Efficacy of *Glycyrrhiza glabra* Roots Extract as an Anti-oxidative Agent Against Oxidative Stress in Diabetic Rats

Amany Ahmed Abd El-Aziz
Assistant Professor of Nutrition and Food Science
Faculty of Specific Education, South Valley University, Egypt

Doaa Ibrahim Mohamed Kabil
Assistant Professor of Nutrition and Food Science
Faculty of specific education-tanta unversity

Abstract: The current study was conducted to investigate the efficacy of *Glycyrrhiza glabra* roots extract as an anti-oxidative agent in diabetic rats. *Glycyrrhiza glabra* roots extract was prepared to assess the presence of antioxidant components. Thirty five albino male rats initially weighing 200-220g were randomly divided into five groups; group (1) represented as negative control group, and other groups were injected with alloxan to induce diabetes. Group (2) represented as positive control group, groups 3, 4 and 5 were treated orally with 200, 400 and 800 mg/kg of *Glycyrrhiza glabra* roots extract for 8 weeks. Results showed that the alcoholic extract of *Glycyrrhiza glabra* was positive for the presence of Saponins, tannins, flavonoids, terpenoids, and steroids, but alkaloids were absent. Injection with alloxan led to severe reduction in body weight despite of increasing feed intake compare to negative control group. The treatment with *Glycyrrhiza glabra* roots extract improved significantly the severe loss of body weight resulted by alloxan. The serum glucose level was measured in the first week after injection by alloxan, in the 4th week, and at the end of the experiment. The most alleviation of serum glucose was scored in group (5) that treated orally with 800 mg/kg b.wt of *Glycyrrhiza glabra* extract. *Glycyrrhiza glabra* roots extract improved significantly lipid profile status. Diabetes led to an increase in malondialdehyde (MDA) level. On the other hand glutathione peroxidase (GSH-PX), catalase (CAT) and superoxide dismutase (SOD) levels were significantly lower due to diabetes. Treatment with *Glycyrrhiza glabra* roots extract modified the oxidative stress status of treated groups 3, 4 and 5. In conclusion, *Glycyrrhiza glabra* roots extract had an effective and powerful antioxidant activity, and is considered as anti-diabetic, anti-atherosclerotic and can be used for various pathologies.

Key words: Diabetic mellitus, *Glycyrrhiza glabra*, Oxidative stress, Bioactive compounds.

فاعلية مستخلص نبات العرقسوس كعامل مضاد للإجهاد التأكسدي على فئران التجارب المصابة بمرض السكر

الملخص: أجريت الدراسة الحالية لتقييم فاعلية مستخلص جذور العرقسوس كعامل مضاد للأكسدة على الفئران المصابة بالبول السكري. تم تحضير مستخلص جذور العرقسوس لتقييم محتواها من المواد المضادة للأكسدة. تم تقسيم خمسة وثلاثين من ذكور الفئران البيضاء يتراوح وزنها من ٢٠٠-٢٢٠ جرام عشوائيا إلى خمس مجموعات كالتالي: المجموعة (١) تمثل المجموعة الضابطة السالبة، والمجموعات الأخرى تم حقنها بمادة الألوكسان لإحداث الاصابة بمرض السكر. المجموعة الثانية تمثل المجموعة الضابطة الموجبة ، وتم معالجة المجموعات ٣ ، ٤ ، ٥ عن طريق الفم بجرعات ٢٠٠ ، ٤٠٠ ، ٨٠٠ ملجم/ كجم من مستخلص جذور العرقسوس لمدة ٨ أسابيع. أظهرت النتائج ايجابية مستخلص جذور العرقسوس لوجود مركبات الفينول والفلافونيد والتربونيدات والاسترويدات وكانت النتائج سلبية لوجود القلويدات. أدى الحقن بعقار الألوكسان إلى انخفاض شديد في وزن الجسم بالرغم من زيادة المستهلك من الغذاء مقارنة بالمجموعة الضابطة السالبة. أدى العلاج بمستخلص جذور العرقسوس إلى تحسن معنوي في كلا من النقص الحاد في الوزن الناتج عن الحقن بالألوكسان ، المستهلك من الغذاء ، ووزن الجسم المكتسب وكذلك نسبة كفاءة الغذاء. تم قياس مستوى الجلوكوز في الدم في الأسبوع الأول بعد الحقن بالألوكسان وفي الأسبوع الرابع وفي نهاية التجربة. سجلت المجموعة (٥) التي عولجت التي عولجت ب٨٠٠ ملجم/ كجم من مستخلص جذور العرقسوس أكبر انخفاض لمستوى السكر بالدم. وكذلك تحسين مستوى دهون الدم. أدت الإصابة بمرض السكري إلى ارتفاع مستوى المالونديالدهيد (MDA). من بينما كانت مستويات الجلوتاثيون بيروكسيديز (GSH-PX)، والكاتلاز (CAT)، وفوق أكسيد الديسموتاز (SOD) أقل بشكل ملحوظ. أدى العلاج بمستخلص جذور العرقسوس إلى تعديل حالة الإجهاد التأكسدي للمجموعات المعالجة ٣ و٤ و٥. وفي الختام، كان لمستخلص جذور العرقسوس نشاط مضاد للأكسدة فعال وقوي، ويعتبر مضادًا لمرض السكري ، كما يمكن استخدامه كعلاج لكثير من الأمراض.

