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Study the Effect of Gurmmar Aqueous Extract on Oxidative Stress of Diabetic Rats

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Abstract

The present study was carried out to analyze the chemical composition of Gymnema sylvestre leaves powder, to identify its total phenolic compounds, also investigate the hypoglycemic effects of different doses of Gymnema sylvestre leaves aqueous extract (GAE) on oxidative stress of diabetic rats . A total of 30 male albino rats weighing(150±5g)were used in this study. Rats were divided into two main group .The first main group (6 rats) fed on basal diet (BD) as a negative control group (NC).The second main group(24 rats) was injected intra-peritoneally with asingle dose of streptozitocin at a dose of 60mg/kg body weight for induction of diabetes. The rats in this group divided into 4 subgroups (n = 6 each), subgroup (1) fed on basal diet, as a (positive control group). Subgroups (2, 3 and 4) fed on basal diet and treated orally with Gymnema water extract at (100 - 200 and 400 mg/kg b.wt/day) respectively. The chemical analysis of Gymnema sylvestre leaves powder showed that each 100 grams contained The highest concentration of total carbohydrate (50.78%) followed by total fiber (14.93 %), total protein (12.8 %) and ash (9.9 %),while the lowest concentration was recorded for total fat (4.39

%).The total phenolic compound (5073.9 ppm gymnenic acid equivalent), has been identified by HPLC.

Oral treating to diabetic rats with different doses of GAE at (100,200 and 400 mg/Kg) improved the mean values of lipid profile, kidney functions, glucose, liver enzymes, malondialdhyde and glutathione peroxidase, as compared to the positive control group.GAE play an important role in decreasing the complications which were resulting from diabetes. In conclusion , *Gymnema sylvestre* extract has high effective antioxidant, hypoglycemic, hypolipidemic, hepatoprotective and nephroprotective effects in STZ-intoxicated rats. The hypoglycemic and antioxidant activity of GAE could be due to presence of many phenolic compounds detected in this study .

Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects of insulin action, insulin secretion or both ***(ADA,2011).*** Diabetes has taken place as one of the most important diseases worldwide, reaching epidemic proportions. Hyperglycemia in the course of diabetes usually leads to the development of micro vascular complications, and diabetic patients are more prone to accelerated atherosclerotic macro vascular disease .These complications account for premature mortality and most of the social and economical burden in the long term of diabetes ***(King et al ., 2010)***. Hyperglycemia increases oxidative stress, which contributes to the impairment of the main processes that fail during diabetes, insulin action and insulin

secretion. In addition, antioxidant mechanisms are diminished in diabetic patients, which may further augment oxidative stress ***(Rains and Jain , 2011)*** and ***(Maritim et al ., 2003)*****.** Increasing evidence suggests that oxidative stress plays a role in the pathogenesis of diabetes mellitus and its complications ***(Brownlee , 2001).***

The herbal medicines are becoming popular due to better results and safe use as compared to marketed drugs and more effective treatment of health problems ***(Smith and Reynard,2011)*** .The bioactive constituents found in many plant species are isolated for direct use as drugs, lead compounds, or pharmacological agents. These traditional approaches might offer a natural key to unlock diabetic complications ***(Babu et al.,2006).*** The chemical structures of a phytomolecule play a critical role in its antidiabetic activity. Several plant species being a major source of terpenoids, flavonoids, phenolics, coumarins, and other bioactive constituents have shown reduction in blood glucose levels ***(Jung et al., 2006)*** and ***(Ji et al.,2009)***. Various antidiabetic plant extracts like **.** ***Gymnema sylvestre (Asclepiadaceae)***a vulnerable species is a slow growing, perennial, medicinal woody climber found in central and peninsular India. The leaves of this plant have been used in India for over 2000 years to treat madhumeha, or “honey urine.” Chewing the leaves destroysthe ability to discriminate the “sweet” taste, giving it its common name, gurmar, or “sugar destroyer.” ***(Kanetkar et al.,2007) .***

Plant constituents include two resins (one soluble in alcohol),gymnemic acids , saponins , stigmasterol,quercitol ***(Zarrelli et al.,2013),*** and the amino acid derivatives betaine, choline and trimethylamine ***(Fletcher et al.,2009).***

Gymnema sylvestre (GS) is considered to have potent anti-diabetic properties. This plant is also used for controlling obesity, in the form of *Gymnema* tea ***(Rachh et al.,2010).*** Extracts of its leaves and roots are used in India and parts of Asiaas a natural treatment for diabetes, as they help lower and balance blood sugar levels ***(Xie et al., 2003) .***

