American Journal of Food Science and Technology, 2021, Vol. 9, No. 4, 161-172
Available online at http://pubs.sciepub.com/ajfst/9/4/8
Published by Science and Education Publishing
DOI:10.12691/ajfst-9-4-8

Vinca (*Catharanthus roseus*) Extracts Attenuate Alloxan-Induced Hyperglycemia and Oxidative Stress in Rats

Yousif Elhassaneen*, Sherif Ragab, Abeer Abd El-Rahman, Safaa Arafa

Department of Nutrition and Food Science, Faculty of Home Economics, Minoufiya University, Shebin El-Kom, Egypt
*Corresponding author: Yousif12@hotmail.com

Received September 27, 2021; Revised October 28, 2021; Accepted November 05, 2021

Abstract The present study was carried out to investigate the effectiveness of *Catharanthus roseus* extracts in modulating the hyperglycemia and oxidative stress (OS) of alloxan-induced diabetic rats. Treatment of rats with alloxan caused a significant ($p \leq 0.05$) increased in serum glucose concentration by the ratio of 273.61% compared to normal control group. Supplementation of the rat diets with 300, 400 and 500 mg/kg of ethanolic *Catharanthus roseus* stems extract (CRSE) and ethanolic *Catharanthus roseus* leaves extract (CRLE) decreased this value which recorded 184.72, 113.47 and 76.39%, and 139.86, 112.91 and 57.36%, respectively. The rate of attenuation exhibited a dose dependent increase with both extracts consumption. Also, CRLE is more effective in the attenuation process than CRSE. The same behavior was recorded for the biomarkers of OS levels in serum i.e. malonaldehyde (MDA). In contrary, significant ($P \leq 0.05$) improving in different antioxidant defense systems in both serum (glutathione fractions, GSH), and RBC's (antioxidant enzymes including glutathione peroxidase, GSH-Px, glutathione reductase, GSH-Rd and catalases, CAT) were recorded. Such biochemical changes observed in the present study were confirmed by the histopathological examination results. In conclusion, *Catharanthus roseus* possess a variety of beneficial activities and have the potential to impart therapeutic effect holistically in complicated disorders like diabetes and its complications.

Keywords: *Catharanthus roseus, leaves, stems, malonaldehyde, glutathione fractions, antioxidant enzymes, histopathology*

Cite This Article: Yousif Elhassaneen, Sherif Ragab, Abeer Abd El-Rahman, and Safaa Arafa, "Vinca (*Catharanthus roseus*) Extracts Attenuate Alloxan-Induced Hyperglycemia and Oxidative Stress in Rats." *American Journal of Food Science and Technology*, vol. 9, no. 4 (2021): 161-172. doi: 10.12691/ajfst-9-4-8.

1. Introduction

Diabetes is defined as a state in which homeostasis of carbohydrate and lipid metabolism is improperly regulated by insulin. This results, decrease in both insulin secretion and insulin action, primarily in elevated fasting and postprandial blood glucose levels. If this imbalanced homeostasis does not return to normalcy and continues for a protracted period of time, it leads to hyperglycemia that in due course turns into a syndrome called diabetes mellitus (DM) [1,2]. There are two main categories of DM. Type 1 diabetes mellitus (T1DM) also called insulin-dependent diabetes mellitus (IDDM), the body does not produce insulin, and daily insulin injections are required. Type 1 diabetes is usually diagnosed during childhood or early adolescence [3]. Type 2 (T2DM), the noninsulin-dependent diabetes mellitus (NIDDM) is the result of failure to produce sufficient insulin and insulin resistance. Early phenomenon of T2DM is insulin insensitivity, which not only has negative metabolic consequences but also contributes subsequent pancreas β-cell exhaustion, resulting in the onset of clinical hyperglycemia [4]. Thus, understanding the regulation of the insulin response and identifying the related mechanisms are important to early treatment and prevention of T2DM.

DM is widely distributed all over the world including Egypt, and nearly one of each 10 person is diabetic. About 415 million people in the world diagnosed with DM and majority of them are due to T2DM. DM incidence predicted to increase 642 million people by the year 2040 [5]. Therefore, the human population worldwide appears to be in the midst of an epidemic of diabetes. Reports from the World Health Organization (WHO) indicate that DM is one of the major killers of our time, with people in Southeast Asia and Western Pacific as well as Middle East being most at risks [1]. DM is one of the world's most common chronic diseases as changing lifestyles lead to reduced physical activity and increased obesity [6]. Also, disease complications, such as cardiovascular symptoms, eye impairment or kidney failure are even fatal for some of patients [7]. Additionally, diabetes induces oxidative stress (OS) and its complications in different experimental animal's models and human patients. OS was initially defined as a serious imbalance between oxidation and

antioxidants leading to potential damage [8]. Reactive species of oxygen, nitrogen and chlorine atoms are represent the most free radicals/oxidants producing by living organisms as a result of normal cellular metabolism [9]. At high concentrations i.e. OS, such oxidants produce adverse modifications to cell components, such as lipids, proteins, and DNA [10]. Therefore, OS is thought to be involved in the development of in several diseases including cancer, diabetes, obesity, atherosclerosis, hypertension, chronic obstructive pulmonary disease, acute respiratory distress syndrome, ischemia/perfusion, idiopathic pulmonary fibrosis, anemia, asthma and neurological disorders [11,12]. Also, it is contributing to tissue injury following irradiation and hyperoxia as well as in diabetes and is likely to be involved in age-related development of cancer. Furthermore, associations between diabetes and markers of OS including lipid oxidative modification have been observed in humans [13]. For example, lipid peroxidation contributes to the development of atherosclerosis Steinberg *et al* [14], Esterbauer *et al* [15] a process known to be accelerated in diabetic patients [16]. Lipid peroxidation process end products such peroxides, malonaldehyde (MDA) etc may be toxic to vascular endothelium in diabetics [17]. Also, free radicals including such end products of lipid peroxidation attack can generate protein peroxides in diabetes, which may decompose to generate other free radicals [18]. Additionally, glycol-oxidation seems to play an important role in the vascular endothelial dysfunction detected in diabetic patients and perhaps in the nephropathy [19]. Hyperglycemia may cause increased generation of reactive oxygen species (ROS) such 0^{-} by endothelium, antagonizing the action of nitrous oxide (NO) and perhaps forming peroxynitrite, ONOO- [20,21,22].

Several strategies have been proposed to improve the all DM complications, because early treatment and prevention play a pivotal role in reducing the population burden of diabetes. Pharmaceutical factors/drugs to treat the disease aggressively early have been recommended, but almost of medications used may have undesirable side effects. Also, the cost of administrating modern antidiabetic drugs is beyond the reach of most people in the low income group and those living in the rural areas [23]. Therefore, the attention of scientists in different countries of the world turned to alternative treatment systems, which are more comprehensive, such as traditional medicinal systems. The medicinal preparations in traditional medicines contain a variety of herbal and non-herbal ingredients that are thought to act on a variety of targets by various modes and mechanisms [1,24-30]. Although a lot of studies established in this medicinal systems the fundamental mechanisms of those are still unexplainable using modern tools. Thus, there has been a growing interest in herbal remedies that can be but have been difficult to maintain over a long term introduced into the general population with the least side effects and the maximal preventive outcome [24]. In this direction, many phytochemicals naturally occurring in plant pats would be desirable options. Amongst all of these bioactive compounds, phenolic compounds, anthocyanins, flavonoids, polysaccharides, alkaloids, and organosulfur compounds are represent the central position. Such compounds have been reported to improve diabetic status by decreasing OS or by reducing the disturbance of hepatic gene expressions [21,31,32,33].