الكلمات المفتاحية: مرض السكري، نبات العرقسوس، الإجهاد التأكسدي، المركبات الحيوية النشطة

INTRODUCTION

Diabetes mellitus (DM) manifests as a global health problem, according to the International Diabetes Federation estimation, an overall prevalence in 2019 is estimated to be 9.3% (463 million people), rising to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045. The prevalence is higher in urban (10.8%) than rural **(Saeedi et al., 2019).** DM is a serious disorder that characterized by impaired glycemic control causing elevation in blood sugar levels that is known as hyperglycemia. The elevation in blood sugar levels leads to oxidative stress, subsequently several of secondary complications **(Wongdee and Charoenphandhu, 2011).** During diabetes, chronic hyperglycemia leads to increased production of free radicals

particularly reactive oxygen species (ROS), for all tissues from glucose auto-oxidation and protein glycosylation **(Robertson, 2004).**

Oxidative stress is known as an imbalance between the generation of oxidants and antioxidant defense capacity of the body. It is suggested as a mechanism underlying diabetes and diabetic complexity like many other diseases **(Atalay and Laaksonen, 2002)**. Most of antioxidant compounds derived from plant source have chemical properties as ability to trap free radicals; and have biological activities such as reducing hyperglycemia **(Mbikay, 2012)**.

Plants regarded as one of the most effective sources for treating common ailments and diseases as diabetes, they have therapeutic advantages, available and have the lowest side effects (**Madhavi et al., 2023)**.

Glycyrrhiza glabra Linne (common name: licorice) is widely used worldwide as one of herbal medicines, treating various diseases and benefiting our health. The genus Glycyrrhiza contains about 30 species, including *Glycyrrhiza glabra* L., *Glycyrrhiza uralensis* Fisch., and *Glycyrrhiza inflata* Bat **(Ding et al., 2022).**

Antioxidant activity is known as the capability of component to inhibit oxidative degradation, such as lipid peroxidation **(Fogliano et al., 1999)**. The predominant bioactive constituents of Glycyrrhiza plants include flavonoids and triterpenoid saponins which responsible for the sweet savor of *Glycyrrhiza glabra* **(Wang et al., 2015).** The phenolic compound glycyrrhizin that has a strong antioxidant activity **(Rackova et al., 2007)** and many flavonoids, involving glycyrrhizic acid, glabridin and isoliquiritigenin have been identified as bioactive compounds for treating diseases **(Deng et al., 2021).** Flavonoids have antioxidant properties responsible for the yellow color of *Glycyrrhiza glabra* **(Rizzato et al., 2017).**

Hence, Glycyrrhiza species can play a vital role as potential therapeutics in managing insulin resistance-affiliated disorders. Glabridin, a naturally occurring antioxidant, is a polyphenolic flavonoid originally isolated from the root of licorice (*Glycyrrhiza glabra* L.) **(Chin et al., 2007).** It helps in adipocyte differentiation and ameliorates glucose and lipid metabolism **(Gaur et al., 2014)**.

Glabridin inhibits glucose intolerance and achieves maximum glucose utilization by translocation of glucose transporter type 4 (GLUT-4) using Adenosine Mono Phosphate Protein Kinase (AMPK) **(Sawada et al., 2014).** Saponin compounds have also garnered attention over the past few decades, for their demonstrated therapeutic potential across various health ailments **(Du et al., 2014)**.