In addition, the plant possesses antimicrobial ***(Chodisetti et al.,2013)*** anti-hyphal ***(Vediyappan et al.,2013)*** anti-hypercholesterolemic ***(Bishayee and Malay,2000)*** and hepatoprotective activities ***(Rana and Avadhoot,2007).*** prevents dental caries caused by Streptococcus mutans ***(Hiji ,20011)*** , and is used in cosmetics ***(Valli and Rao ,2008).*** In addition, it is also used in the treatment of rheumatism, cough, ulcer, jaundice, dyspepsia, constipation, asthma, eye complaints, inflammations and snake bites**.**

Gymnema's antidiabetic activity appears to be due to a combination of mechanisms. Two animal studies on beryllium nitrate- and streptozotocin-diabetic rats found *Gymnema* extracts doubled the number of insulin-secreting beta cells in the pancreas and returned blood sugars to almost normal ***(Paliwal et al.,2009) .*** *Gymnema* increases the activity of enzymes responsible for glucose uptake and utilization ,and inhibits peripheral utilization of glucose by somatotrophin and corticotrophin ***(Nigur et al.,2008)*** .Plant extracts have also been found to inhibit epinephrine-induced hyperglycemia. ***(Singh et al., 2008).***

The present study aimed to analyze the Chemical composition of *Gymnema sylvestre* leaves powder, to indentify its total phenolic compounds , and to evaluate the effect of concurrent

administration of *Gymnema sylvestre* leaves aqueous extract on oxidative stress Of Diabetic Rats.

Materials and Methods

Plant

The dried *Gymnema sylvestre* leaves were purchased as crude dried material from a local company for folk Medicinal Plants and Herbs, Cairo, Egypt. leaves were ground into a fine powder using a coffee mixer and stored in an air-tight contained, kept in a desiccators until analyzed and preparation.

Rats

Thirty mature male albino rats of Sprague Dawley strain weighing (150±5g) and 10–12 weeks old were purchased from Laboratory of Animal Colony Helwan Egypt. Rats were maintained under controlled hygienic conditions.

Chemicals and biochemical kits

Biochemical kits for determination of liver enzymes aspartate amino transferase (AST) , alanine amino transferase (ALT) , alkaline phosphatase (ALP) and urea nitrogen, uric acid, creatinine, total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), Malondialdehyde (MDA) and Glutathione peroxidase (GPx) were purchased from the gamma trade company for pharmaceutical and chemicals, Dokki, cairo ,Egypt. And chemicals were purchased from EL - Gomhorya company Cairo, city, Egypt. Streptozotocin (STZ) (Sigma-Aldrich, Germany) was purchased from sigma chemical company, St Louis, Missouri. USA.

Preparation of *Gymnema sylvestre*tea:

The dried leaves were milled using a coffee grinder into a fine powder. *Gymnema sylvestre* tea was prepared by using 25,50 and 100g fine powder /100ml distilled water and boiling for 5 min at100 ºC The solution was kept to stand for 10min before being filtered , cooled to room temperature and adjusted to 100ml water before using ***(Renno et al., 2006) .***

Sensory evaluation:

Tea was prepared freshly in boiled water at the concentration 10g/100 ml and kept in thermo bottles, and was served warm during the different tests .Then sensory acceptance test expressed as taste, color, aroma, appearance and overall acceptability was evaluated by ten randomized volunteers ***(Ekissi et al., 2014).***

Chemical analyses of *Gymnema sylvestre*:

Moisture, protein, fat , ash of *Gymnema sylvestre* (leaves) fine powder were determined separately according to the methods of the **(*A.O.A.C. 2000)*** ,while total carbohydrates were calculated by differences as following: Carbohydrate % = 100 - (Moisture% + protein % + fat% + Ash%).

Determination of phenolic compounds:

The ethanolic extract of total phenolic compounds were determined by HPLC according to ***(Singleton and Rossi ,1965).***

Preparation of Gymnema sylvestre Extracts :-

One kg of dried, powdered leaves of *Gymnema sylvestre* leaves .were dissolved in 4 litres of distilled water and allowed to soak overnight. The suspension were centrifuged at 5000 rpm for 20

minutes and filtered through a Whatman No. 1 filter paper. The supernatant fluid was allowed to evaporate in sterile, glass petri dishes under tube light. When completely dry, the extract was collected by scraping and stored. Stock solution of aqueous extract was prepared by dissolving 500 mg of the extract in 5 ml of distilled water ***(Venugopaland Venugopal, 1994).***

\\\\Preparation of basal diet:

Basal diet was prepared according to the method of ***(Reeves et al.,1993).*** It was consisted of 20 % protein (casein), 10 % sucrose , 4.7% fat (corn oil), 0.2% choline chloride, 1% vitamin mixture, 3.5 % salt mixture and 5% fibers. The remainder was corn starch up to 100%.