Vinca (*Catharanthus roseus*) is known as Madagascar periwinkle. It is a perennial herb of the *Apocynaceae* family originally native to Madagascar [34]. It measures about two feet in height and has dark green glossy leaves and pale pink or white flowers. *C. roseus* (seeds, stems, leaves and flowers) contains several categories of bioactive compounds including phenolic compounds, flavonoids, saponins, terpenes, alkaloids antocyanines, flavonols, glucosides and steroids [35,36,37]. Also, the extracts of the sprouts of *C. roseus* are used as a potential source of natural available antioxidants and with excellent pharmaceutical applications [38]. Furthermore, the plant contains about seventy alkaloids, some of which include catharanthine, lochnenine, vindoline vindolinenine, vincristine, vinblastine, tetrahydroalstonine, reserpine, serpentine, etc. [39]. *Catharanthus roseus* has very powerful medicinal properties which are due to the bioactive compounds present in it. The organic extracts of *Catharanthus roseus* is used in the folklore treatment of diabetes, malaria, leukemia, wasp stings, sore throat, eye irritation, astringent, diuretic and expectorant infections [40,41,42].

Figure 1. Vinca (Catharanthus roseus)

All of the previous studies with others confirmed the use of *Catharanthus roseus* for the treatment of common diseases such as diabetes is very common. In line with the WHO [43] expert committee on diabetes which recommends that traditional methods of management of diabetes should be further investigated. Also considering the economic resource constraints and cheapness of this phyto-product, this present study was designed to investigate the potentials effects of *Catharanthus roseus* extracts on attenuation of the alloxan-induced hyperglycemia and oxidative stress in rats.

2. Materials and Methods

2.1. Materials

2.1.1. Vinca

Vinca *(Catharanthus roseus*) plant was obtained from Nurseries of the Agricultural Plant Department, Faculty of Agriculture, Kafrelsheikh University, Kafrelsheikh, Egypt. The plant was verified by the Faculty Stuff of Plant Taxonomy in the same Department.

2.1.2. Chemicals

Alloxan, used for induction of DM among rats, and reduced glutathione (GSH) and thiobarbituric acid (TBA) were obtained from Sigma Chemical Co., St. Louis, Mo. Casein, as main source of protein was purchased from Morgan Company for Chemicals. Cairo, Egypt. Vitamins and salts mixtures, organic solvents and other chemicals in analytical grade were purchased from El-Ghomhorya Company for Trading Drugs, Chemicals and Medical instruments, Cairo, Egypt.

2.2. Methods

2.2.1. Preparation of *Catharanthus roseus* Parts Powder

Vinca parts, stems and leaves, were separated manually and washed with water. The resulted stems and leaves were collected and dried in a hot air oven (Horizontal Forced Air Drier, Proctor and Schwartz Inc., Philadelphia, PA) at 55°C until arriving by the moisture in the final product to about 10%. The dried parts were ground into a fine powder in high mixer speed (Moulinex Egypt, Al-Araby Co., Egypt). The material that passed through an 80 mesh sieve was retained for use.

2.2.2. Preparation of *Catharanthus roseus* Parts Extracts

Stems and leaves of Vinca powders were used for the preparation of ethanol extract as follow: A 20 g from dried plant powder plus 180 ml ethanol (80%, v/v) were homogenized and transferred to a beaker and stirred at 200 rpm in an orbital shaker (Unimax 1010, Heidolph Instruments GmbH Co. Germany) for 1 h at room temperature. The extract was then separated from the residue by filtration through Whatman No. 1 filter paper. The remaining residue was re-extracted twice, and then the two extracts were combined. The residual solvent of was removed under reduced pressure at 45°C using a rotary evaporator (Laborata 4000; Heidolph Instruments GmbH Co., Germany) and the extract stored at -18°C for the further experiments.

2.2.3. Biological Experiments

2.2.3.1. Ethical Approval

Biological experiments for this study were ethically approved by the Scientific Research Ethics Committee (Animal Care and Use), Faculty of Home Economics, Menoufia University, Shebin El-Kom, Egypt (Approval no. 06- SREC- 011-2019).

2.2.3.2. Experimental Animals and Basal Diet

Normal male albino rats (150±10g) were obtained from Research Institute of Ophthalmology (RIO), Medical Analysis Department, Giza, Egypt. The basic diet prepared according to the formula as mentioned by AIN [44] the used vitamins and minerals mixtures were formulated according to [45].

2.2.3.3. Induction of Diabetes

Diabetes was induced in thirty six normal healthy rats by injection into operationally with freshly prepared alloxan monohydrate in saline at a dose level of 150 mg/ kg body weight [46]. Immediately after injection animals were received 5% glucose solution over night to overcome drug induced hypoglycemia [47,48]. After five days fast blood glucose (FBG) was analyzed using a specific kit (AlGomhoryia Company for Trading Drugs, Chemicals and Medical Instruments, Cairo, Egypt) by a drop of blood was obtained from tail vein and subjected to a strip of haemogluco test. All rats with FBG >126 mg/dl were considered to be diabetics and included in the study.

2.2.3.4. Experimental Design

Total of 48 rats were divided in two main groups. Main group (1), six rats, served as normal controls were administered with saline intraperitoneally (IP), which was used as a vehicle for the treatment of animals, in alloxan (diabetic) group and fed basal diet (BD). Main Group (2), 42 rats were given alloxan to induce diabetes and divided into equal seven subgroups as follow: group (2), fed on BD and served as positive control; groups (3, 4 and 5) fed on BD containing 300, 400 and 500 (mg/kg, w/w) CRSE, and groups (6, 7 and 8), fed on BD containing 300, 400 and 500 (mg/kg, w/w) CRLE. The treatment with plant parts extracts to the animal belonging to groups (3) to (8) was started 14 days prior to diabetes induction. All the rats had free access to the diet and water as well as the treatments continued for a total duration of 8 weeks.

2.2.3.5. Biological Evaluation

During the experimental period (28 days), the diet consumed was recorded every day and body weight was recorded every week. The body weight gain (BWG, %), feed intake (FI, g/day/rat) and food efficiency ratio (FER) were determined according to Chapman *et al*., (1959) using the following equations: BWG (%) = (Final weight – Initial weight)/ Initial weight ×100 and FER = Grams gain in body weight (g/28 day)/Grams feed intake (g/28 day).

2.2.3.6. Blood Sampling

At the end of experiment period, 4 weeks, blood samples were collected after 12 hours fasting using the abdominal aorta and rats were scarified under ether anesthetized. Blood samples were received into glass centrifuge tubes, containing oxalate solution (1.34%) as anticoagulant. After centrifugation at 3000 rpm for 10 min., serum was with drown and used for the analysis of blood parameters. The erythrocyte residue was washed with three successive portions of sodium chloride solution (0.9 %) and then haemolysed with deionized water for 30 min. Haemolysate was then centrifuged at 30,000 rpm for 30 min and the supernatant fractions was transferred to a clean test tube and analyzed of antioxidant enzymes [49].

2.2.3.7. Hematological Analysis

Serum glucose

Enzymatic determination of serum glucose was carried out colorimetrically according to [50].

Glutathione fractions

Reduced (GSH) and oxidized (GSSG) glutathione in serum were determined according to the method of [51]. Samples were prepared, extracted, purified, derivatized and injected onto HPLC system (SP Thermo Separation Products Liquid Chromatograph (Thermo Separation products, San Jose, CA).

Antioxidant enzymes

GSH-Px and CAT activities were measured as described by Splittgerber and Tappel [52] and Aebi [53] respectively. GSH-Rd activity was determined according to the method recommended by the International Committee for Standardization in Haematology [54]. Activities of GSH-Px enzyme was expressed in international unit per milliliter erythrocyte sediment.

MDA determination

MDA was measured as described by [55]. Half milliliter of plasma were added to 1.0 ml of thiobarbituric acid reagent, consisting of 15% TCA, 0.375% thiobarbituric acid (TBA) and 0.01% butylated hydroxytoluene in 0.25 N HCl. Twenty-five microliters of 0.1 M $FeSO_4.7H_2O$ was added and the mixture was heated for 20 min in boiling water. The samples were centrifuged at 1000 xg for 10 min and the absorbance was read at 535 nm using Labo-med. Inc., spectrophotometer against a reagent blank. The absorbance of the samples was compared to a standard curve of known concentrations of malondialdehyde.

