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Research Article

Biochemical and Clinicopathological Studies on Some Antihyperglycaemic Herbal Preparations in Alloxan induced Diabetic Rats

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Abstract: The present study investigated the effect of aqueous extracts of chamomile and/or oregano on diabetic rats. Fifty rats were assigned into five groups equally. Groups (2-5) were made diabetic by intra-peritoneal administration of alloxan while group (1) was kept as normal control. Groups 3, 4& 5 were administered chamomile and/or oregano for six weeks. The significant improvement in the body gain appeared gradually after extracts administration by the six week. All of the extracts had significant ($P \le 0.05$) hypoglycaemic effect on diabetic treated rats compared to control or diabetic non treated groups manifested by lowering blood glucose, glycosylated hemoglobin (HbA₁C), amylase activity with an increase of insulin. Renal functions represented by blood urea, creatinine, uric acid and total protein showed significant improvement on all of the treated groups especially in oregano and mixture group. While, High Density Lipoprotein-Cholesterol (HDL-c) was increased among the treated groups compared with the non treated one. Total Cholesterol (TC), Triglyceride (TG), Low Density Lipoprotein-Cholesterol (LDL-c) and Very Low Density Lipoprotein-Cholesterol (VLDL-c) were significantly ($P \le 0.05$) lowered in the groups treated with all extracts when compared with those untreated. This effect appeared to be abolished with chamomile extract as evidenced by lower decreases in the lipid fractions.

Keywords: Diabetes, Chamomile, Oregano, Glucose, Insulin, Lipid Profile.

INTRODUCTION

Diabetes mellitus (DM), is a metabolic disease growing rapidly throughout the world [1]. As a very common chronic disease, diabetes is becoming the third 'killer' of mankind, after cancer, cardiovascular and cerebrovascular diseases, because of its high prevalence, morbidity and mortality [2]. Diabetes mellitus characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and protein. Also it leads to serious complications such as nephropathy, retinopathy, neuropathy, cardiovascular disease, impotence and gangrene [3].

Diabetes mellitus is classified into two main types, type-1 diabetes, insulin-dependent diabetes mellitus (IDDM), which is a state of insulin deficiency because of defect in the function of islet β -cell and type-2 diabetes, non-insulin-dependent diabetes mellitus (NIDDM). About 90% of patients are NIDDM where insulin resistance playing a key role in the development of the disease [4].

Diabetes mellitus cannot be cured, but can be controlled. The plants with anti-diabetic activities provide important sources for the development of new drugs in the treatment of diabetes mellitus. Many traditional medicinal herbs and spices have been used in most developing countries as a valuable alternative for treating diabetes mellitus [5]. The major advantages of herbal medicine seem to be their efficacy, low incidence of side effects, and the low cost [6].

Amongst such plants which have been reported to have beneficial effects in the treatment of diabetes, spices such as cinnamon, cloves, ginger, turmeric [7], bitter melon [8], gurmar [9], Korean ginseng [10], onions and garlic [11], holy basil [12], oregano [13], and chamomile [14].

Chamomile (*Matricaria chamomilla L.*), is one of the most ancient medicinal herbs known in ancient Egypt, Greek and Rome [15]. It has been widely used as a herbal tea all over the world [16]. Latterly, chamomile has been well known for its pharmaceutical properties such as anti-inflammatory, immune-modulatory activity, anticancer, arcaricidal property and antipruritic effect [17].

Furthermore, the recent Studies recorded that chamomile ameliorates the hyperglycemia and diabetic complications via suppressing blood sugar levels and increasing liver glycogen storage [18]. The pharmacological activity of chamomile extract has shown to be independent on insulin secretion [19] and further studies reveal its protective effect on pancreatic beta cells in diminishing hyperglycemia-related oxidative stress [17]. Oregano (*Origanum vulgare*), is a native plant widely distributed throughout the Mediterranean, Euro-Siberian and Irano-Siberian regions [20]. Oregano leaves traditionally used as a potent hypoglycaemic agents in diabetes control and treatment [21]. The main known pharmacological activities of oregano were antibacterial [22], antifungal [23], anti-parasitic [24], antioxidant [25], anti-thrombin [26] and antiinflammatory [27]. Also, there are some reports regarded the anti-mutagenic and anti-carcinogenic effect of oregano which in turn represents an alternative for the potential treatment and/or prevention of certain chronic ailments, like cancer [28].

This study was planned to elucidate the antihyperglycemic effect of chamomile and oregano either as a single treatment or in combination together in alloxan induced-diabetic rats besides estimation of some other related biochemical profiles.

