and/or their families have genetic diseases. Diabetes is found in 66.7% of those with genetic diseases (n=14), cancer in 9.5% (n=2), heart disease in 28.6% (n=6) and other diseases in 14.3% (n=3).

90.4% of participants (n=113) don't use medicines regularly while 9.6% (n=12) do. 37.6% of the workers (n=47) did not have an x-ray in the past year but 62.4% of the other workers (n=78) did. 43.6% of the workers had an x-ray in the past month, 16.7% within 3-6 months, 20.5% within 6-12 months and 19.2% had an x-ray 12 months ago and more.

22.4% of workers (n=28) don't wash their clothes at home while 77.6% (n=97) do. 16.5% of the workers (n=16) that wash their clothes at home, wash their clothes by hand, 83.5% (n=81) use a washing machine, 41.2% (n=40) wash the clothes by themselves, 36.1% (n=35) have them washed by their wives/husbands, 21.6% (n=21) by friends/relatives and 1.0% (n=1) by other relatives.

96.0% of the workers' families (n=120) have no family members with asbestos related diseases while 4.0% of other workers (n=5) do. 40.0% of family members with asbestos related diseases (n=2) have lung cancer, 20.0% (n=1) have stomach/bowel cancer, 20.0% (n=1) have other tumors and 20.0% (n=1) have shortness of breath.

Table 2. Dispersions of diseases.

	n	%
Presence of genetic diseases in the worker and/or in his/her family		
Yes	104	83.2
No	21	16.8
Type of genetic diseases[a] (n=21)		
Diabetes mellitus	14	66.7
Cancer	2	9.5
Heart diseases	6	28.6
Chromosomal disorders	0	0.0
Others	3	14.3
Regular use of medications		
No	113	90.4
Yes	12	9.6
X-Ray extraction in the previous year		
No	47	37.6
Yes	78	62.4
Time of X-Ray extraction in the previous year (n=78)		
Within the last 3 months	34	43.6
3-6 months	13	16.7
6-12 months	16	20.5
> 12 months	15	19.2
Washing clothes at home		
No	28	22.4
Yes	97	77.6
Washing style of clothes		
By hand	16	16.5
In washing machine	81	83.5
Who washes the clothes? (n=97)		
Worker	40	41.2
Wife/Husband	35	36.1
Friend, relatives	21	21.6
Other	1	1.0
Family member with asbestos related diseases		
Does not exist	120	96.0
Exists	5	4.0
Asbestos related diseases in family (n=5)		
Lung cancer	2	40.0
Stomach/bowel cancer	1	20.0
Other tumors	1	20.0
Shortness of breath	1	20.0

[a]*Seen more than once*

Table 3. Dispersion of answers given to questions about asbestos.

	Yes		No	
	n	%	n	%
Asbestos is a dust that cause lung cancers, and is considered as an occupational hazard.	85	68.0	40	32.0
Duration of responsibility about asbestos is 10 years	9	7.2	116	92.8
Wearing gloves and protective masks are sufficient to prevent harm from asbestos related works.	11	8.8	114	91.2
Materials that contain asbestos can be disposed of alongside domestic waste.	2	1.6	123	98.4
Asbestos is a fire-resistant material and has low permeability to electricity.	6	4.8	119	95.2
Asbestos shows it's effects after many years.	22	17.6	103	82.4
Smoking doesn't trigger the negative effects of asbestos.	2	1.6	123	98.4
There isn't any legislation about asbestos in our country.	5	4.0	120	96.0
The site chief can carry out works after educating the asbestos workers.	9	7.2	116	92.8
Asbestos is resistant to acids and bases.	8	6.4	117	93.6
The most used asbestos type is chrysotile.	15	12.0	110	88.0
Equipment used in asbestos works such as overalls, gloves etc. is disposable.	19	15.2	106	84.8
Asbestos waste is directly disposed of in water-resistant polyethylene (PE), polyproplyene (PP) and 'Big-Bag' bags.	21	16.8	104	83.2

When we look at the Table 3, 68.0% of workers (n=85) answered yes when asked whether 'Asbestos is a dust that causes lung cancer and considered as an occupational hazard.' while 32.0% (n=40) answered 'No'.