MATERIAL AND METHODS:

- **Preparation of the *Glycyrrhiza glabra* root alcoholic extracts**

Glycyrrhiza glabra roots were obtained from local Qena market, Qena governorate, Egypt. Roots were dried and grinded mechanically. 50g of the roots powder was dissolved in 500 ml of 70% ethanol for 24hrs, then put on the shaker for 6hrs. The extracts were sterilized by Millipore filter units. The filtrated extract was dried at 57-60°C in oven and the extract powder was used to prepare alcoholic crude extract and crude aqueous extract as described by **Ajagannavar et al. (2014).**

- **Detection of the presence of alkaloids, saponins and tannins** by the method described by **Kodangala et al. (2010)**.
- **Detection the presence of flavonoids, terpenoids and Sterols** by the method described by **Sharma et al. (2013).**
- **Determination of total flavonoids**

  The flavonoids content was determined by the method described by **Zhishen et al. (1999).**
- **Determination of total phenolic content**

  Total phenolic content was measured by using Folin's reagent according to the method of **Singleton and Rossi (1965).**
- **Assay of antioxidant activity**

The antioxidant activity of *Glycyrrhiza glabra* extract against the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical was assayed according to the method of **(Elslimani et al., 2013)**.

Experimental design:

Thirty five albino male rats (Sprague Dawley strain) initially weighing 200-220g were housed individually in wire cages under the normal laboratory conditions and fed on the basal diet for a week as adaptation period. Rats were divided into two main groups as following:

Group 1: was kept as a negative control group (NCG) (7 rats), that fed on basal diet, which was consisted of 20% casein, 10% corn oil, 1% vitamins mixture, 4% salt mixture, 5% fibers and 0.2% choline chloride as described by **(Reeves et al., 1993)**.

Second main group (diabetic rats): the second main group (28 rats) were injected by under skin injection of alloxan as described by **(Misra and Aiman, 2012 & Ananthan et al., 2004).** This group was divided into 4 groups as the following:

Group 2: was kept as a positive control group (PCG) fed on basal diet.

Group 3: was fed on basal diet and given orally *Glycyrrhiza glabra* extract at a dose of 200 mg/kg b.wt.

Group 4: was fed on basal diet and given orally *Glycyrrhiza glabra* extract at a dose of 400 mg/kg b.wt.

Group 5: was fed on basal diet and given orally *Glycyrrhiza glabra* extract at a dose of 800 mg/kg b.wt.

- **Induction of diabetes:**

Diabetes was induced in the rats by a single under the skin injection of freshly prepared alloxan monohydrate of 150 mg/kg as a 5% solution in normal saline). The animals were allowed to drink 5% glucose solution over night to overcome alloxan induced hypoglycemia **(Misra and Aiman, 2012 & Ananthan et al., 2004)** .

At the end of the experimental period (8 weeks), rats were fasted overnight and blood samples were collected from the reto-orbital plexus from all animals of each group into clean, dry and labeled centrifuge tubes. Blood samples were left to clot at room temperature, then serum was separated by cooling centrifugation (-3°C) at 5000 rpm for 10 minutes. Serum samples were stored at -20°C until biochemical assays.

Biological evaluation:

Rat's body weight (BW) was recorded weekly, the diet consumed was recorded every day; accordingly the body weight gain% (BWG %) and feed efficiency ratio (FER) was calculated according to **Chapman *et al*., (1959)**. Using the following formulas:-

BWG % = [(Final weight - initial weight) / initial weight] × 100

FER = Gain in body weight (g) / feed intake (g)

- **Determination of serum glucos**e level was determined in serum at the 1st, 4th and 8th weeks according to **(Trinder, 1969)**.
- **Determination of biomarker for lipids profile:** total cholesterol according to **Richmond, (1973),** triglycerides (T.G) according to **Fossati and Prencipe (1982**), very low, low and high density lipoprotein cholesterols (VLDL-c, LDL-c and HDL-c, respectively according to **Lopes et al. (1977).**
- Atherogenic index of plasma (AIP) was calculated according to **Castelli and Levitra (1977).**

  Atherogenic index (AIP) = LDLc / HDL
- **Determination of lipid peroxide malondialdehyde (MDA):** Lipid peroxide was determined according to the method of **(Draper and Hadley, 1990)**.