Experimental design and grouping of rats:

A total of 30 male albino rats weighing (150±5g) fed one week on basal diet for adaptation.

After adaptation period the rats were divided into two main groups as follows:

The first main group (6 rats): fed on basal (control negative group).

The second main group (24 rats): injected with streptozitocin to induce diabetes, animals was injected with a single dose of freshly prepared solution of streptozitocin monohydrate (dissolved in ice cold water) intra -peritoneally at a dose of 60mg/kg body weight ***(Rao et al., 2001).*** Since streptozitocin is able to produce hypoglycemia as a result of massive pancreatic insulin release, rats were given 20% glucose solution , after 6 hours. The rats were kept for the next 24 hours on 5% glucose solution. 5% Glucose containing water bottles \were kept in the cages to prevent hypoglycemia ***(Gupta et al.,***

2009). Control group rats were treated identically and served as diabetic control.

After 48 hours blood samples were drawn in order to ensure that hyperglycemia has been induced. The levels of blood glucose considered to be in normal ranges from 50-135mg/dl. Animals with fasting glucose levels >120mg/dl were considered as diabetic and chosen for experimental study ***(Pari and Maheswari, 1999).***

This groups divided into 4 subgroub as follows:

The experimental groups were as follows:-

Subgroup(1): Diabetic rats fed on basal diet only, “Positive control”.

Supgroup(2): Diabetic rats fed on basal diet and treated orally with Gymnema water extract in a dose of 100 mg/kg b.wt/day.

Subgroup(3): Diabetic rats fed on basal diet and treated orally with Gymnema water extract in a dose of 200 mg/kg b.wt/day.

Subgroup(4): Diabetic rats fed on basal diet and treated orally with Gymnema water extract in a dose of 400 mg/kg b.wt/day.

At the end of the experimental period (4 weeks), rats were fasted over night before sacrificing. Blood samples collected from each rat and centrifuged at 3000 r.p.m. to separate the serum. Serum was carefully separated and transferred into dry clean Ebendorf tubes and kept frozen at - 20° C till analysis.

Liver and kidney were removed by careful dissection and blotted free of adhering blood immediately, after sacrificing the rats. The organs were washed with cold saline and dried between two filter papers, then weighed (10%) according to ***(Drury and Wallington, 1980).***

Biological evaluation:

Biological evaluation of the different tested diets was carried by determination of feed intake (FI), body weight gain% (BWG %) and organs weight body weight% according to ***(Chapman et al., 1959)*** using the following equation:

BWG% = Final body weight – Initial body weight / Initial body weight × 100

Biochemical analyses

Serum total cholesterol was determined according to ***(Allain et al., 1974),*** triglycerides ***(Fossati and Principe, 1982)*** and high density lipoprotein ***(Virella , 1977)*** were chemically measured. Low density lipoprotein (LDL) was calculated ***(Friedewald et al.,1972) .*** Serum glucose levels were determined according to the methods of ***(Trinder ,1969).*** Activities of serum liver enzymes aspartate amino transferase (AST), alanine amino transferase (ALT)and alkaline phosphatase (ALP) were chemically determined according to ***(Reitman and Frankel ,1975).*** Blood urea nitrogen was determined using Bio Meraux kits according to ***(Patton and Crouch ,1977)***, Serum uric acid ***(Haisman and Muller ,1977),*** Urea nitrogen in serum was determined calorimetrically according to ***(Henry et al., 1974).***and creatinine concentrations were chemically determined ***(Bartels and Bohmer, 1971).*** Serum activity of GPX enzyme was determined according to the methods described by ***(Paglia and***

Valentaine ,1967), Serum MDA level as µmoles/dL. was determined as described by ***(Draper and Hadley, 1990).***

Statistical analysis

The statistical analysis was carried out by using SPSS, PC statistical software ***(version 10.0; SPSS Inc, Chieago, USA).***The results were expressed as mean ± SD. Data were analyzed by one way analysis of variance **(ANOVA).** The differences between means were tested for significance using least significant difference (LSD) test at (P<0.05) ***(Steel and Torri, 1980).***