MATERIALS AND METHODS Chemicals

Kits of glucose, glycosylated hemoglobin (HbA₁C), Total cholesterol (TC), Triglycerides (TG), High density lipoprotein cholesterol (HDL-c), urea, creatinine, uric acid, total protein and amylase were obtained from Human Co. All other chemicals are purchased from Sigma.

Preparation of Plant extract

Chamomile and oregano were obtained from local market, Egypt. Dry chamomile aerial parts and the oregano leaves were weighed and crushed to powder and a 5% suspension was prepared in a flask by adding hot boiled water. The flask was then placed on a shaker (200 rpm) for four hours. After shaking, the suspension was filtered. The filtrate was dried on a rotary evaporator to give a semi-solid residue from which appropriate weight was measured and dissolved in 2.0 ml of physiological saline to obtain the desired concentration of 200 mg/ml of the extract [29,30].

Experimental Animals

Fifty male albino rats weighing 150-200 (g) were purchased from the animal house of National Research Center, Cairo-Egypt. Rats were housed for one week under controlled condition and provided with standard diet and water ad libitum to allow them acclimatize. All of the rats received human care in accordance with the National Institute of Health guidelines for the care and use of laboratory animals.

Induction of diabetes

Diabetes mellitus was induced in rats of groups (2, 3, 4& 5) by intra-peritoneal injection of 120mg/Kg body weight of alloxan dissolved in normal saline while group (1) was not induced. The development of diabetes was checked by measuring blood glucose level after Four days of alloxan injection.

Diabetes mellitus was confirmed by elevated fasting serum glucose over 300 mg/dl [31].

Experimental Design

Rats divided into five groups of ten rats per each one. Group (1): was kept as normal control, non- diabetic group.

Groups (2), (3), (4) and (5): were served as alloxan diabetic groups.

Group 2: was a diabetic non-treated rat.

Group (3): Diabetic rats were given aqueous extract of chamomile at dose level of 200 mg/kg body weight by stomach tube for 6 weeks. No evidence of side effects was observed after chamomile administration.

Group (4): Diabetic rats were given aqueous extract of oregano at dose level of 200 mg/kg body weight by stomach tube for 6 weeks.

Group (5): Diabetic rats were given aqueous extract from a combination of chamomile and oregano at a dose equivalent to 200 mg /kg B.W by stomach tube for 6 week.

After 3 and 6 weeks of treatment, five rats of each group were sacrificed and blood samples were collected in plain centrifuge tube to separate serum for measurement of blood glucose and other biochemical parameters.

Biochemical analysis

Glucose was determined according to Tietz method [32]. Glycosylated hemoglobin (HbA₁C) was estimated according to the method of Eross et al., [33]. Amylase was assayed according to the method of Henry & Chiamori [34]. Total cholesterol was determined according the method described by Allain et al., [35]. Triglycerides were determined according to the method described by Trinder & Ann [36]. Determination of HDL-c was carried out according to the method of Gordon & Amer [37]. The calculation of serum very low density lipoprotein cholesterol (VLDL-c) and low density lipoprotein cholesterol (LDL-c) were carried out according to the method of Lee & Nieman [38], from the equations: VLDL-c (mg/dl) = TG/5 and LDL-c (mg/dl) = TC - HDL - VLDL. Serum creatinine was determined according to the method described by Henry [39]. Serum uric acid was determined according to the method described by Fossati et al., [40]. Blood urea nitrogen was determined according to the method described by Patton & Cruch [41]. Total protein was measured according to Bradford method [42]. The serum insulin level was assayed with an ELISA kit (Linco Research Inc., USA).

Statistical analysis

The data were analyzed by using SPSS for Windows. Data are shown as mean \pm Standard Error. The significance of differences was calculated by using one-way analysis of variance (ANOVA) followed by Tukey's procedure for multiple comparisons. Any value

of $P \le 0.05$ was considered significant. All experiments were conducted twice to ensure consistency [43].

RESULTS AND DISCUSSION

The use of natural products for medicinal application in their natural available form is the general trend now [44]. Plants have been effectively prescribed for the treatment of many diseases including diabetes mellitus. The demand for herbal remedies is growing as various types of hypoglycemic agents, may produce a number of side effects at the same time with controlling blood sugar [45]. These herbs may lower blood glucose however; their test results are subjected to several factors. Firstly, each herb contains thousands of components, only a few of which may be therapeutically effective. Secondly extraction of active component is not easy [46,47]. The hypoglycemic activity of some plants extracts has been evaluated and confirmed in animals and in human beings [48].