- **Determination of glutathione peroxidase (GSH-PX):** The activity of GSH-Px was determined according to the method of **(Beutler et al., 1963).**
- **Determination of superoxide dismutase (SOD):** The activity of SOD was measured according to **(Sun and Oberley, 1988).**
- **Determination of catalase (CAT):** The activity of **CAT** was measured according to **(Aebi, 1984).**

Statistical analysis

The results were expressed as mean±standard deviation. The methods of statistical analysis were done using SPSS Computer Program. Statistical significance of differences ($p<0.05$) was evaluated using Dunnett's multiple comparisons test **(Snedecor and Cochran, 1980)**

RESULTS AND DISCUSSION

Total phenolic compound, flavonoids and antioxidant activity hpof *Glycyrrhiza glabra* roots alcoholic extractions

Glycyrrhiza glabra roots are rich source of various phenolic antioxidants which are considered as the main antioxidant components. As illustrated in Table (1) results revealed that the alcoholic extract of *Glycyrrhiza glabra* was positive for the presence of saponin, tannins, flavonoids, terpenoids, and steroids, but alkaloids were absent. Such result agreed with that obtained by **Husain et al. (2015); Deng et al. (2021) and Al Moousawi et al. (2022)** where they isolated alkaloids from their samples.

Table (1): The phytochemicals screening of *Glycyrrhiza glabra* roots alcoholic extract

phytochemicals	presence
Alkaloids	–
Saponins	+
Tannins	+
Flavonoids	+
Terpenoids	+
Sterols	+

As shown in Table (2) the total phenolic content (TPC) of *Glycyrrhiza glabra* roots alcoholic extract was (73.59 mg GAE/100g), the result was agreed with those obtained by **Tohma and Gulçin (2010).**

Glycyrrhiza glabra root had a plentiful content of flavonoids, which is considered to be the most important component as the biological activities. The total content of flavonoids was 4.6 mg/g, this result was similar to that obtained by **Awad (2017)** who referred to the values of flavonoid content in ethanol and aqueous extracts of *Glycyrrhiza glabra* L. root were (4.2 and 5.1 µg QE/mg, respectively).

Glycyrrhiza glabra roots alcoholic extract recorded potent free radical scavenging activity (68.91%) at a concentration of 500µg/ml. The current result was nearly with those obtained by **Chopra et al. (2013) and Karahan et al. (2016).** From the results listed in Table (2) *Glycyrrhiza glabra* roots extract rich in bioactive component such as phenolic compounds and flavonoids. **Shrestha and Dhillion (2006)** reported that there were a linear correlation between phenolic compounds and flavonoid content with antioxidant capacity.

Table (2): Total phenolic compound, flavonoids and antioxidant activity of *Glycyrrhiza glabra* roots alcoholic extract

Sample	Total phenolic mg GAE/100g	Total flavonoids (mg/g)	Antioxidant activity %
G. glabra	73.59±0.03	4.6±0.1	68.91±1.01

Biological experiment

Effect of oral consumption with *Glycyrrhiza glabra* roots extract on body weight gain (BWG), feed intake (FI) and feed efficiency ratio (FER) of rats

As observed from Table (3), there were no significant differences among groups in the beginning of experiment (initial body weight), the mean of body weight of group 1,2,3,4 and 5 was 209.6, 211.3, 213.1, 212.6 and 2.10.8 g, respectively. The body weight of rats was recorded weekly, results showed that there was a severe loss of body weight for positive control group (PCG), which was injected by alloxan and was not treated by *Glycyrrhiza glabra* roots extract where, the mean of final body weight of group 3, 4 and 5 was 221.3, 229.6 and 236.4 g, respectively.

Table (3) showed that the mean of BWG% was -11.2% although rats of that group scored a high significant increase in FI as reported by **Lucchesi *et al.* (2015).** The treatment with *Glycyrrhiza glabra* roots extract improved significantly the severe loss of body weight resulted by alloxan, therefore the mean value of BWG% in groups 3, 4 and 5 was 3.8, 7.9 and 12.14%, respectively. Such results were in harmony

with **Badr et al (2013).** The improvement of body weight may be attributed to the presence of antioxidant components (phenolic and flavonoid compounds) that isolated from *Glycyrrhiza glabra* roots extract as showed in Table (2). Similar finding was obtained by **Ahn et al. (2013)** who stated that glycyrrhizin and glabridin were the most effective in *Glycyrrhiza glabra* roots extract.

Biological results showed that the mean of feed intake (FI) of negative control group (NCG) was (13.6 g/day), while the FI of positive control group was (14.7 g/day). *Glycyrrhiza glabra* consumption caused significant reduction in FI, the FI of diabetic groups (3,4,5) that treated with 200, 400, 800 mg/kg b.wt *Glycyrrhiza glabra* roots extract were 13.9, 12.7 and 12.3 g/day, respectively. The improvement of FI in treated diabetic groups may be due to the bioactive components of *Glycyrrhiza glabra* roots, similar finding by **(Shalaby et al. 2004)** who reported that the decrease of feed intake may be due to glycoside glycyrrhizin.