2.2.3.8. Histopathological Examination

Specimens of the internal organ (pancreas) were taken immediately after sacrificing rats and immersed in 10% neutral buffered formalin. The fixed specimens were then trimmed and dehydrated in ascending grades of alcohol, cleared in xylene, embedded in paraffin, sectioned (4-6 µm thickness), stained with hematoxylin and eosin and examined microscopically [56].

2.2.3.9. Statistical Analysis

All measurements were done in triplicate and recorded as mean± standard deviation (SD). Statistical analyses were performed using Student *t*-test and MINITAB 12 computer program statistical software (Minitab Inc., State College, PA). *P* values ≤ 0.05 were considered significant.

3. Results and Discussions

3.1. Effect of *Catharanthus roseus* Ethanolic Extracts on Body Weight Gain (BWG), Feed Intake (FI) and Feed Efficiency Ratio (FER) of Diabetic Rats

BWG, FI and FER of rats injected by alloxan and consumed CRSE and CRLE were shown in Table 1. From such data it could be noticed that the alloxan-treated rats exhibited significantly ($p \leq 0.05$) decreased in BWG (-49.75%) and FER (-50.57%) compared to the normal group. The opposite direction was recorded in FI which increased by the rate of 9.19%. However, supplementation of the rat diets with 300, 400 and 500 mg/kg of CRSE and CRLE for 28 days significantly ($p \leq 0.05$) increased the levels BWG, FI and FER with the positive control group by different rates. The rate of increasing in all those parameters exhibited a dose dependent increase with *Catharanthus roseus* extracts consumption. Furthermore, CRLE is more effective in rasing the BWG, FI and FER level than CRSE.

Such data are in agreement with that observed by Hasani- Ranjbar *et al* [57], Ajuru et al [58] who found that the body weight of the treated animals with leaf extracts of *cathaeanthus roseus* were observed to increase significantly ($p \leq 0.05$) in comparison with the natural control group after 30 days of treatment. Also, Hamzawy *et al* [59], Elhassaneen et al [60] reported that liver rat's disorders probably induced by diabetes reveal significant reduction of the body weight and feed intake. Furthermore, several studies showed that diabetes and liver disorders can lead to malnutrition and the major causes of malnutrition in patients with diabetes and liver disease are poor dietary/feed intake, maldigestion, malabsorption and abnormalities in the metabolism and storage of macro and micro nutrients [22,60,61,62]. Additionally, Elhassaneen et al [63] found that the decreasing in both FER and BWG in experimental animals was improved by consumption plant parts contains bioactive compounds such as found in *cathaeanthus roseus* extracts.

3.2. Effect of *Catharanthus roseus* Ethanolic Extracts on Serum Glucose Concentration of Diabetic rats

Data in Table 2 were shown the serum glucose concentration of alloxan-induced diabetic rats consumed CRSE and CRLE. From such data it could be noticed that treatment of animals with alloxan caused a significant increased ($p \leq 0.05$) in serum glucose concentration by the ratio 273.61% compared to normal animals (negative control group). Supplementation of the rat diets with 300, 400 and 500 mg/kg of CRSE and CRLE decreased this value which recorded 184.72, 113.47 and 76.39%, and 139.86, 112.91and 57.36%, respectively. Also, in both extracts, the rate of attenuation was raised with the increasing of the extract consumption concentration. Furthermore, CRLE is more effective in lowering the serum glucose level than CRSE. Such data in accordance

with Ahmed et al [64] who found that the methanolic whole *Catharanthus roseus* extract at high dose (500 mg/kg) exhibited significant antihyperglycemic activity than whole plant extract at low dose (300 mg/kg) in diabetic rats. In similar study, Vega-Avila et al [65] found that the aqueous extracts from *C. roseus* reduced the blood glucose of both healthy and diabetic mice. The aqueous stem extract (250 mg/Kg) and its alkaloid-free fraction (300 mg/Kg) significantly ($P \leq 0.05$) reduced blood glucose in diabetic mice by 52.90 and 51.21%. The best hypoglycemic activity was presented for the aqueous extracts and by alkaloid-free stem aqueous fraction. This fraction is formed by three polyphenols compounds. Also, Islam et al [66] found that the ethanolic extract of the *Catharanthus roseus* lowered blood glucose levels in oral glucose tolerance tests in glucose induced hyperglycemic rats. Furthermore, the antihyperglycemic activity has been reported following administration of *Catharanthus roseus* leaf powder in STZ-diabetic rats [67]. Additionally, the aqueous extracts have the capacity to decrease the blood glucose in 20 % in trials with diabetic rats, while the reductions of the levels of glucose in the blood with dichloromethane and methanol extracts are of 49 and 58 %, respectively [68].

In line with the results of the present study, several researches have been done on the effect of plant parts extract including *Catharanthus roseus* consumption on diabetic conditions. Such activity may be related to diverse bioactive compounds present in *Catharanthus roseus* including phenolics, flavonoids, lycopene, polysaccharides, terpenoids, tannins and alkaloids [69,70]. These compounds are known for their properties in scavenging free radicals, inhibiting lipid oxidation; improve glucose response, alleviating metabolic dysregulation of free fatty acids and insulin resistance associated with type 2 diabetes [21,22,29,71,72]. Also, such bioactive compounds have been reported to improve damages/complications caused by many diseases including DM [31,32]. Furthermore, Tiwari and Madhusudana [1] reviewed that bioactive compounds determined in *Catharanthus roseus* such polyphenolics, apart from their much-cited antioxidant activities, also have been reported to inhibit a-amylase and sucrase, and have been shown to be the principle substance for suppressing postprandial hyperglycemia (PPHG). Additionally, these compounds inhibit glucose transport across the intestine by inhibiting sodium glucose co-transporter-1 (S-GLUT-1). Another mechanism for the effect of *Catharanthus roseus* on serum glucose attenuation have been proposed by Ahmed *et al* [64] which could be due to the possibility that some β-cells are still surviving to act upon by *such plant* extract to exert its insulin releasing effect i.e. β cell regeneration.

Table 1. Effect of *Catharanthus roseus* ethanolic extracts on body weight gain (BWG), feed intake (FI) and feed efficiency ratio (FER) of diabetic rats

Value	Control (-) Std diet	Control (+) Diabetic	CRSE Conc. (w/w, mg/kg)			CRLE Conc. (w/w, mg/kg)		
			300	400	500	300	400	500
BWG (%)								
Range	28.96-55.21	8.09-33.49	20.32-40.11	43.75-71.88	7.07-61.62	21.91-91.53	29.61-98.86	20.00-84.24
Mean*	40.8^{a}	20.5^{a}	29.2^{a}	52.4^{a}	34.9^{a}	42.8^{a}	44.9^{a}	59.4^{a}
SD	10.80	10.26	9.98	11.31	20.56	28.28	30.73	25.15
% of change	--------	-49.75	-28.43	28.43	-14.46	4.90	10.05	45.59
FI (g/day/rat)								
Range	14-16	16-18	14-16	15-16	15-17	13-14	13-17	12-15
Mean*	15.4^{a}	16.8^{a}	15^{ab}	15.6^{a}	15.6^{a}	13.4^{b}	15^{ab}	13.4^{b}
SD	0.89	0.84	1.00	0.55	0.89	0.55	1.58	1.14
% of change	---------	9.19	-2.60	1.30	1.30	-12.99	-2.60	-12.99
FER								
Range	3.75-7.07	1.06-4.25	2.25-5.21	5.60-9.20	0.88-7.60	3.00-12.46	2.93-12.36	2.54-10.69
Mean*	5.26^{a}	2.6^{a}	3.9^{a}	6.7^{a}	4.3^{a}	5.8^{a}	5.7^{a}	6.7^{a}
SD	1.327	1.30	1.33	1.46	2.53	3.88	3.82	2.98
% of change	---------	-50.57	-25.86	27.38	-18.25	10.27	8.37	27.38

* CRSE, *Catharanthus roseus* stems extract, CRLE, *Catharanthus roseus* leaves extract. Means in the same row with different superscript letters are significantly different at $p \leq 0.05$.