Results and Discussion

Data presented in table (1) showed that drink tea prepared from dried *Gymnema sylvestre* leaves using different concentration (2.5, 5 or 10%) of as preliminary study to evaluate its sensory characteristics. A significant difference in taste, color and overall acceptance were found between the different concentrations, while there was no significant different in aroma .Tea prepared using 2.5% concentration was more acceptable to all ten volunteers.These results are in agreement with ***(Sharmaet al .,2017)*** who showed that**,** *Gymnema sylvestre* liquid extract had a brownish green color, no specific odor and bitter in taste while dried extract was brown in color and also bitter in taste.

The result of chemical composition of dried *Gymnema sylvestre* leaves is recorded in table (2).The chemical analysis of dried *Gymnema sylvestre* leaves revealed that, The concentration of total carbohydrate (50.78%) was the highest followed by total fiber

(14.93%), total protein (12.8%), ash (9.9%). While the lowest concentration was recorded for total fat (4.39%).

These results are in agreement with ***(Sharma et al ., 2017)*** who found that, the proximate composition of *Gymnema sylvestre* powder like moisture ,crude fat, crude protein , crude fiber ,total ash and total carbohydrate contents were 7.38, 5.80, 10.94, 11.50, 9.49 and 54.89 per cent respectively.

Total phenolic compound in Gymnema sylvestre leaves extract is recorded in table (3).it is clear from table (2) that ,the concentration of total phenolic compound in *Gymnema sylvestre* leaves extract was 5073.9ppm(gymnenic acid equivalent). These results are in agreement with ***(Kritikar and Base , 2011)*** who showed that the major bioactive constituents of *Gymnema sylvestre* are a group of oleanane-type triterpenoid saponins known as gymnemic acids.

The gymnenic acid is made up of molecules whose atom arrangement is similar to that of glucose molecules .Those molecules fill the receptor locations on the taste buds for a period of one to two hours, thereby preventing the taste buds from being activated by any sugar molecules present in the food. Similarly the glucose-like molecules in the gymnemic acid fill the receptor locations in the absorptive external layers of the intestine thereby preventing the intestine from absorbing the sugar molecules ***(Sujin et al.,2009)***. Presence of gymnemic acid in leaves suppresses the transport of glucose from the intestine into the blood stream resulting lowering of blood sugar level***,(Saneja et al,.2010).***

Table(4) showed that intraperitoneal injection of streptozotocin in a single dose (60 mg/kg b.wt) to rats caused significant decreases (P< 0.05) in body weight gain, but caused an increase in feed intake, liver and kidney weight when compared to the negative control group. Oral administration of *Gymnema sylvestre* leaves extract (GAE) (400 mg/kg) to rats inflicted with oxidative stress caused significant (P<0.05) increases in body weight gain, feed intake and as compared to the positive control group fed on(BD) . All treated groups with oral administration of GAE at (100 , 200 and 400 mg /kg/d) caused, non-significant changes in feed intake and BWG% , as compared the positive control group. Oral administration of GAEat (100, 200 and 400 mg /kg/d) caused significant decreases (p<0.05), in liver and kidney weights/body weights %, as compared to the positive control group.

These results are in agreement with ***(Pothuraju et al.,2013)*** different extracts (aqueous, methanol, ethanol and acetone) of *Gymnema sylvestre* have a role in the treatment of body weight gain and accumulation of lipids in epididymal fat tissue, liver and muscle. On the other hand***(Reddy et al ., 2012)*** demonstrated that , feeding of aqueous extract of saponins rich in *Gymnema sylvestre* (100mg/ kg/d) for 8weeks reduced body weight gain and organ weight such as liver, kidney and heart.Also***(Kang et al ., 2012)*** concluded that , an ethanol extract of *Gymnema* sylvestre(100mg/kg/d) fed for 4 weeks to STZ diabetic rats showed reduction in body weight, slightly increased liver weight and no change in kidney weight.

Data in table (5)showed that there were significant elevations in glucose levels of all the animals administered streptozotocin after three days as compared to normal control. On the other hand, there

were significant increases in blood glucose levels in the untreated diabetic rats compared to the normal control group were continuous until the end of the study. Treated diabetic rats with different doses of gurmmar aqueous extract at (100 , 200 and 400 mg/kg b wt) caused significant decrease in glucose level compared to the untreated diabetic rat (+ve). The treated group with gurmmar aqueous extract at (400 mg/kg b.wt) normalized blood glucose level compared to control negative group.