Results illustrated in Table 1 showed the changes in body weight, blood levels of glucose, glycosylated haemoglobin (HbA1C), insulin and amylase activity in all of the experimental groups. The body weight of the diabetic rats was significantly decreased as compared to normal control and diabetic treated rat groups. The diabetic rats treated with oregano and/or chamomile extract relatively maintained the body gain especially in the mixture treated group. Weight loss has been known to be one of the DM symptoms [49, 50]. Similar observation was obtained by Nema & Omimah [51], who reported that the administration of oregano leaves extract into diabetic group kept its body weight. Also, Manal [6] revealed that the diabetic rats treated with the plant extract showed significant gain in body weight as compared to the diabetic control, which may be due to its protective effect in controlling muscle wasting. Where loss of body weight associated with diabetes may be due to increased muscle wasting and to the loss of tissue proteins. Moreover, Kato et al., and Nema & Omimah [18, 51] postulated that, the deficiency of insulin in the diabetic rats led to decreased amino acids uptake by tissues with a consequent reduction in the level of protein synthesis. Also, insulin deficiency results in lipolysis in adipose tissues and protein breakdown [52].

The intra-peritoneal injection of alloxan to normal rats effectively induced diabetes as reflected by significant elevation of blood glucose, glycosylated haemoglobin (HbA₁C) and amylase activity with a fall of insulin level when compared to control group. Treatment of diabetic rats with chamomile or oregano separately or as a mixture of both showed significant reduction of blood glucose, glycosylated haemoglobin (HbA₁C) and amylase activity with an increased insulin as compared to diabetic group (group 2). Our results revealed that the mixture of both extracts significantly decreased the blood glucose when compared with the effect of each extract alone on diabetic animals (group 3 & 4). On the same hand, the mixture of both plants significantly decreased glycosylated haemoglobin (HbA₁C) and amylase activity and increased insulin level when compared with the effect of chamomile on diabetic animals (group 3). These results are in accordance with Jouad et al., [53] who observed that the anti-hyperglycaemic effect of oregano was very potent and cumulative. Also, Manal [6] investigated that the extract of rosemary and chamomile exhibited a potent blood glucose lowering property in diabetic rat. Moreover, Darvishpadok et al., [54] revealed that the administration of chamomile in diabetic rats significantly decreased the level of blood glucose and HbA₁C after 17th days. In addition to, these results agreed with that obtained by Kato et al., [18] who investigated the effect of chamomile extract and its major components on the prevention of hyperglycemia and the protection or improvement of diabetic complications in diabetes mellitus. Where they attributed these effects to the inhibition of amylase activity and lowering the blood glucose level. The hypoglycaemic activity of the oregano extract may, therefore, be due to the inhibition of hepatic glucose production and/or stimulation of glucose utilization by peripheral tissues, especially muscle and adipose tissue [55]. Our results revealed that there was a significant decrease in the amylase activity in all treated groups (3,4&5) but the effect of oregano was more prominent either alone or as a mixture. These results agree with Patrick et al. [56] who concluded that the antioxidantrich oregano extracts inhibit α -amylase activity which explain how these constituents of traditional antidiabetes medicines could confer their therapeutic The results in table (1) investigated a benefits. significant increase of serum insulin in all of diabetic treated rats (group 3, 4 &5). These results agreed with the study of Kholoud & Manal [57] who noticed that the treatment with chamomile showed significant increase in serum insulin and C-peptide levels in diabetic rats. So, the possible mechanism by which both extracts brings about its hypoglycemic action in diabetic rats may be due to potentiating the effect of insulin in serum or by increasing either the pancreatic secretion of insulin from the existing beta cells or its release from the bound form. On the other hand, Lemhardi et al., [13] proved that, the oregano extract had no effect on the basal plasma insulin concentrations in both normal and diabetic rats.