Table 2. Effect of *Catharanthus roseus* ethanolic extracts on serum glucose concentration (mg/dL) of diabetic rats

Value	Control (-) Std diet	Control (+) Diabetic	CRSE Conc. (w/w, mg/kg)			CRLE Conc. (w/w, mg/kg)		
			300	400	500	300	400	500
Range	69-74	259-284	198-209	149-157	118-134	164-179	131-148	111-117
Mean*	72^{g}	269^{a}	205^{b}	153.7^{d}	127^{ef}	172.7^{c}	153.3 ^{de}	113.3^{f}
SD	2.65	13.23	6.18	4.16	8.19	7.77	8.50	3.21
% of change	----------	273.61	184.72	113.47	76.39	139.86	112.91	57.36

* CRSE, *Catharanthus roseus* stems extract, CRLE, *Catharanthus roseus* leaves extract. Means in the same row with different superscript letters are significantly different at $p \leq 0.05$.

3.3. Effect of *Catharanthus roseus* Ethanolic Extracts on Serum Oxidant Concentration (Malonaldehyde, MDA) of Diabetic Rats

Oxidants concentration (i.e. oxidative stress) in diabetic rats serum feeding some selected CRSE and CRLE was assessed by measuring lipid peroxidation (malonaldehyde, MDA) (Table 3). From such data it could be noticed that diabetes induced a significant increased ($p \leq 0.05$) in MDA concentration in serum by 31.41% compared to normal rats. Supplementation of the rat diets with 300, 400 and 500 mg/kg of CRSE and CRLE decreased this value which recorded 184.72, 113.47 and 76.39%, and 139.86, 112.91and 57.36%, respectively. Also, in both extracts, the rate of attenuation was raised with the increasing of the extract consumption concentration. Furthermore, CRLE is more effective in lowering the serum MDA level than CRSE.

In similar studies, clinical evidences for diabetes-associated oxidative stress have been provided by measurement of either biomarkers or end-products of free radical-mediated oxidative processes [21,22,61]. For instance, lipid peroxidation markers such as MDA one of the most major products of the oxidation of polyunsaturated fatty acids, lipid hydroperoxides and conjugated dienes are found to be increased in plasma from diabetic subjects in many clinical studies [22,61]. Such data are in accordance with that observed by Zheng and Wang [73] who reported that here was far more increase in lipid peroxidation i.e.MDA in the negative control group than in the group treated with *C. roseus* extract. This shows that *C. roseus* has antioxidant activity, an attribute required in the treatment of diseases. Also, Rzaeizadeh et al [74] in a study of MDA inhibition methanol extract of Momodia charantia fruit reported that this extract inhibited 32.5 % of MDA by the end of experiment (8^{th} day). But, *C. roseus* ethanol extract had stronger performance than this plant. On the other side, interest in the possible significance of MDA, has been stimulated by reports that are mutagenic and carcinogenic compound [75]. The observed positive effects of *C. roseus* extracts on oxidants formation/concentration of diabetic rats could be attributed to several mechanisms induced by their bioactive components content. In this context, Coskun et al [32] found that phenolic compounds such as found in *C. roseus* have antioxidative and anti-inflammatory activities. Such dietary phenolics found in *C. roseus* are metabolized in liver, inhibiting liver injury induced by diabetes i.e. enhancing lipid metabolism, reducing OS may be particularly effective, consequently.

3.4. Effect of *Catharanthus roseus* Ethanolic Extracts on Antioxidative Defense Systems of Diabetic Rats

3.4.1. Glutathione Fractions Concentration in Plasma

Biological antioxidant macromolecules i.e. glutathione fractions concentration in serum of diabetic rats consumed *Catharanthus roseus* extracts were assessed (Table 4). From such data it could be noticed that diabetes induced a significant decreased ($p \leq 0.05$) in reduced glutathione (GSH) and oxidized glutathione (GSSG) concentrations in serum by -39.53 and -24.76% compared to normal controls, respectively. Supplementation of the rat diets with 300, 400 and 500 mg/kg of CRSE and CRLE increased this value which recorded -28.45, -13.59 and -10.73%; and -25.10, -10.58 and -7.70% for GSH and -23.02, -18.47 and -15.17; and -19.96, -13.97 and -11.03% for GSSG, respectively. Also, in both extracts, the rate of attenuation was raised with the increasing of the extract consumption concentration. Furthermore, CRLE is more effective in elevation the serum GSH fractions level than CRSE. The same behavior was observed for the GSH/GSSG ratio.

Glutathione (GSH), a tripeptide (L-glutamyl-L-cysteinyl-glycine) synthesized in the cytoplasm, is the most abundant intracellular non-protein thiol involved in antioxidant elimination [76]. The antioxidant function of GSH includes its role in the activities of GSH enzymes family including glutathione peroxidase (GSH-Px), glutathione reductase (GSH-Rd) and peroxiredoxins (PRXs). In addition, GSH can apparently serve as a nonenzymatic scavenger of oxyradicals [25,27,28]. Several oxidative injuries have been associated with glutathione depletion [77]. A marked decreased level of GSH is reported in the serum of diabetic patients [78]. GSH systems may have the ability to manage OS stress with adaptational changes in enzymes regulating GSH metabolism. By other meaning, the link between hyperglycemia and GSH depletion has been reported [22,60,61,79].

Decreasing in GSH fractions observed in diabetic rats group generally accompanied by a concomitant decreased in the ratio of GSH/GSSG. In such direction, Bedard et al [80] mentioned that a more fundamental effect of oxyradical-generating compounds as the diabetes development, however, is their effect on what can be referred to as the redox status (GSH/GSSG) of cells or tissues. Few studies have been addressed directly the issue of effects of pro-oxidants on redox status. For example, Elhassaneen et al [81] mentioned that increased fluxes of oxy-radicals might be decreased in the GSH/GSSG ratio, due either to direct radical scavenging or to increased peroxidase activity. This effect could also occur indirectly due to reduced NADPH availability [necessary for glutathione reductase (GSH-Rd) activity] resulting, for example, from oxidations in the first step of the redox cycle [82]. Also, Bedard and Krause [83] reported that various enzymes inside the cells can also produce ROS. Particularly, the family of NAPDH oxidases (NOX) is considered to be an important source of ROS generation. Such effect could be one of the most important reasons for reducing the GSH/GSSG ratio in diabetic rats.

The *Catharanthus roseus* extracts consumption are rich in different bioactive compounds including phenolics, flavonoids, lycopene, polysaccharides, terpenoids, tannins and alkaloids [69,70]. These compounds are known to exhibit antioxidant effects against oxidants/ROS formation as the diabetes development through several mechanisms of action including raising of redox status (GSH/GSSG ratio) in the living cells, and/or increasing the GSH synthesis.

Table 3. Effect of *Catharanthus roseus* ethanolic extracts on serum oxidant concentration (malonaldehyde, MDA, nmol/mL) of diabetic rats

Value	Control (-) Std diet	Control (+) Diabetic	CRSE Conc. (w/w, mg/kg)			CRLE Conc. (w/w, mg/kg)		
			300	400	500	300	400	500
Range	216.83-228.61	283.50-299.01	262.27-278.66	255.07-269.22	236.98-249.77	257.48-270.83	250.09-262.61	230.14-253.01
Mean*	222.47^{d}	292.34^{a}	269.92^{b}	262.76^{b}	244.50^{c}	265.14^{b}	257.22^{b}	239.63^{c}
SD	5.56	7.20	6.95	6.40	6.05	6.38	6.00	9.50
% of change	------	31.41	21.33	18.11	9.90	19.18	15.62	7.71

* CRSE, *Catharanthus roseus* stems extract, CRLE, *Catharanthus roseus* leaves extract. Means in the same row with different superscript letters are significantly different at p≤ 0.05.