These results are in agreement with ***(El Shafey et al., 2012)*** and ***(Daisy et al.,2009)*** who showed that ,there are a significant reduction in plasma glucose level by (20.20%) in diabetic rats treated with *Gymnema sylvestre* leaves extract at (18 mg/kg body weight) when compared with untreated diabetic rats. On the other hand***(Sathya et al.,2008)*** reported that, oral administration with (2ml/kg) of the water extract of *Gymnema sylvestre* leaf to both normal and diabetic rats caused a significant reduction in blood glucose level in diabetic rats.

Vaidya , (2011) demonstrated that ,Gymnemic acid has been found to interfere with the ability of the taste buds on the tongue to taste sweet and bitter. It is believed that by inhibiting the sweet taste sensation, it will limit their intake of sweet foods, and this activity may be partially responsible for its hypoglycemic effect .

Agarwal et al .,(2000) explained the mechanisms by which gymnemic acids exert its hypoglycemic effects by several ways like, it increases secretion of insulin, promotes regeneration of islet cells, increases utilization of glucose: it is shown to increase the activities of enzymes responsible for utilization of glucose by insulin-dependent pathways, an increase in phosphorylates activity, decrease in

gluconeogenic enzymes and sorbitol dehydrogenase, and it causes inhibition of glucose absorption from intestine by binding the glucose binding sites on transport receptors .

As shown in Table (6), the rats injected intraperitoneally with streptozotocin had significant increases in ($P< 0.05$) serum levels of total cholesterol (TC), triglycerides (TG) and low density lipoprotein (LDL) when compared to the negative control group. Oral administration of gurmmar aqueous extractat (100,200 and 400 mg/kg) to diabetic rats inflicted with oxidative stress resulted in significant decreases ($P< 0.05$) in the elevated serum TC, TG and LDL levels and an increase in serum HDL when compared to the positive control (+ve) group.

These results are in agreement with***(Kim et al.,2016), (Li et al.,2015),(Aralelimath and Bhise , 2012) , (Kang et al.,2012) and (Daisy et al., 2009)***who concluded that, Oral administration with GS at (100 mg/kg body weight daily) to STZ diabetic rats decreased TG ,TC,LDL-C levels and caused an increased in HDL-C levels in blood serum . ***Aralelimath and Bhise , (2012)*** explained the reasons for decreasing levels of triglyceride, cholesterol and LDL-cholesterol and increasing level of HDL-cholesterol might be due to an increase in insulin which caused an increased activity of lipoprotein lipase (Facilitated chylomicron transport through cell membranes) and a decreased activity of hormone-sensitive lipase (converted neutral fats into free fatty acids) . Also ***(Mallet al.,2009)*** reported that ,G. sylvestre decreases total cholesterol, LDL-cholesterol, VLDL-cholesterol and triglyceride levels in diabetic rats and that could be due to the presence of hypolipidemic agent such as sitosterol in the aqueous leaf extract.

Data presented in table (6) showed that, intraperitoneal injection of streptozotocin (STZ) in a single dose at 60 mg/kg/day to rats caused hyperglycemia manifested by significant (*P*< 0.05) increases in blood urea nitrogen , uric acid , creatinine, when compared to normal control group (–ve) .Oral administration with different doses of of gurmmar aqueous extract (GAE)at 100,200 and 400 mg/kg b.wtto diabetic rats for 4 weeks induced significant (*P*< 0.05) decreases in elevated blood urea nitrogen , uric acid , creatinine, when compared to positive control diabetic rats. Oral administration with gurmmar aqueous extract (GAE)at had the best effect in all kidney biomarker in serum near to the normal control group.

These results are in agreement with ***(Kishore and Singh,2017)*** who illustrated that, supplementation with homeopathic preparation of *Gymnema sylvestre* showed protective effect against Diabetic nephropathy (DN), since they exhibited beneficial effects on the blood glucose level, associated biomarkers of DN and advanced glycation end products (AGEs) in kidney, Moreover, biomarkers of diabetic nephropathy (uric acid, urea and creatinine level) were also improved after the administration of *Gymnema sylvestre* in diabetic animals. Also ***(Sathya et al.,2008)*** showed that, kidney biomarkers(Urea, uric acid and creatinine) levels were increased in untreated diabetic rats .But After oral administration treatment with water extract of *Gymnema sylvestre* leaf at (2ml/kg .b.wt) the levels of these biomarker were altered near to normal level .