The mean of feed efficiency ratio (FER) of (NCG) was (1.14) compared with the FER of (PCG) which was (-0.7). FER mean of group 3, 4 and 5 was 0.26, 0.62 and 0.82, respectively. The increase of FER of treated groups was accompanied with the increased dose of *Glycyrrhiza glabra* roots extract and this reversed the weight loss associated with alloxan injection. The findings of current study agreed with those by **(Abd El-Lahot et al., 2017)** who reported that FER of rats fed on *Glycyrrhiza glabra* extract showed the highest FER value.

Table (3): Effect of consumption on *Glycyrrhiza glabra* roots extract on BW, BWG%, FI and FER of rats

Rats group	n	Body Weight (g)		BWG%	FI (g/day)	FER
		Initial weight	Final weight			
		Mean (±)SD	Mean (±)SD	Mean (±)SD	Mean (±)SD	Mean (±)SD
Group 1	7	209.6^{a}±3.4	242.2^{a}±4.2	15.5^{a}±3.0	13.6^{b}±0.51	1.14^{a}±1.22
Group 2	7	211.3^{a}±4.2	187.6^{a}±6.3	-11.2^{a}±1.4	14.7^{a}±0.26	-0.7^{d}±0.56
Group 3	7	213.1^{a}±3.8	221.3^{b}±3.1	3.8^{c}±2.8	13.9^{b}±0.61	0.26^{c}±1.10
Group 4	7	212.6^{a}±1.9	229.6^{b}±6.5	7.9^{b}±4.2	12.7^{c}±0.52	0.62^{b}±2.62
Group 5	7	210.8^{a}±2.1	236.4^{c}±7.9	12.14^{a} ±5.1	12.3^{c}±0.63	0.82^{b}±0.66

Data followed by different letters in the same column are significantly different at $p \leq 0.05$
BWG: body weight gain **FI:** feed intake **FER:** feed efficiency ratio

Effect of oral consumption with *Glycyrrhiza glabra* roots extract on blood glucose level

Data listed in Table (4) showed the effect of oral consumption with *Glycyrrhiza glabra* roots extract on serum glucose level. The serum glucose level was measured in the first week after injection by alloxan,

in 4th week and at the end of the experiment. The level of serum glucose of NCG remained stable until the end of experiment. Serum glucose levels increased significantly in PCG, it was 268.6, 274.3 and 279.1 mg/dl in the first week after injection by alloxan, in 4th week and at the end of experiment, respectively.

The treatment with *Glycyrrhiza glabra* roots extract improved significantly the diabetogenic disorders resulted from alloxan, where it improved blood glucose level. The level of serum glucose of treated groups 3, 4 and 5 was 211.3, 178.6 and 146.2 mg/dl, respectively. This finding was similar to those obtained by **Kataya et al., (2011).** The improvement in serum glucose level increased with prolong of treatment, in addition to the increase amount of *Glycyrrhiza glabra* extract consumption. The most alleviation of serum glucose was scored in group (5) that treated orally with 800 mg/kg b.wt of *Glycyrrhiza glabra* extract, the level of serum glucose was 266.8, 201.1 and 146.2 mg/dl in the first week after injection by alloxan, in 4th week and at the end of the experiment, respectively. Similar finding was obtained by **Takii et al. (2001); Sen et al. (2011), Mitra Mazumder et al. (2012)** who reported that Glycyrrhizin treatment particularly, the presence of the phenolic compounds, improved the diabetogenic effects.

Table (4): Effect of oral consumption with *Glycyrrhiza glabra* roots extract on serum glucose level

Rats group	n	serum glucose (mg/dl) Mean (±)SD		
		1st week	4th week	8th week
Group 1	7	99.3^{a}±1.6	99.4^{a}±2.16	99.7^{a}±1.2
Group 2	7	268.6^{b}±3.1	274.3^{d}±4.3	279.1^{e}±6.4
Group 3	7	265.1^{b}±4.1	243.8^{c}±2.6	211.3^{d}±3.05
Group 4	7	267.3^{b}±2.6	227.7^{c}±2.05	178.6^{c}±4.7
Group 5	7	266.8^{b}±1.7	201.1^{b}±4.05	146.2^{b}±2.6

Data followed by different letters in the same column are significantly different at p≤0.05

Effect of oral consumption with *Glycyrrhiza glabra* roots extract on biomarkers of lipid profile