7.2% of workers (n=9) answered yes when asked whether 'Duration of responsibility about asbestos is 10 years' while 92.8% (n=116) answered 'No'.

8.8% of workers (n=11) answered yes when asked whether 'Wearing gloves and protection masks are sufficient to prevent harm from asbestos related works.' while 91.2% (n=114) answered 'No'.

1.6% of workers (n=2) answered yes when asked whether 'Materials that contain asbestos can be disposed of alongside domestic waste.' while 98.4% (n=123) answered 'No'.

4.8% of workers (n=6) answered yes when asked whether 'Asbestos is a fire-

resistant material and has low permeability to electricity.' while 95.2% (n=119) answered 'No'.

17.6% of workers (n=22) answered yes when asked whether 'Asbestos shows it's effects after many years.' while 82.4% (n=103) answered 'No'.

1.6% of workers (n=2) answered yes when asked whether 'Smoking doesn't trigger negative effects of asbestos.' while 98.4% (n=123) answered 'No'.

4.0% of workers (n=5) answered yes when asked whether 'There isn't any legislation about asbestos in our country.' while 96.0% (n=120) answered 'No'.

7.2% of workers (n=9) answered yes when asked whether 'Site chief can carry out works by educating the asbestos workers.' while 92.8% (n=116) answered 'No'.

6.4% of workers (n=8) answered yes when asked whether 'Asbestos is resistant to acids and bases.' while 93.6% (n=117) answered 'No'.

Table 4. Dispersion of knowledge of asbestos removing and regulations.

	n	%
Information about the 28539 numbered regulation of Health and Social Security Protections in Asbestos Works which was published in official newspaper on 25 January 2013		
No	100	80.0
Yes	25	20.0
Have any education sessions about asbestos removal been organized?		
No	93	74.4
Yes	32	25.6
Frequency of sessions (n=32)		
Once a year	1	3.1
Twice a year	1	3.1
At the beginning of every asbestos removal	10	31.3
Unknown	20	62.5
Who is/are leading the education sessions?[a]		
Site chief	3	9.4
Staff	-	-
Asbestos removal worker	7	21.9
Job Security Specialist	21	65.6
Asbestos removal specialist	3	9.4
Project manager	-	-
Other	1	3.1

[a]*Checked more than one option.*

12.0% of workers (n=15) answered yes when asked whether 'The most used asbestos type is chrysotile.' while 88.0% (n=110) answered 'No'.

15.2% of workers (n=19) answered yes when asked whether 'Equipments used in asbestos works such as overalls, gloves etc. are disposable.' while 84.8% (n=106) answered 'No'.

16.8% of workers (n=21) answered yes when asked whether 'Asbestos waste is directly disposed of in water-resistant polyethylene (PE), polyproplyene (PP) and 'Big-Bag' bags.' while 83.2% (n=104) answered 'No'.

In Table 4, 80.0% of participants (n=100) have no information about the 28539 numbered regulation of Health and Social Security Protections in Asbestos Works which was published on 25 January 2013, while 20.0% of others (n=25) have knowledge about it.

74.4% of workers (n=93) said that they don't receive any education about asbestos removal, but 25.6% of other workers (n=32) are getting education about asbestos removal. 3.1% (n=1) said that the education sessions are organized once a year, 3.1% (n=1) said twice a year, 31.3% (n=10) said that there is a session at the beginning of every asbestos removal, 62.5% (n=20) don't know the frequency. When a question about who leads the education sessions was asked, 9.4% of participants answered 'the site chief', 21.9% answered 'an asbestos removal worker', 65.6% answered 'the job security specialist', 9.4% answered 'an asbestos removal specialist' and 3.1% of answered 'other people'.