As shown in Table (7), the rats injected intraperitoneally with streptozotocin (STZ) in a single dose at 60 mg/kg/day to rats had significant increases(P< 0.05) in serum levels of AST, ALT, ALP enzymes, when compared to the negative control group. Oral

administration of gurmmar aqueous extract at (100,200 and 400 mg/kg) to rats inflicted with oxidative stress resulted in significant decreases ($P< 0.05$) in the elevated serum AST, ALT and ALP when compared to the positive control (+ve) group. The mean value of liver enzymes decreased gradually with increasing the three dosages of (GAE).

These results are in agreement with ***(El Shafeyet al.,2012)*** who concluded that , treated diabetic rats with *Gymnema* sylvestre leaves extract at (18 mg/kg body weight) led to significant decrease in ALT and AST compared to untreated diabetic rats and ***(Pothurajuet al.,2013)*** who showed that different extracts (aqueous, methanol, ethanol and acetone) of *Gymnema sylvestre* play an important role in the treatment of liver diseases .

Data illustrated in table (8)showed that, rats subcutaneously injected with a single dose of STZ (positive control) had significant decrease in antioxidant enzymes activity glutathione peroxidase (GPx) in blood serum and enhanced the end product of lipid peroxidation (MDA) level in blood serum as compared with the negative control group .

Oral administration of gurmmar aqueous extractat (100, 200 and 400 mg/kg) group for four weeks after injection with a single dose of STZ showed significant increase in the (GPX) enzyme in blood serum, while the elevated (MDA) levels were found to be reduced back towards the normal level in the treated rats given the highest dose of (GAE). The level of antioxidant enzyme was significantly improved by administration of (400mg/kg b.wt) of gurmmar aqueous extract in STZ diabetic.

These results are in agreement with ***(Ohmoriet al.,(2005)*** Discovered the antioxidant ability of *Gymnema sylvestre* when study the antioxidant activity of six teas against free radicals and LDL oxidation **.**

Vasi and Austin,(2009) concluded that, administration of *Gymnema sylvestre* extract to diabetic rats increased superoxide dismutase activity and decreased lipid peroxide by either directly scavenging the reactive oxygen species, due to the presence of various antioxidant compounds, or by increasing the synthesis of antioxidant molecules (albumin and uric acid) .

Kang et al., (2012) reported that, orall *Gymnema sylvestre* extract to diabetic rats decreased lipid peroxidation levels in serum, in liver and in kidney and decreased the activity of glutathione peroxidase in cytosolic liver and glutamate pyruvate transaminase in serum to normal levels.

Al-Rejaieet al.,(2012) concluded that , *Gymnema sylvestre* pretreatment at doses of 100, 200 and 400 mg/kg/d for 4weeks decreased the malondialdehyde levels by increasing the dose.

Kang et al .,(2012) confirmed that,Oral feeding of ethanolic extract of *Gymnema sylvestre*showed a reduction in lipid peroxidation product (e.g. malonaldehyde) in serum , in liver and in kidney in diabetic rats. Moreover, the extract increased glutathione content and also increased the activity of enzymes such as glutathione peroxidase (GSH-Px) , glutathione-S-transferase (GST) and catalase in rat liver.

Table (1): Sensory Evaluation of tea drink prepared from dried Gymnema sylvestre leaves

Samples	Taste	Color	Aroma	overall acceptance
2.5 gm/100ml	13.800[a] ± 2.347	24.100[a] ± 1.595	25.00[a] ± 0.00	24.400[a] ± 0.966
5 gm/100ml	8.100[c] ± 2.558	21.600[b] ± 1.837	25.00[a] ± 0.00	21.800[b] ± 1.549
10 gm /100ml	3.900[d] ± 2.378	16.100[c] ± 2.183	25.00[a] ± 0.00	15.700[d] ± 2.162

All results are expressed as mean ± SD

Values in each column, which have different litters, are Significant different (P <0.05)

Table 2: chemical composition of dried *Gymnema sylvestre* leaves

Parameters %	Dry matter (gm/100g)
Ash content %	9.9
Total fat %	4.39
Total protein%	12.8
Total carbohydrate%	50.78
Total fiber%	14.93
Total moisture%	7.2

Table 3: Concentrations of total polyphenolic compounds in Gymnema sylvestre leaves extract