As observed from Table (5) diabetic rats had higher level in both of total cholesterol (TC), triglyceride (TG), very low density lipoprotein (VLDL) and low density lipoprotein (LDL) than negative control group; caused by alloxan injection as reported by **(Maritim et al., 2003)** who found that injected rats with alloxan led to increase in

thiobarbituric acid reactive substances. The levels of TC, TG, VLDL and LDL in PCG were 139.39, 137.6, 27.5 and 75.9 mg/dl, respectively. On the other hand, the level of HDL in PCG reduced to 30.2 mg/dl. **Alvarado-Vasquez et al. (2003)** illustrated that diabetes stimulates increasing cholesterol and triglyceride level in blood. As cleared from Table (5), atherogenic index (AIP) of diabetic group was higher in PCG compared to other groups and that is may be an indicator as a strong risk predictor of cardiovascular, similar finding was obtained by **Poznyak et al. (2020) and Li et al. (2021)** who demonstrated that AIP revealed significant correlation with DM.

The treatment with *Glycyrrhiza glabra* roots extract improved significantly lipid profile biomarkers. Results in Table (5) showed a significant reduction in TC, TG, LDL and VLDL-cholesterol and significant increase in HDL-cholesterol levels in all treated groups. These results were similar to that obtained by **Reda et al. (2021).** The more consumption of *Glycyrrhiza glabra* roots extract, the more improvement in lipid profile, where the rate of lipid profile improvement in group (5) was better than the rate of lipid profile improvement of both groups (4 & 3) because of the increasing of *Glycyrrhiza glabra* roots extract consumption. *Glycyrrhiza glabra* roots extract effect resulted in the antioxidant effect of glabridin (the most active flavonoid) that inhibits the oxidation of low-density lipoproteins (LDL) as stated by **Fuhrman et al. (2000) and Kang et al. (2015).** The current results showed that the reduction of LDL and the increase in HDL in treated groups caused reduction in AIP ratio, subsequently, reduced the risk of coronary heart disease, that finding was corresponded with that obtained by **Superko and Krauss (1994).**

Table (5): Effect of oral consumption with *Glycyrrhiza glabra* roots extract on biomarker for lipids profile of rats

Rats group	n	CHO. (mg/dl)	TG (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	AIP
		Mean (±)SD	Mean (±)	Mean	Mean	Mean	
Group 1	7	99.27^{a}±4.4	98.7^{a}±3.6	19.7^{a}±4.5	46.5^{a}±5.8	49.4^{a}±6.2	0.94^{a}±6.2
Group 2	7	139.39^{c}±3.5	137.6^{c}±5.3	27.5^{c}±4.8	75.9^{d}±5.7	30.2^{d}±4.5	2.51^{d}±6.2
Group 3	7	112.77^{b}±5.6	117.3^{b}±7.1	23.5^{b}±3.9	69.1^{c}±3.7	36.8^{c}±6.1	1.87^{c}±6.2
Group 4	7	104.88^{b}±4.6	110.8^{b}±4.7	22.1^{b}±6.6	55.4^{b}±7.2	41.2^{b}±5.6	1.34^{b}±6.2
Group 5	7	99.47^{a}±3.8	101.1^{a}±6.1	20.4^{a}±4.6	48.9^{b}±4.6	45.7^{b}±4.3	1.07^{a}±6.2

Data followed by different letters in the same column are significantly different at $p \leq 0.05$

Effect of *Glycyrrhiza glabra* roots extract on serum oxidative stress enzymes of rats

Diabetes led to increase in Malondialdehyde (MDA) level, where, MDA was significantly higher in the diabetic rats (group 2) as compared to control group (group 1). On the other hand Glutathione peroxidase (GSH-PX), Catalase (CAT) and Superoxide dismutase (SOD) levels were significantly lower in the PCG as compared to NCG. Such finding was in line with that obtained by **Piconi et al. (2003) and Moussa (2008)** who showed that there is a close link between hyperglycemia, oxidative stress and diabetic complications. **Rosen et al. (2001)** explained that the reactive oxygen species (ROS) was increased in diabetes and that related with oxidative stress. **El Sheikh and Othman (2019)** reported that the increase in MDA levels and the decrease in CAT, GSH.px and SOD activities may be related to the action of oxidative stress resulting from hyperglycemia.