Table 4. Effect of *Catharanthus roseus* ethanolic extracts on serum antioxidant biological molecules (glutathione fractions) of diabetic rats

Value	Control (-) Std diet	Control (+) Diabetic	CRSE Conc. (w/w, mg/kg)			CRLE Conc. (w/w, mg/kg)		
			300	400	500	300	400	500
Reduced glutathione concentration (GSH, µmol /L)								
Range	9.80-10.33	5.96-6.29	6.99-7.43	8.43-8.90	8.70-9.17	7.31-7.69	8.74-9.18	9.05-9.53
Mean*	10.05^{a}	6.08^{e}	7.19^{d}	8.69^{c}	8.98^{bc}	7.53^{d}	8.99^{bc}	9.28^{b}
SD	1.25	1.15	0.99	1.11	2.01	1.18	1.54	2.23
% of change	------	-39.53	-28.45	-13.59	-10.73	-25.10	-10.58	-7.70
Oxidized glutathione concentration (GSSG, µmol /L)								
Range	0.81-0.86	0.61-0.64	0.62-0.66	0.67-0.70	0.69-0.72	0.65-0.68	0.70-0.73	0.72-0.76
Mean*	0.83^{a}	0.63^{g}	0.64^{fg}	0.68^{de}	0.71^{cd}	0.67^{ef}	0.72^{bc}	0.74^{b}
SD	0.08	0.04	0.08	0.03	0.09	0.07	0.06	0.05
% of change	------	-24.76	-23.02	-18.47	-15.17	-19.96	-13.97	-11.03
GSH/GSSG ratio								
Range	10.56-14.88	8.89-14.78	9.04-13.05	11.50-13.99	9.56-14.97	10.56-13.88	10.67-13.67	10.67-14.30
Mean*	12.01	9.65	11.23	12.78	12.65	11.24	12.49	12.54
SD	1.56	2.01	1.34	0.99	2.04	1.59	1.07	1.29

* CRSE, *Catharanthus roseus* stems extract, CRLE, *Catharanthus roseus* leaves extract. Means in the same row with different superscript letters are significantly different at p≤ 0.05.

3.4.2. Antioxidant enzymes Activities in Red Blood Cells (RBCs)

Antioxidant defense system in RBCs in diabetic rats consumed *Catharanthus roseus* extracts were assessed by measuring antioxidant enzymes activities including GSH-Px, GSH-Rd and CAT (Table 5). From such data it could be noticed that diabetes induced a significant decreased (p≤0.05) in GSH-Px, GSH-Rd, and CAT activities in RBC's by -43.49, -48.24 and -36.436 % compared to normal controls, respectively. Supplementation of the rat diets with 300, 400 and 500 mg/kg of CRSE and CRLE increased those values which recorded -32.07, -25.92 and -14.09%, and -26.56, -17.65 and -9.06% for GSH-Px, -31.85, -24.93 and -8.59, and -26.40, -19.70 and -6.93% for GSH-Rd, and -26.915, -15.685 and-9.653%, and -18.46, -14.85 and -7.04% for CAT, respectively. Also, in both extracts, the rate of attenuation was raised with the increasing of the extract consumption concentration. Furthermore, CRLE is more effective in elevation the serum GSH fractions level than CRSE.

Generally, to prevent free radical damages (OS activities), the organism has developed antioxidant defense systems largely based on antioxidant enzymes able to scavenge ROS. Superoxide dismutase (SOD) enzymes are responsible for the reduction of $O_2^{\cdot-}$ to H_2O_2 and multiple enzymes will remove H_2O_2 including GSH-Px and CAT. Also, the GSH-Rd reduces the Se and the reduced form of the enzyme then reacts with H_2O_2. The ratio of nine *GSH/GSSG* in normal cells is kept high. So there must be a mechanism of reducing GSSG back to GSH. This is achieved by GSH-Rd enzymes which catalyze the reaction: GSSG + NADPH + H^+ → 2GSH + $NADP^+$. GSH-Rd can also catalyze reduction of certain mixed disulphides such as that between GSH and Co-enzyme A [84].

Mammalian RBC's operate the pentose phosphate pathway in order to provide NADPH for GSH reduction. Many studies such Galinier et al [85] and Cao [86] reported that antioxidant enzymes systems are active in liver cells. Decreasing the activity of the antioxidant enzymes results in increased ROS production and mitochondrial dysfunction [87]. The *Catharanthus roseus* extracts in the present study consuming are rich in bioactive compounds such phenolics, flavonoids, lycopene, polysaccharides, terpenoids, tannins and alkaloids etc which exhibited antioxidant activities in different biological systems [13,88]. Such antioxidant properties are important in manipulation of the diabetes development through ROS scavenging processes in RBC's. i.e stimulate GSH related antioxidant enzymes activity i.e. GSH-peroxidase and GSH-reductase ae well as CAT.

Table 5. Effect of *Catharanthus roseus* ethanolic extracts on RBC's antioxidant enzymes of diabetic rats

Value	Control (-) Std diet	Control (+) Diabetic	CRSE Conc. (w/w, mg/kg)			CRLE Conc. (w/w, mg/kg)		
			300	400	500	300	400	500
Glutathione peroxidase (GSH-Px, U/g Hb)								
Range	22.23-23.43	12.64-13.33	15.05-15.99	16.40-17.31	18.99-20.01	16.26-17.11	18.26-19.17	19.87-20.93
Mean*	22.80^{a}	12.89^{f}	15.49^{e}	16.89^{d}	19.59^{bc}	16.75^{d}	18.78^{c}	20.74^{b}
SD	2.47	1.32	2.39	2.71	3.49	2.41	2.24	3.51
% of change	------	-43.49	-32.07	-25.92	-14.09	-26.56	-17.65	-9.06
Glutathione reductase (GSH-Rd, U/g Hb)								
Range	13.42-14.15	6.99-7.37	9.12-9.69	10.04-10.59	12.20-12.86	9.84-10.35	10.75-11.29	12.76-13.16
Mean*	13.77^{a}	7.13^{f}	9.38^{e}	10.34^{d}	12.59^{b}	10.14^{d}	11.06^{c}	12.82^{d}
SD	2.34	1.17	1.24	2.25	2.31	1.24	2.26	3.02
% of change	------	-48.24	-31.85	-24.93	-8.59	-26.40	-19.70	-6.93
Catalase (CAT, U/g Hb)								
Range	186.21-196.33	117.77-124.21	135.67-144.15	156.37-165.05	167.30-176.33	151.28-159.12	158.15-166.06	173.18-182.41
Mean*	191.05^{a}	121.44^{e}	139.63^{d}	161.09^{c}	172.61^{c}	155.78^{c}	162.68^{c}	177.61^{b}
SD	4.77	2.99	3.59	3.92	4.27	3.75	3.41	4.42
% of change	------	-36.436	-26.915	-15.685	-9.653	-18.46	-14.85	-7.04

* CRSE, *Catharanthus roseus* stems extract, CRLE, *Catharanthus roseus* leaves extract. Means in the same row with different superscript letters are significantly different at $p \leq 0.05$.