Parameters	Concentrations (ppm)
Total phenols	5073.9ppm gymnenic acid equivalent

Table (4): Effect of different doses of gurmmar aqueous extract on feed intake, body weight gain % and some organs weight/body weight% of diabetic rats

Parameters / Groups	Feed Intake g/day/rat	BWG%	Organs weight/body weigh %	
			Liver	Kidney
Control (-ve) "Healthy rats"	17.700[a] ±1.122	33.690[a] ± 2.208	2.483[e] ± 0.095	0.479[d] ± 0.041
Control (+ve) "Diabetic rats"	18.637[a] ± 0.725	17.208[b] ± 1.186	3.565[a] ± 0.132	0.792[a] ± 0.064
100 mg GAE/ kg b.w	18.385[a] ± 1.032	16.352[b] ± 1.100	3.283[b] ± 0.106	0.703[b] ± 0.024
200 mg GAE/ kg b.w	18.235[a] ± 0.878	16.00[b] ± 0.768	3.036[c] ± 0.059	0.597[c] ± 0.020
400 mg GAE/ kg b.w	17.976[a] ± 0.817	15.360[b] ± 0.642	2.725[d] ± 0.060	0.520[d] ± 0.029

GAE: gurmmar aqueous extract

Mean values in each column with same letters are not significantly different.

LSD: Least significant differences (P<0.05).

Table (5): Effect of different doses of gurmmar aqueous extract on lipid profile of diabetic rats

Groups	TC (mg/dL)	TG (mg/dL)	LDL (mg/dL)	HDL (mg/dL)
Control (-ve) "Healthy rats"	70.695 [d] ± 4.237	39.480 [c] ± 3.594	20.521 [e] ± 2.149	42.278 [a] ± 2.370
Control (+ve) "Diabetic rats"	134.065 [a] ± 7.882	67.567 [a] ± 5.805	102.590 [a] ± 6.014	17.962 [e] ± 0.813
100 mg GAE/ kg b.w	124.907 [a] ± 8.213	62.206 [a b] ± 5.849	88.616 [b] ± 5.779	23.850 [d] ± 1.527
200 mg GAE/ kg b.w	108.500 [b] ± 7.328	54.819 [b] ± 5.253	68.431 [c] ± 4.359	29.105 [c] ± 2.569
400 mg GAE/ kg b.w	92.879 [c] ± 5.550	45.359 [c] ± 3.862	51.314 [d] ± 5.281	34.754 [b] ± 2.236

GAE: gurmmar aqueous extrac

Mean values in each column with same letters are not significantly different. LSD: Least significant differences (P<0.05).

Table (6): Effect of different doses of gurmmar aqueous extract on kidney functions of diabetic rats.

Parameters / Groups	Uric acid	Urea Nitrogen	Creatinine
	mg/dl		
Control (-ve) "Healthy rats"	1.319 ^{e} ± 0.064	22.187 ^{e} ± 1.705	0.541 ^{e} ± 0.017
Control (+ve) "Diabetic rats"	2.510 ^{a} ± 0.082	59.918 ^{a} ± 1.225	1.364 ^{a} ± 0.044
100 mg GAE/ kg b.w	2.260 ^{b} ± 0.085	54.418 ^{b} ± 1.459	1.195 ^{b} ± 0.033
200 mg GAE/ kg b.w	2.095 ^{c} ± 0.115	44.806 ^{c} ± 2.194	0.953 ^{c} ± 0.057
400 mg GAE/ kg b.w	1.701 ^{d} ± 0.095	37.073 ^{d} ± 1.867	0.766 ^{d} ± 0.060

GAE: gurmmar aqueous extract

Mean values in each column with same letters are not significantly different.

LSD: Least significant differences (P<0.05).

Table (7): Effect of different doses of gurmmar aqueous extract on liver enzymes of diabetic rats

Parameters / Groups	AST	ALT	ALP
	U/l		
Control (-ve) "Healthy rats"	52.392 ^{d} ± 3.103	17.902 ^{e} ± 1.873	84.950 ^{e} ± 2.073
Control (+ve)"Diabetic rats"	93.160 ^{a} ± 4.992	55.484 ^{a} ± 1.675	156.940 ^{a} ± 8.798
100 mg GAE/ kg b.w	87.801 ^{a} ± 3.936	50.517 ^{b} ± 1.364	145.082 ^{b} ± 7.271
200 mg GAE/ kg b.w	76.551 ^{b} ± 4.659	40.439 ^{c} ± 1.181	133.188 ^{c} ± 5.537
400 mg GAE/ kg b.w	66.991 ^{c} ± 4.076	32.914 ^{d} ± 1.449	120.382 ^{d} ± 4.588

GAE: gurmmar aqueous extract

Mean values in each column with same letters are not significantly different.