In Table 5, There were no precautions taken at the pre and post-demolition stages in 15.2% of demolitions (n=19), in 84.8% of other demolitions (n=106), precautions were taken. When analyzing the security precautions, it can be seen that 46.2% (n=49) are risk assessments, 23.6% (n=25) are measurements of environment, 64.2% (n=68) are health scans, 25.5% (n=27) are isolated areas, 63.2% (n=67) are personal protections, 12.3% (n=13) are asbestos removal education sessions, 11.3% (n=12) are spraying water on asbestos-containing material, 51.9% (n=55) are alert/warning signs and 1.9% of them (n=2) are other security precautions.

84.8% of personal protection equipment (n=106) are helmets, 49.6% (n=62) protective clothes, 69.6% (n=87) gloves, 79.2% (n=99) work shoes, 58.4% (n=73) breathing masks and 14.4% (n=18) are other precautions.

68.8% of workers (n=86) were involved in 1-3 urban transformation projects, while 21.6% of them (n=27) were involved in 3-10, and 9.6% (n=12) were involved in more than 10 projects.

Table 5. Dispersion of precautions at pre and post-demolition stages of buildings.

	n	%
Precautions before and after demolition of buildings		
Not taken	19	15.2
Taken	106	84.8
Precautions that were taken at demolition (n=106)[a]		
Risk assessments	49	46.2
Measurement of environment	25	23.6
Health scans	68	64.2
Isolating the area	27	25.5
Personal protection	67	63.2
Asbestos removal education session	13	12.3
Spraying water on asbestos-containing materials	12	11.3
Alert/Warning Signs	55	51.9
Other precautions	2	1.9
Personal protections[a]		
Helmets	106	84.8
Protective clothes	62	49.6
Gloves	87	69.6
Work shoes	99	79.2
Breathing masks	73	58.4
Others	18	14.4
Number of Urban Transformation projects in which workers were involved		
Between 1-3	86	68.8
Between 3-10	27	21.6
>10	12	9.6
Removal of Asbestos-containing material before demolition		
No	90	72.0
Yes	35	28.0
Complete Precaution at the demolition stage		
No	33	26.4
Yes	92	73.6
Complete Precautions that were taken (n=92)[a]		
Heating the building/material	59	64.1
Putting netting around the building	54	58.7
Sending workers away from building	64	69.6
Other precautions	6	6.5

[a]*Checked more than one option.*

72.0% of participants (n=90) state that asbestos-containing materials weren't removed before demolition, while 28.0% of them (n=35) stated the opposite.

26.4% of the workers (n=33) expressed that complete precautions weren't taken at the demolition phase, 73.6% of other workers (n=92) said that complete precautions were taken. When evaluating complete precautions, 64.1% (n=59) are

heating the building/material, 58.7% (n=54) are putting a netting around the building, 69.6% (n=64) are sending workers away from building and 6.5% (n=6) are the other precautions.

Table 6. Dispersion of health scans and sharing of health concerns.

	n	%
Frequency of Health Scans		
When entering a job	41	32.8
When entering a job and periodical	82	65.6
Never	2	1.6
Sharing health concerns with workplace physician		
Everytime	87	69.6
Sometimes	28	22.4
No workplace physician	7	5.6
I don't share	3	2.4

When evaluating the frequency of health scans on workers in Table 6, 32.8% of workers (n=41) were scanned when they started their job, 65.6% (n=82) were scanned when they started their job and periodically and 1.6% (n=2) were not scanned.

69.6% of participants (n=87) always share their health concerns with their workplace physician, 22.4% (n=28) share sometimes, 2.4% (n=3) never share and 5.6% (n=7) state that there is no workplace physician at their workplace.