Table 1: Effect of Chamomile, Oregano and Their Mixture on Body Weight, Glucose, Insulin, Serum α-Amylase					
and HbA ₁ C in Alloxan Induced Diabetic Rats (mean ±S.E)					

Groups	Control	Diabetic	Diabetic + Chamomile	Diabetic + oregano	Diabetic + Mixture
Parameters					
Body weight	180.52±4.92	158.62±6.13	164.10±5.25	162.25±4.13	168.65±2.64
(g)					
Glucose	75.69±0.56	349.30±6.09	275.33±4.02	246.21±5.67	208.91±4.03 abcd
(mg/dl)		a	ab	abc	
Insulin	12.45±0.37	4.75±0.21	6.31±0.34	7.59±0.32	8.17±0.25
(IU/ml)		a	а	ab	abc
	121.38±0.66	184.48±1.27	170.80±0.47	160.59±1.51	158.25±0.23
α-Amylase (U/l)		a	ab	abc	abc
HbA ₁ C	3.95±0.16	10.39±0.51	7.55±0.20	6.30±0.26	5.38±0.23
(%)		а	ab	ab	abc
		6 v	veeks post treatmen	t	
Body weight	210.52±4.08	156.71±4.82	171.84±4.81	168.90±3.50	176.81±2.55
(g)		а	а	а	а
Glucose	79.68±2.40	363.21±7.11	254.45±9.42	230.29±5.81	194.81±5.56 ^{abcd}
(mg/dl)		а	ab	ab	
Insulin	13.88±0.33	6.17±0.21	7.73±0.34	9.02±0.32	9.59±0.25
(IU/ml)		а	а	ab	abc
	120.64±0.90	161.76±0.75	146.68±0.36	135.91±0.98	131.90±0.74
α-Amylase (U/l)		a	ab	abc	abc
HbA ₁ C	5.15±0.22	11.48±0.58	8.44±0.14	7.24±0.21	5.80±0.19
(%)		a	а	ab	abc

a, b, c & d Significantly difference at $P \le 0.05$. a : compared to control, b : compared to diabetic non treated. C : compared to chamomile & d: compared to oregano group

On regarding the results in Table 2, we found a significant increase ($P \le 0.05$) in the levels of blood urea, uric acid and creatinine, with a decrease in the total protein as a markers of renal dysfunction in the diabetic group compared to control group and treated diabetic groups. On the other hand, treatment of diabetic rat with each extract alone or in a mixture nearly renormalized the levels of blood urea, uric acid, creatinine and total protein as compared to diabetic rats (group 2). The results revealed that the mixture of both extracts on diabetic rats (group5) was more effective in the improvement of renal parameters compared to the effect of each extract on diabetic animals (Group 3 & 4). Similar effect was recorded previously by Alarcon et al. [58] and Jaya et al., [59]. Also, Najla et al., [15] indicated that, the treatment of diabetic rats with

chamomile extract decreased both of serum creatinine and blood urea to the normal level while uric acid level remained high. The difference in uric acid may be returned to the different dose. The decrease in blood urea and serum creatinine in chamomile treated diabetic rats may be due to the antioxidant activity of chamomile [15]. Moreover, Nema & Omimah [51] revealed that, the oral administration of the aqueous extract of oregano leaves improved the elevation in blood urea, uric acid and creatinine levels in diabetic rats. On the same hand, Khan *et al.* [60] stated that, oregano leaves showed anti-urolithic activity both *in vitro* and *in vivo* models in addition to its antioxidant, renal epithelial cell protective, antispasmodic and diuretic activities.

Groups Parameters	Control	Diabetic	Diabetic + Chamomile	Diabetic + oregano	Diabetic + Mixture	
	3 weeks post treatment					
BUN	18.02±0.42	37.74±0.23	28.43±0.21	26.83±0.26	25.33±0.34	
(mg/dl)		а	ab	abc	abc	
Creatinine	0.31±0.01	0.91±0.10	0.68±0.02	0.66±0.01 ^{ab}	0.63±0.01	
(mg/dl)		а	ab		ab	
Uric Acid	3.84±0.04	9.48±0.17	7.51±0.11	6.42±0.13 ^{abc}	5.90±0.20	
(mg/dl)		а	ab		abc	
Total protein	8.35±0.09	6.14±0.12	6.62±0.40	7.21±0.10 ^{ab}	7.36±0.08	
(g/dl)		а	а		abc	
		6 w	eeks post treatmen	t		
BUN	17.79±0.37	43.44±0.23	26.75±0.21	25.15±0.26	23.46±0.33	
(mg/dl)		а	ab	ab	ab	
Creatinine	0.34±0.01	1.02±0.02	0.51±0.01	0.44±0.01	0.40±0.01	
(mg/dl)		а	ab	abc	abc	
Uric Acid	3.24±0.18	10.74±0.33	6.84±0.15	5.76±0.27	5.16±0.31	
(mg/dl)		а	ab	а	ab	
Total Protein	7.67±0.37	5.68±0.18	6.14±0.09	6.59±0.11	6.98±0.21	
(g/dl)		а	а	ab	b	