LSD: Least significant differences (P<0.05).

Table (8): Effect of different doses of gurmmar aqueous extract on malonialdehyde and glutathione Peroxidase of diabetic rats

Parameters / Groups	Malonialdehyde MDA mmol/l	Glutathione Peroxidase (GPx)(ng/ml)
Control (-ve) "Healthy rats"	8.00 [e] ± 0.318	0.538 [e] ± 0.010
Control (+ve) "Diabetic rats"	18.660 [a] ± 0.754	0.202 [a] ± 0.007
100 mg GAE/ kg b.w	16.832 [b] ± 0.598	0.286 [b] ± 0.022
200 mg GAE/ kg b.w	14.221 [c] ± 0.859	0.413 [c] ± 0.013
400 mg GAE/ kg b.w	12.128 [d] ± 0.592	0.468 [d] ± 0.013

GAE: gurmmar aqueous extract

Mean values in each column with same letters are not significantly different.

LSD: Least significant differences (P<0.05).

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دراسة تأثير المستخلص المائي للجورمار على الأجهاد التاكسدى فى الفئران المصابة بالسكر

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المستخلص العربى

استهدفت هذه الدراسة التحليل الكيميائى وتقدير المركبات الفينولية فى مسحوق أوراق الجورمار و معرفة تأثير المستخلص المائى للجورمار على الاجهاد التأكسدى فى ذكور الفئران التي أُحدث بها مرض السكر، استخدمت فى هذه التجربه 30 فأر من نوع الالبينو ، اوزانهم (150 ± 10) جم ، تم تقسيم الفئران عشوائياً الى مجموعتين رئيسيتين . المجموعة الاولى (6فئران) تم تغذيتها على غذاء اساسى واستخدمت كمجموعة ضابطة سالبة سليمة . المجموعة الرئيسية الثانية وعددها (24فأر) تم تقسيمهم الى اربعة مجموعات فرعية (6فئران لكل منهم) وتم حقن الاربعة مجموعات من هذه الفئران بجرعة واحدة من محلول محضر حديثا من مادة الاستربتوزيتوسين (مذابة في ماء بارد مثلج) داخل الغشاء البريتوني بجرعة 60 مجم /كجم من وزن الجسم لاحداث مرض السكر . المجموعة الفرعية (1) تم تغذيتها على غذاء اساسى واستخدمت كمجموعة ضابطة موجبة مصابه بالسكر ، المجموعة الفرعية (2 ، 3 ، 4) تم تغذيتهم على غذاء اساسى مع اعطاؤها عن طريق انبوب الفم منقوع مستخلص الجورمار بجرعات (100 ، 200 ، 400 ملجم / كجم / يوميا) على التوالى ، وأوضحت نتائج التحليل الكيمائى أن كل 100 جرام من مسحوق اوراق الجورمار احتوت على اعلى نسبة من الكربوهيدرات % (50.78%) ويليها الألياف (14.93%), , البروتين(12.8%) , الرماد ,(9.9%) والتركيز المنخفض كان للدهون (4.39%), .وكان التقدير الكلى للمركبات الفينولية (5073.9 ppm gymnenic acid equivalent) والتى تم فصلها باستخدام جهاز كروماتوجرافيا السائل ذو الضغط المرتفع و أشارت النتائج الى ان العلاج الفموى بجرعات مختلفة من مستخلص الجورمار (100 , 200 , 400 ملجم / كجم) حدث تحسن ف بالدهون الكلية ، ومستوى جلوكوز الدم ، وظائف الكلى ،

انزيمات الكبد ، ومالونديالدهيد , الجلوتاثيون بيروكسيداز فى الفئران المصابة بالسكر مقارنة بالمجموعة الضابطة المصابة , وأحدث اعطاء خلاصة اوراق الجورمار تأثيرات مضادة للأكسدة ومخفضة للسكر و لدهون الدم وواقية للكبد والكلى فى الفئران المصابة بالسكر وتقليل المشاكل الناجمة عن السكر . ويعزى التأثير المضاد للسكروللاكسدة من اوراق الجورمار الى وجود العديد من المركبات الفينولية التى تم فصلها فى هذه الدراسة .