3.5. Effect of *Catharanthus roseus* Ethanolic Extracts on Pancreas Histopathological Disorders of Diabetic Rats

The effect of *Catharanthus roseus* ethanolic extracts on pancreas histopathological disorders of diabetic rats. From such microscopically examination (Figure 2) it could be noticed that pancreas of rats from group 1 revealed normal pancreatic acini and normal islets of Langerhan's (Photos 1 and 2). On the other hand, pancreas of rats from group 2 showed marked vacuolations of cells of islets of Langerhan's and congestion of blood capillaries (Photos 3 and 4). Examined sections from group 3 revealed congestion of pancreatic blood vessel (Photo 5) and vacuolations of some cells of islets of Langerhan's (Photo 6). Moreover, pancreas from group 4 exhibited vacuolations of some cells of islets of Langerhan's (Photos 7 and 8). However, some sections from group 5 revealed no histopathological alterations (Photo 9), whereas, other sections showed vacuolations of some cells of islets of Langerhan's (Photo 10). Meanwhile, pancreas of rats from group 6 showed vacuolations of some cells of islets of Langerhan's (Photo 11), congestion of pancreatic blood vessels (Photo 12) and dilatation of pancreatic duct (Photo 13). On the other hand, pancreas of rats from group 7 showed no histopathological alterations except vacuolations of sporadic cells of islets of Langerhan's (Photos 14 and 15). Examination of pancreas from group 8 exhibited vacuolations of some cells of islets of Langerhan's (Photos 16 and 17). Such histopathological changes observed in the present study were confirmed by the biochemical results. In general, no significant lesions were observed in pathological sections of the control; however, the lesions in diabetic group showed massive lesions in the islets of Langerhans and reduced dimensions of islets due to the damage of beta-cells. Data of the present study are going well with that showed in similar studies. For example, Eman et al [89] reported that the damage of pancreas in alloxan-treated diabetic rats and regeneration of beta-cells by *Catharanthus roseus* treatment was observed. Also, *Catharanthus roseus* extract at this dose (300 mg/kg bw) was effective in this direction and showed normal results. This effect may be due to the possibility that some β-cells one still surviving to act upon by *Catharanthus roseus* extract to exert its insulin releasing effect. Histopathological studies reinforce the healing of pancreas, by *Catharanthus roseus* extracts, as possible mechanism of their antidiabetic activity. Other study by Ahmed et al [64] found that histopathological studies reinforce the healing of pancreas, by *Catharanthus roseus* extracts with dose response behavior, as a possible mechanism of their antidiabetic activity i.e to act by beta- cells regeneration. Furthermore, Eman et al [90] noticed that histopathological sections of the pancreas, which treated by ethanolic extracts of *Catharanthus roseus*, illustrated increasing number of beta-cells hypercellularity. Therefore, ethanolic extracts of *Catharanthus roseus* could be beneficial for the diabetes.

3.6. Correlation Studies

In the correlation analysis, important differences were found between OS parameter (MDA) and antioxidant defense systems (GSH fractions, antioxidant enzymes) in diabetic rats feeding *Catharanthus roseus* extracts (Table 6). From such data it could be noticed that there was a strong negative significant ($p \leq 0.05$) relationship between GSH concentration in serum ($r^2 = - 0.9213$), GSSG concentration in plasma ($r^2 = - 0.8834$), antioxidant enzymes in RBC's [GSH-Px ($r^2 = - 0.9051$), GSH-Rd ($r^2 = - 0.8473$), and CAT ($r^2 = - 0.8512$)] and MDA concentration in serum. These correlations confirm that if there were no change in the antioxidant defense systems of diabetes rats, it would be difficult to observe high concentrations of MDA. Such data are confirmed by several studies those are reported that high levels of lipid peroxidation i.e. MDA in serum of diabetic rats were associated with rather low levels of antioxidant enzymes including GSH-Px, GSH-Rd, SOD and CAT [22,61].

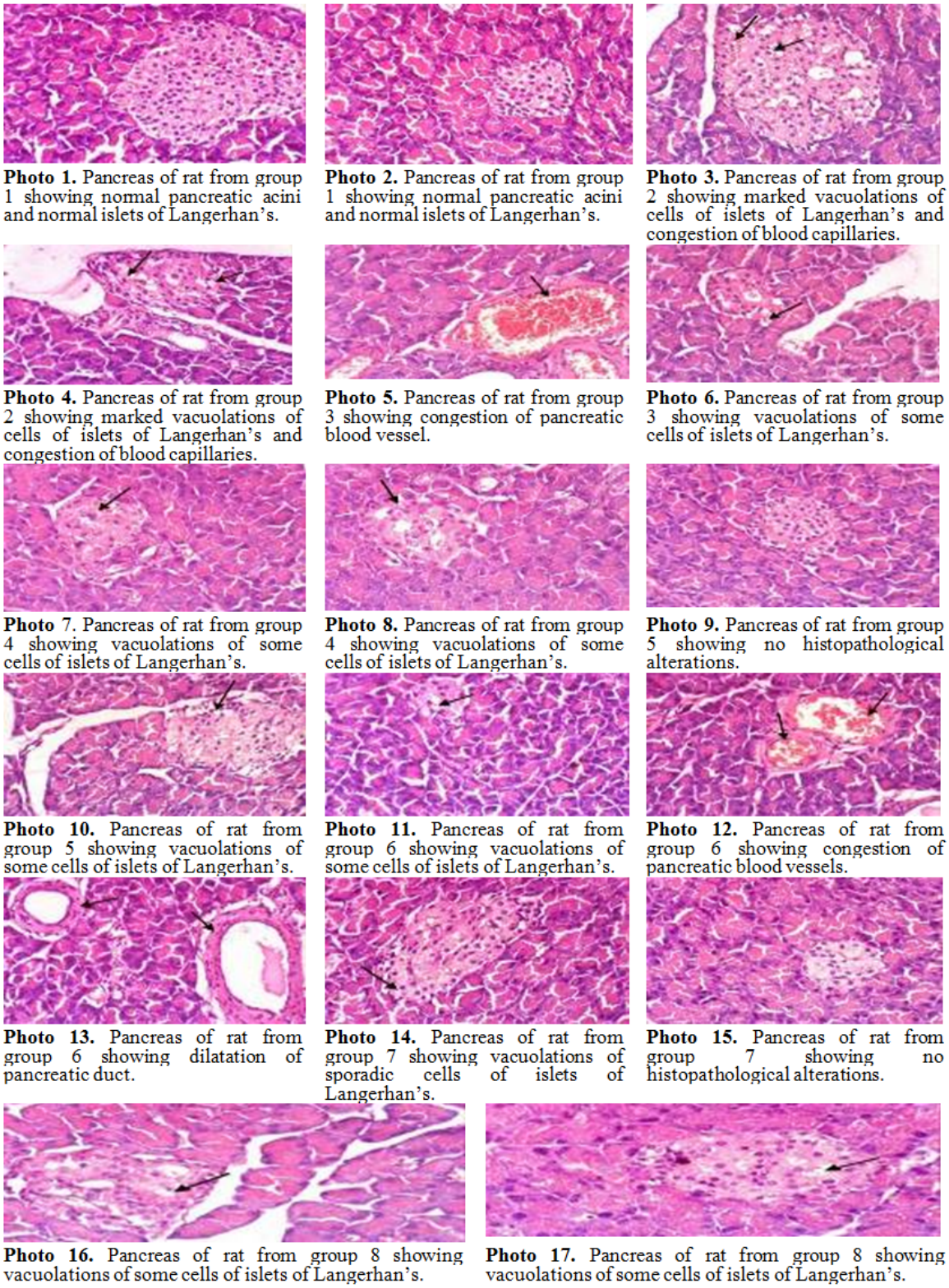

Photo 1. Pancreas of rat from group 1 showing normal pancreatic acini and normal islets of Langerhan's.

Photo 2. Pancreas of rat from group 1 showing normal pancreatic acini and normal islets of Langerhan's.

Photo 3. Pancreas of rat from group 2 showing marked vacuolations of cells of islets of Langerhan's and congestion of blood capillaries.

Photo 4. Pancreas of rat from group 2 showing marked vacuolations of cells of islets of Langerhan's and congestion of blood capillaries.

Photo 5. Pancreas of rat from group 3 showing congestion of pancreatic blood vessel.

Photo 6. Pancreas of rat from group 3 showing vacuolations of some cells of islets of Langerhan's.

Photo 7. Pancreas of rat from group 4 showing vacuolations of some cells of islets of Langerhan's.

Photo 8. Pancreas of rat from group 4 showing vacuolations of some cells of islets of Langerhan's.

Photo 9. Pancreas of rat from group 5 showing no histopathological alterations.

Photo 10. Pancreas of rat from group 5 showing vacuolations of some cells of islets of Langerhan's.

Photo 11. Pancreas of rat from group 6 showing vacuolations of some cells of islets of Langerhan's.

Photo 12. Pancreas of rat from group 6 showing congestion of pancreatic blood vessels.

Photo 13. Pancreas of rat from group 6 showing dilatation of pancreatic duct.