Score of Asbestos Knowledge

A total score was obtained from 8 informational questions about asbestos; 'Asbestos is a dust that causes lung cancers and considered as an occupational hazard.', 'Duration of responsibility about asbestos is 10 years', 'Asbestos is a fire-resistant material and has low permeability to electricity.', 'Asbestos shows its effects after many years.', 'Asbestos is resistant to acids and bases.', 'The most used asbestos type is chrysotile.', 'Equipment used in asbestos works such as overalls, gloves etc. is disposable.', 'Asbestos waste is directly disposed of in water-resistant polyethylene (PE), polyproplyene (PP) and 'Big-Bag' bags.' and the question regarding 'Information about the 28539 numbered regulation of Health and Social Security Protections in Asbestos Works which was published in official newspaper on 25 January 2013'.

The score of asbestos knowledge is calculated from 9 questions, 1 point for each correctly answered question and 0 points for a wrong answer. The obtained score is converted to 100 point scale. According to this scale, participants who answered all questions correctly will receive 100 points, and participants who answered all questions incorrectly 0 points (Table 7).

Table 7. Dispersion of asbestos knowledge scores.

Score of asbestos knowledge	*Minimum-Maximum (median)* *Average±Standard Deviation*	0-88.9 (11.11) 18.67*±20.86*
Number of correctly answered questions; *n (%)*	0 1 2 3 4 5 6 7 8	27 (21.6%) 61 (48.8%) 11 (8.8%) 7 (5.6%) 5 (4.0%) 5 (4.0%) 3 (2.4%) 5 (4.0%) 1 (0.8%)

The scores of the participants in this study range from 0 to 88.9 and average score is 18.67±20.86.

Table 8. Evaluation of asbestos knowledge score according to occupation.

		Occupation		***P***
		Blue Collar Workers (n=90)	**White Collar Workers (n=35)**	
Asbestos knowledge score	*Minimum-Maximum (Median)* *Average±Standard Deviation*	0-77.8 (11.11) 13.83±15.65	0-88.9 (22.22) 31.11±26.92	***0.001****

*Mann Whitney U Test **p<0.01*

Blue collar workers' asbestos knowledge score is 13.83±15.65 points, and the white collar workers' is 31.11±26.92 points. There is a statistically significant difference between blue and white collar workers (p=0.001; p<0.01). The asbestos knowledge score of white collar workers is significantly higher than the blue collars workers' score (Table 8).

Table 9. Age and level of asbestos knowledge score relationship.

	r	***p***
Level of Asbestos Knowledge Score - Age Relationship	0.142	***0.114***

r: Spearman's Correlation Coefficient

There is no significant relationship between age and level of asbestos knowledge (p>0.05) (Table 9).

Result of the reliability analysis on survey questions is 0.79 (Kuder Richardson-20).

DISCUSSION AND CONCLUSION

Asbestos is the general term used for the fibrous silicates such as actinolite, grunerite (amosite), anthophyllite, chrysotile, crocidolite, tremolite. It was generally used in buildings for insulation purposes. [9] There has been steep rise in the production and use of asbestos in the last 100 years. Asbestos consumption has levelled off in recent years to about 4 million tones (1983).[10] However, all forms of asbestos are known human carcinogens and classified as group 1 human carcinogen by International Agency for Research on Cancer (IARC).[11] It has been shown that asbestos can iduce transformation of cells in culture, including mesothelial cells and fibroblasts.[12] Therefore, asbestos is a reason for mesothelioma alongside with lung, larynx, and ovary cancer.[11]

This study carried out with urban transformation workers aims to raise awareness about asbestos exposure to workers and to create sensitivity to asbestos.

As a result of the survey evaluations, 70.4% of the workers have no information about asbestos. 21.6% of the workers didn't give the correct answer to all 9 questions in the survey study and only 1 question in 9 was answered correctly by 48.8% of the workers. Workers who gave at least 1 correct answer to questions, were able to give the right answer thanks to the preliminary information given before the survey study. A lot of workers learned in the preliminary information that asbestos is carcinogen and that they can be exposed to it in urban transformation projects. Thus, our study raised awareness regarding the toxic effects of asbestos.