 Table 2: Effect of Chamomile, Oregano and Their Mixture on BUN, Creatinine , Uric Acid and Total Protein in

 Alloxan Induced Diabetic Rats (mean ±S.E)

a, b, c & d Significantly difference at $P \le 0.05$. a : compared to control, b : compared to diabetic non treated. C : compared to chamomile & d: compared to oregano group

Concerning the results of total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein (LDL-c) and very low density lipoprotein (VLDL-c) in all groups were showed in Table (3). Serum cholesterol were triacylglycerol (TG) and LDL-c (TC), significantly (P≤ 0.05) increased in diabetic group compared to the control one while HDL-c was decreased. Treatment of diabetic rats with oregano and /or chamomile extracts lowered the level of total cholesterol, triglycerides and LDL-c and elevates HDLc as compared to the diabetic rats (group 2). The results of TC and LDL-c showed that the treatment of diabetic rats with the mixture of both extracts (group 5) were significantly effective as anti-hyperlipidemic compared to the effect of each extract alone on the diabetic animals (group 3 & 4). Our results are in agreement with that obtained by Pimple et al., [61] who observed that the extract of oregano was more effective in reducing the serum lipid levels significantly in NIDDM rats. Also, Najla et al., [15] reported that the increases in TC, TG and LDL-c with fall in HDL-c were reversed towards normal level after administration of the water extract of chamomile in streptozotocin induced diabetic. Moreover, Ozdemir et al., [62] reported that the consumption of Origanum onites distillate had a beneficial effects on lipid profile where it significantly highly increased HDL-c and significantly highly decreased LDL-c. The increase in serum levels of TC and TG may be due to the uninhibited actions of lipolytic hormones on the fat depots [63] or to the increase in the metabolism of free fatty acids from the peripheral fat depots [64]. On the same direction, Estakhr & Javdam [65], reported that the cause of depletion of glucose, TC and TG in hyperglycemia condition after using Matricari recutita extract is the inhibition of the enzyme glycogen phosphorylase catalyzes glycogenolysis.

Groups Parameters	Control	Diabetic	Diabetic + Chamomile	Diabetic + oregano	Diabetic + Mixture		
	3 weeks post treatment						
Cholesterol	92.65±1.11	161.14±2.70	146.27±2.52	143.64±1.79	133.59±1.884		
(mg/dl)		а	ab	ab	abcd		
Triglycerides	94.45±2.50	167.66±2.35	138.87±2.01	132.80±4.06	128.67±6.58		
(mg/dl)		а	ab	ab	ab		
HDL-C	59.09±1.78	37.91±0.60	42.83±0.30	43.94±0.13	50.65±0.42		
(mg/dl)		а	ab	ab	bcd		
LDL-C (mg/dl)	14.66±2.49	89.69±2.44	75.65±2.49	73.13±2.15	57.21±1.34		
		а	ab	ab	abcd		
VLDL-C	18.89±0.50	33.53±0.47	27.77±0.40	26.56±0.81	25.73±0.85		
(mg/dl)		а	ab	ab	ab		
		6 w	eeks post treatmen	t	I		
Cholesterol	93.40±0.51	170.17±3.17	136.45±3.12	132.28±3.50	124.19±4.40		
(mg/dl)		а	ab	ab	ab		
Triglycerides	99.34±3.25	170.96±2.01	132.20±3.51	127.50±2.12	121.68±3.13		
(mg/dl)		а	ab	ab	ab		
HDL-C	62.52±0.83	31.26±0.87	46.83±0.30	50.29±0.15	54.40±1.55		
(mg/dl)		а	ab	ab	ab		
LDL-C (mg/dl)	11.01±1.38	104.72±3.08	63.18±3.05	56.49±3.75	45.44±3.61		
		а	ab	ab	ab		
VLDL-C	19.86±0.65	34.19±0.40	26.44±0.70	25.50±0.42	24.34±0.62		
(mg/dl)		а	ab	ab	ab		

Table 3: Effect of Chamomile, Oregano and Their Mixture on TC, TG, HDL-c, LDL-c and VLDL-c in Alloxan Induced Diabetic Rats (mean ±S.E)

a, b, c & d Significantly difference at $P \le 0.05$. a : compared to control, b : compared to diabetic non-treated. C : compared to chamomile & d: compared to oregano group.

CONCLUSION:

In conclusion, the present study demonstrated that daily oral consumption of chamomile and/or oregano could be potentially useful in the self-medication of hyperglycemia and diabetic complications. We should take inconsideration that both plant extracts have accumulative effect and oregano results seems to be better than chamomile.

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