Photo 14. Pancreas of rat from group 7 showing vacuolations of sporadic cells of islets of Langerhan's.

Photo 15. Pancreas of rat from group 7 showing no histopathological alterations.

Photo 16. Pancreas of rat from group 8 showing vacuolations of some cells of islets of Langerhan's.

Photo 17. Pancreas of rat from group 8 showing vacuolations of some cells of islets of Langerhan's.

Figure 2. Effect of *Catharanthus roseus* ethanolic extracts on pancreas histopathological disorders of diabetic rats (H and E X 400)

Table 6. Correlation between oxidative stress and antioxidant defense systems in diabetes rats consuming *Catharanthus roseus* extracts*

Parameters	r^{2*}
MDA/GSH	- 0.9213
MDA/GSSG	- 0.8834
MDA/GSH-Px	- 0.9051
MDA/GSH-Rd	- 0.8473
MDA/CAT	- 0.8512

* $P \leq 0.05$.

4. Conclusion

Data of the present study has demonstrated the efficiency of the *Catharanthus roseus* extracts to relatively attenuate hyperglycemia and oxidative stress in diabetic rats. All of these amelioration effects could be attributed to the high antioxidant activities due to the high levels of many bioactive compounds found in *Catharanthus roseus* extracts which exhibited antioxidant

effects against oxidants/ROS formation as the diabetes development through several proposed mechanisms including: 1) increasing the GSH synthesis, 2) raising of redox status (GSH/GSSG ratio), 3) stimulate antioxidant enzymes activity i.e. GSH-Px, GSH-Rd, CAT and SOD in the living cells, and reinforce the histopathological healing of pancreas. These findings provide a basis for the use of *Catharanthus roseus* as a promising plant for the prevention and/or treatment of type-2 Diabetes mellitus.

Acknowledgements

All the authors of this manuscript are thankful to Research Support Unit (RSU), Minoufiya University, Shebin El-Kom, Egypt for partially financial support.

References

[1] Tiwari A, Madhusudana J, Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. Current Science, 2002; 83 (1): 30-38.

[2] Wadkar KA, Magdum CS, Patil SS, Naikwade NS. Antidiabetic potential and Indian medicinal plants. J Herbal Med and Toxicol, 2008, 2: 45-50.

[3] Fabregat M, Fernandez M, Javiel G, Vitarella G, Mimbacas A, The Genetic Profile from HLA and Non-HLA Loci Allows Identification of Atypical Type 2 Diabetes Patients. J Diabetes Res, vol. 2015; 2015 (6): 485132.

[4] Stumvoll M, Goldstein BJ, van Haeften TW, Type 2 diabetes: principles of pathogenesis and therapy. Lancet, 2005; 365: 1333-1346.

[5] IDF, International Diabetes Federation, Diabetes Atlas. Global estimates for the prevalence of diabetes 7th edition Diabetes Res. Clin. Pract, 2015; 128: 40-50.

[6] Wild S, Roglic G, Green A, Sicree R, King H, Global prevalence of diabetes. Diabetes Care 2004; 27: 1047-1053.

[7] Lu DY, Che JY, Rethink of diabetes treatment and drug development. Cell & Developmental Biology, 2014; 3: e125.

[8] Sies H, Introductory remarks. In: Sies H (Ed) Oxidative stress. Academic, London, 1985; pp 1-8.

[9] Esra B, Cansin S, Omer K, Oxidative Stress and Antioxidant Defense. WAO Journal, 2012; 5: 9-19.

[10] Stadtman ER, Role of oxidant species in aging. Curr Med Chem, 2004; 11:1105-1112.

[11] Chaitanya KV, Pathan AA, Mazumdar SS, Charavarthi GP, Parine N, Bobbarala V, Role of oxidative stress in human health: An overview. Journal of Pharmacy Research. 2010; 3: 1330-1333.

[12] Rahman T, Ismail H, Towhidul I, Hossain U, Shekhar T, Oxidative stress and human health. Advances in Bioscience and Biotechnology, 2012; 3: 997-1019.

[13] Elhassaneen Y, Salem A. Biochemical/Nutritional Studies on some Obesity Cases in Egypt. Journal of Home Economics 2014; 24(1): 121-137.

[14] Steinberg D, Parthasarathy S, Carew TE, beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. N Engl J Med, 1989; 320: 915-924.

[15] Esterbauer H, Gebicki J, Puhl H, Jurgens G, The role of lipid peroxidation and antioxidants in oxidative modification of LDL. Free Rad Biol Med 1992; 13: 341-390.

[16] Tesfamariam B, Cohen RA, Free radicals mediate endothelial cell dysfunction caused by elevated glucose. Am J Physio, 1994; 263:H321-H326.

[17] Pieper GM, Siebeneich W, Diabetes-induced endothelial dysfunction is prevented by long-term treatment with the modified iron chelator, hydroxyethyl starch conjugated-deferoxainine. J Cardiovasc Pharni, 1997; 30: 734-738.

[18] Davies MJ, Dean RT, Radical-Mediated Protein Oxidation. From Chemistry to Medicine. 1997; Oxford, England: Oxford University Press.

[19] Mayhan WG, Superoxide dismutase partially restores impaired dilatation of the basilar artery during diabetes mellitus. Brain Res, 1997; 760: 204-209.

[20] Cosentino F, Hishikawa K, Katusic Z, Liischer T. High glucose increases nitric oxide synthase expression and superoxide anion generation in human aortic endothelial cells. Circulation, 1997; 96: 25-28.

[21] Elhassaneen Y, Ragab S, Thoraya M, Azza E, Abeer A. Effect of Sweet Violet (*Viola odorata* L.) Blossoms Powder on Liver and Kidney Functions as well as Serum Lipid Peroxidation of Rats Treated with Carbon Tetrachloride. The Journal of American Science, 2012; 9 (5): 88-95.

[22] Aly A, Elbassyouny GM, Elhassaneen YE. Studies on the antioxidant properties of vegetables processing by-products extract and their roles in the alleviation of health complications caused by diabetes in rats. Proceeding of the 1st International Conference of the Faculty of Specific Education, Kafrelsheikh University, "Specific Sciences, their Developmental Role and Challenges of Labor Market" 24-27 October, 2017, PP 1-24, Sharm ElSheikh, Egypt.

[23] Jevas O, Anti-Diabetic effects of Allium cepa (ONIONS) aqueous extracts on alloxan-Induced diabetic Rattus Novergicus, Pharmacologyonline, 2011; 1(7): 270-281.

[24] Matsui T; Ogunwande LA, Abesundara KJ, Matsumoto K, Antihyperglycemic potential of natural products. Mini-Rev Med Chem., 2006; 6: 109-120.

[25] Elhassaneen Y, Ragab S, Saleh A, Effect of Selected Plant Parts Extracts on Liver Injuries Induced by CCl4 in vitro. Pyrex Journal of Medicinal Plant Research, 2016-a; 2 (2): 8-20.

[26] Elhassaneen Y, Ragab S, Khater O, Fati G, Functional foods extracts applied in breads ameliorates liver disorders induced by CCl4 in rats. Journal of Home Economics, 2016-b; 26 (4): 31-49.

[27] Elhassaneen Y, El-Waseef S, Fathy N, Sayed Ahmed S, Bioactive compounds and antioxidant potential of food industry by-products in Egypt. American Journal of Food and Nutrition, 2016-c; 4 (1): 1-7.

[28] Elhassaneen Y, El-Wasef S, Elkhamisy A, Fayez S, Phytochemicals for polycyclic aromatic hydrocarbon formation, toxicity and carcinogenicity control: A review. 4th International-18th Arab Conference of Home Economics "Home Economics and Development Issues" 5-6 April, Faculty of Home Economics. Minoufiya University, Egypt. Journal of Home Economics (Special issue), 2016-d; 26 (2): 43-71.

[29] Elhassaneen Y, Samia G. Ryeaan S, Mayada Y, Onion, orange and prickly pear peel extracts mixed with beef meatballs ameliorates the effect of alloxan induced diabetic rats. American Journal of Pharmacology and Phytotherapy, 2016-e; 1 (1): 15-24.