Results in Table (6) indicated that *Glycyrrhiza glabra* roots extract had an effective and powerful antioxidant activity, treatment with *Glycyrrhiza glabra* roots extract modified the oxidative stress status of treated groups 3, 4 and 5. As observed from the same table the more consumption of *Glycyrrhiza glabra* roots extract increase, the more level of GSH-PX, CAT and SOD increase. While there was a significant inverse relationship ($p < 0.001$) between consumption of *Glycyrrhiza glabra* roots extract concentration and status of MDA. Previous studies explained the role of different active compounds in *Glycyrrhiza glabra* roots extract which are responsible for modifying the oxidative stress status as the following: **Haraguchi et al. (1998)** clarified that phenolic compounds had biological against oxidative stress, **Biondi et al. (2003)** revealed that the strong scavenging activity on DPPH radical of *Glycyrrhiza glabra* roots extract had the ability to inhibit lipid peroxidation, **Rui-Min et al. (2012) and Varsha and Sonam (2013)** illustrated that flavonoids reduce oxidizing free radicals.

Malondialdehyde level is known as an oxidative stress index and the antioxidant status **(Gaweł et al. 2004).** Injection by alloxan led to increase in MDA level. The MDA level of group (1) and group (2) was 11.18 to 26.26 nmol/mL, respectively. The treatment with *Glycyrrhiza glabra* roots extract improved significantly MDA level of group 3, 4 and 5, the level MDA of previous groups were 19.32, 16.53 and 13.66 nmol/mL, respectively.

From Table (6) where showed that GSH is an important antioxidant, its reduction caused the increase of oxidative stress,

which confirms the link between hyperglycemia and GSH depletion. The mean values of GSH-PX of group 1, 2, 3, 4 and 5 were 19.43, 9.03, 11.47, 13.02 and 16.52 µmol/L. The statistical showed that there was a significant reduction between NCG and PCG. The GSH-PX level of treated groups 3, 4 and 5 which consumed *Glycyrrhiza glabra* roots extract with different concentration increased significantly than diabetic group, that was due to its high antioxidant content as confirmed by **(Seghrouchni et al. 2002) and Randhir and Shetty (2007).**

As cleared in the same table the CAT level in diabetic group (PCG) was lower than negative group (NCG), that means diabetes-related effects of catalase deficiency may be increased levels of hydrogen peroxide in blood cells due to decreased blood catalase may favor insulin signaling via inactivation of the oxidation-sensitive tyrosine phosphatases that could not dephosphorylate insulin receptors as stated by **Goth (2008).** Treatment with *Glycyrrhiza glabra* roots extract resulted in significant improvement of catalase activity in diabetic rats, the CAT level of group 3, 4 and 5 was 14.49, 17.32 and 20.36 µmol/L, respectively, such result was in agree with that obtained by **(Sen and Singh, 2021).**

The SOD level was significantly diminished in diabetic rats compared to normal control rats, **Dominguez et al. (1998) and Singh et al. (2015)** reported that glucose autoxidation in diabetic led to formation of hydrogen peroxide that inactivates SOD. Treatment with *Glycyrrhiza glabra* roots extract resulted in significant modified the SOD level in diabetic rats, SOD level in group 3, 4 and 5 was 12.5, 15.9 and 33.4 µmol/L, respectively. That improvement in SOD activity probably resulted from the presence of phenolic and flavonoids that showed in Table (1, 2), such results compatible with results of **Yehuda et al. (2011)**.

Table (6): Effect of *Glycyrrhiza glabra* roots extract on serum oxidative stress indicators in diabetic rats

Rats group	MDA (nmol/mL)	GSH-PX (µmol/L)	CAT (µmol/L)	SOD (µmol/L)
	Mean (±)SD	Mean (±)SD	Mean (±)SD	Mean (±)SD
Group 1	11.18^{a}±3.9	19.43^{a}±5.36	32.37^{a}±5.91	40.7^{a}±4.32
Group 2	26.26^{d}±6.32	9.03^{d}±5.33	12.39^{d}±6.41	9.2^{c}±5.08
Group 3	19.32^{c}±4.98	11.47^{c}±6.22	14.49^{c}±4.24	12.5^{c}±6.58
Group 4	16.53^{bc}±5.02	13.02^{c}±4.66	17.32^{bc}±2.33	15.9^{b}±4.46

Group 5	13.66^{ab}±4.39	16.52^{b}±3.9	20.36^{b}±6.42	33.4^{a}±6.21

Data followed by different letters in the same column are significantly different at p≤0.05
MDA: Malondialdehyde **GSH–PX**: Glutathione peroxidase **CAT**: Catalase
SOD: Superoxide dismutase

As cleared from Figure (1) there was a significant direct correlation (p <0.001) between level of GSH-PX, CAT and SOD. On the contrary, there was inverse correlation between MDA level and both of GSH-PX, CAT and SOD in diabetic case. Where, MDA level increased while, GSH-PX, CAT and SOD levels decreased. On the contrary, when serum glucose decrease in treated groups the MDA level decrease, while GSH-PX, CAT and SOD levels increased.