It is observed that the number of asbestos removal workers is too low and generally, the same workers do the asbestos removal jobs. Even these workers have not shown the expected success in the asbestos knowledge survey. As a result, it is observed that even asbestos removal workers who are educated regularly about this subject need more education about asbestos.

When evaluating the results of the survey, it is thought that the least aspirated dust is asbestos with a ratio of 25.6%, after iron dust with 35.2%, wood dust with 31.2%, lime dust with 34.4% and other dusts with 41.6%. It is thought that reason for low ratio of asbestos dust aspiration is the failure to measure it, and that fact that many workers don't know what asbestos is.

Another evaluation is the number of smokers. The percentage of non-smokers is approximately 49.6%. 83.2% of workers don't think that they and their family have genetic diseases. 90.4% of workers regularly use medicines. Despite their work conditions, construction workers think that they are healthy.

62.4% of workers appear to have had an x-ray in the last year because they had

lung x-rays before asbestos and heavy works.

We thought that family members may also have been exposed to asbestos due to contact with construction clothes, so we asked a question about how the clothes are washed. 22.4% of workers don't wash their clothes at home while 77.6% do. Of workers who wash their clothes at home, 16.5% of them wash their clothes by hand, 83.5% wash them in a machine. 41.2% of workers wash their clothes by themselves, 36.1% have them washed by their wives/husbands, 21.6% by friends/relatives and 1.0% by other relatives.

In a question for evaluating the asbestos consciousness, 74.4% of workers said that education on asbestos removal was not organized. When asked a question about who gives the education, workers said that 9.4% of educators are site chiefs, 21.9% asbestos removal staff, 65.6% job security specialist, 9.4% asbestos removal specialist and 3.1% other educators. 80.0% of workers never heard about asbestos regulations.

It is understood that precautions were taken mostly for personal protection. 63.2% of precautions are personal protections and 64.2% are health scans. Risk assessment regulations suggest on-site protection precautions firstly and make personal protections the lowest priority. 84.8% of personal protective equipments are helmets, 79.2% are work shoes and gloves, masks, protective clothes come after them. Because the most used personal protective equipment was a helmet, it is understood that these personal protections weren't selected for protection from asbestos dust.

We grouped personnel like engineers, architects, job security specialist as white collar workers and other personnel as blue collar workers and made an awareness test. 28% of personnel are white collar workers and 72% are blue collar workers. It is observed that white collar workers seem like they have heard about what asbestos is, but they received only 26.92 points in a 88.9-point survey. This result shows that site chief or job security specialist are not qualified enough to give information and education to workers even if they know about asbestos. There is no meaningful relationship between age and asbestos awareness level.

Setting up a contamination unit and ventilation system for asbestos is very expensive. Therefore, audits conducted by official institutions are extremely important.

Studies about asbestos reveal that asbestos related occupational diseases should be reported and records should be kept. Employers and workers should be informed about legal regulations and rights in the case of an occupational disease. Some arrangements should be made for asbestos removal workers such as work-

ers compensation fees and early retirement packages, by analyzing the studies and legislations in other countries such as Croatia and Slovenia.

It is unknown whether there is asbestos in many Istanbul buildings because there is no inventory information about buildings. All urban transformation workers should have information about asbestos. It should be added to current education regulations that all workers must be educated about asbestos whether there is asbestos in urban transformation site or not. This education can be given to workers by asbestos removal specialists before starting at job. Education about the health effects of asbestos by workplace physician should be provided.

It is obvious that employers also need to be aware of asbestos and measurements about asbestos should be done in all urban transformation sites. Workers should not enter construction sites without taking the necessary precautions and risk assessment reports.

We think that importance should be given to asbestos because it shows its effect after many years. Our current regulations about asbestos guide us about what needs to be done, but it was observed that there isn't any application and awareness about asbestos. It is obvious that carrying out more scientific research, creating public advertisements by government and organizing education sessions about asbestos are needed to increase awareness.

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