In addition to, there was a significant modification in MDA, GSH-PX, CAT and SOD activity resulted from consumption of *Glycyrrhiza glabra* roots extract. That improvement increased significantly with the dose of *Glycyrrhiza glabra* roots extract increased.

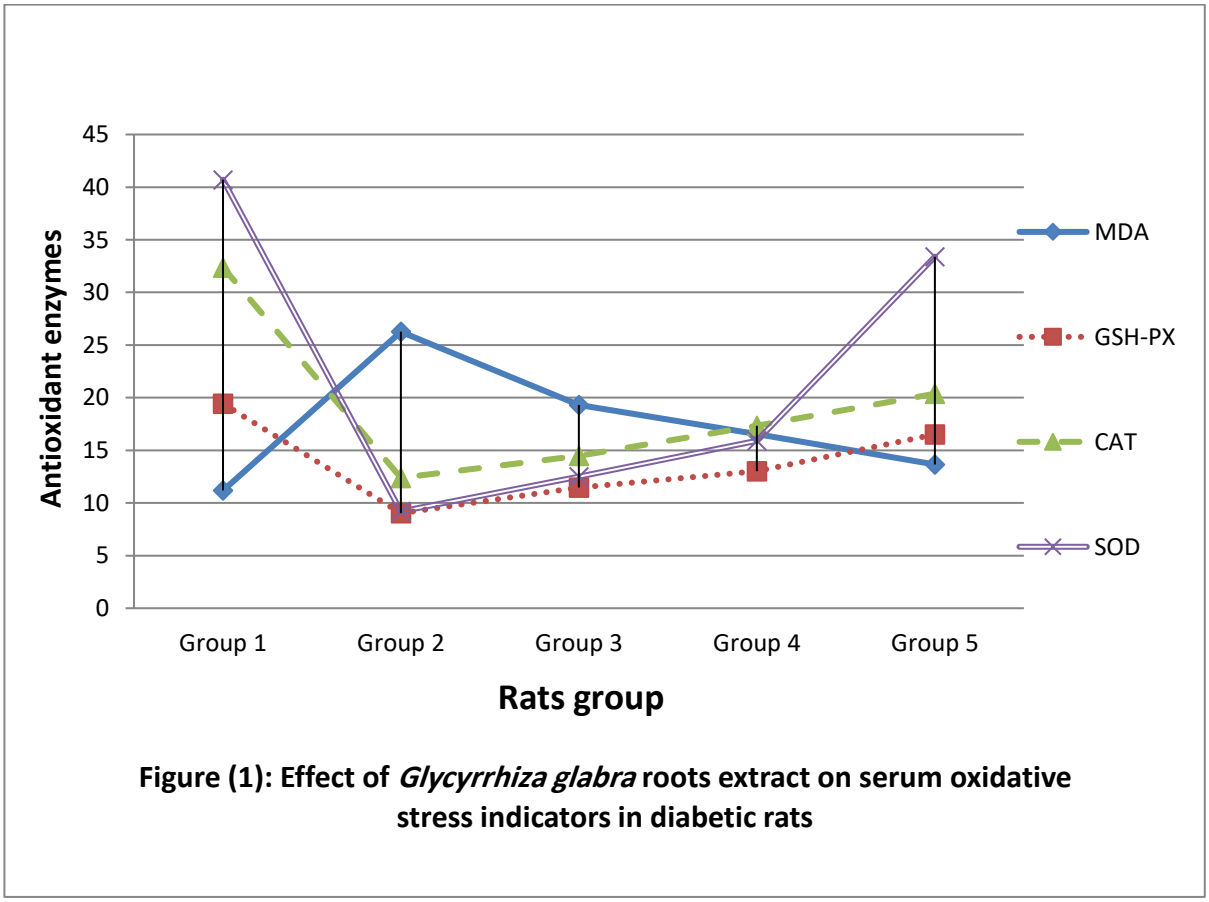

Figure (1): Effect of *Glycyrrhiza glabra* roots extract on serum oxidative stress indicators in diabetic rats

CONCLUSION

According to this study *Glycyrrhiza glabra* roots extract had an effective and powerful antioxidant activity, it had therapeutic effects, it considered as an anti-diabetic and an anti-oxidative agent. It can improve the oxidative stress disorder accompanied with the pathogenesis of diabetes and its complications. *Glycyrrhiza glabra* roots extract had positive effects on body weight status and lipids profile. So, it can recommended to use *Glycyrrhiza glabra* roots as a natural food additive and as a functional food for diabetics.

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