[30] Elhassaneen Y, Sherif R, Badawy R, Antioxidant activity of methanol extracts from various plant parts and their potential roles in protecting the liver disorders induced by benzo (*a*) pyrene. *Public Health International*, 2016-f; 2 (1): 38-50.

[31] Dias AS, Porawski M, Alonso M, Marroni N, Collado PS, Gonzalez-Gallego J, Quercetin decreases oxidative stress, NF-kappaB activation, and iNOS overexpression in liver of streptozotocin-induced diabetic rats. J Nutr, 2005, 135: 2299-2304.

[32] Coskun O, Kanter M, Korkmaz A, Oter S. Quercetin, a flavonoid antioxidant, prevents and protects streptozotocin-induced oxidative stress and beta-cell damage in rat pancreas. Pharmacol Res, 2005; 51: 11.

[33] Kobori M, Masumoto S, Akimoto Y, Takahashi Y, Dietary quercetin alleviates diabetic symptoms and reduces streptozotocin-induced disturbance of hepatic gene expression in mice. *Mol. Nutr. Food Res*, 2009; 53, 859-868.

[34] Hall N, Botanists of the Eucalypts. Commonwealth Scientific and Industrial Research Organization, Melbourne, 1978; pp: 160.

[35] Piovan A, Filippini R, Anthocyanins in Catharanthus roseus in vivo and in vitro: a review. Phytoche. Rev, 2007; 6(2): 235-242.

[36] Ferreres F, Pereira DM, Valentao P, Andrade PB, Seabra RM, Sottomayor M, New phenolic compounds and antioxidant potential of Catharanthus roseus. J. Agric. Food Chem, 2008; 56(21): 67-74.

[37] Yadav PD, Bharadwaj N S, Yedukondalu M, Methushala CH, Kumar AR Phytochemical evaluation of Nyctanthes arbortristis, Nerium oleander and Catharanthus roseus. Ind. J. Res. Pharm. Biotechnol. 2013; 1(3): 333-338.

[38] Mallik A, Arhsad I, Qurban A, Muhammad R, Evaluation of Catharanthus roseus Plant Extract Grown under Multiple Stress

Environmental, Plant Cell Biotechnology and Molecular Biology, 2020; 21(53-54), 71-78.

[39] Chabner BA, Longo DL, Cancer chemotherapy and biotherapy: Principles and practice, 4th Edn, Lippincott Williams and Wilkins, Philadelphia, PA, USA. 2005; PP: 879.

[40] Gaston KJ, Spicer JI, Biodiversity: An Introduction. 2nd Edn. Wiley-Blackwell Science, Oxford, UK, 2004; pp: 191.

[41] Iweala EEJ, Okeke CU, Comparative study of the hypoglycemic and biochemical effects of Catharanthus roseus (Linn) g. apocynaceae (Madagascar Vinca) and chlorpropamide (diabenese) on alloxan-induced diabetic rats. Biokemistri, 2005; 17: 149-156.

[42] Mostofa MME, Choudhury MA, Hossain MZ, Islam MS, Islam M, Sumon H, Antidiabetic effects of Catharanthus roseus, Azadirachta indica, Allium sativum and Glibenclamide in experimentally diabetic rats. Bangladesh J. Vet. Med, 2007; 5: 99-102.

[43] WHO, Definition, diagnosis and classification of diabetes mellitus and its complications. World Health Organization Department of Nonncomunicable Disease Surveillance. 1999; (http://whglibdoc.who. int/hg/1999/WHO-NCD-NCS-99.2pdf). 60 p. Retrieved on 7/6/2007.

[44] AIN, American Institute of Nutrition Purified diet for laboratory Rodent, Final report. J. Nutrition. 1993; 123: 1939-1951.

[45] Reeves PG, Nielsen FH, Fahey JrGC, AIN-93 Purified Diets for Laboratory Rodents: Final Report of the American Institute of Nutrition Ad Hoc Writing Committee on the Reformulation of the AIN-76A Rodent Diet. J. Nutr., 1993; 11(123): 1939-1951.

[46] Lazarow A, Palay B, "Experimental Diabetes and its relation to the Disease" Asymposium. Black wells scientific Publication, 1954; 14: 66 – 69.

[47] Godin DV, Wohaieb SA, Alterations in free radical tissue defense mechanisms in streptozotocin induced diabetes in rat. Effects of insulin treatment. Diabetes, 1987, 36:101418.

[48] Kakkar R, Mantha SV, Radhi J, Prasad K, Increased oxidative stress in rat liver and pancreas during progression of streptozotocin – induced diabetes. Clinical Science, 1998; 94: 623-632.

[49] Stroev EA, Makarova VG, Laboratory Manual in Biochemistry, MIR Publishers, Moscow, USSR. 1989.

[50] Yound DS. Determination of GOT. Clin. Chem, 1975; 22(5): 1-21.

[51] McFarris MW, Reed DJ, HPLC of thiols and disulfides: dinitrophenol derivatives. Methods Enzymol, 1987; 143: 101-109.

[52] Splittgerber AG, Tappel AL, Inhibition of glutatione peroxidase by cadimium and other metal ions. Arch Biochem Biophys, 1979; 197: 534-42.

[53] Aebi H. Catalase. In: Bergmeyer HU. Ed. Methods of enzymatic analysis. New York, London: Academic Press, 1974; pp. 673-677.

[54] ICSH, International Committee for Standardization in Haematology Recommended methods for red cell enzyme analysis. British Journal of Haematology, 1979; 35: 331-340.

[55] Buege JA, Aust SD, Microsomal lipid peroxidation in Packer L., (ed), Methods in enzymology, New York, NY, Academic, 1978; 52: 302-310.

[56] Carleton H, Histological Techniques, 1978, 4th Ed., London, Oxford, New York, Tornoto.

[57] Hasani- Ranjbar S, Nayebi N, Larijani B, Abdollahi M, Asystematic review of the efficancy and safety of Teucrium species from anti- oxidant to anti- diabetic effects. Int. J. Pharmacol., 2010; 6: 315-325.

[58] Ajuru, MG, Ajuru G, Nmom FW, Worlu CW, Igoma, PG. Acute Toxicity Study and Determination of Median Lethal Dose of Catharanthus roseus in Wistar Albino Rats. Journal of Applied Sciences, 2019; 19: 217-222.

[59] Hamzawy MA, El-Denshary ES, Mannaa FA, AbdelWahhab MA, Dietary supplementation of Calendula officinalis counteracts the oxidative stress and liver damage resulted from aflatoxin. ISRN Nutrition, 2013; 9(10), 5402-538427.

[60] Abd EL-Rahman A, Elhassaneen Y, Badran H, Badawy N. Potential Effect of Milk Thistle on Liver Disorders Induced by Carbon Tetrachloride. Journal of Home Economics, 2021; 31 (1): 83-93.

[61] Elhassaneen Y, El-Sayed M, Hassan H, The effect of some food products mixed with plant parts on blood sugar levels of rats. 3rd International-17 Th Arab Conference of Home Economics "Home Economics in the Service of Science, Industry and Society Issues" 9-11 September, 2014, Faculty of Home Economics. Minoufiya University, Egypt. *Journal of Home Economics* (Special issue), 2014; 24(4): 85-109.

[62] Sayed Ahmed S, Yousif Elhassaneen, Safaa El-Waseef, Naglaa Fathy, The effect of Plant by-products on liver functions and plasma glucose in rats fed a high fat diet induced obesity. Port Saied Specific Education Journal (PSSRJ), 2016; 19 (1): 649-659.

[63] Elhassaneen Y, Badran H, Abd EL-Rahman A, Badawy N, Potential Effect of Milk Thistle on Liver Disorders Induced by Carbon Tetrachloride. Journal of Home Economics, 2021; 31 (1): 83-93.

[64] Ahmed I, Lakhani MS, Gillett M, John A, Raza H. Hypotriglyceridemic and hypocholesterolemic effects of antidiabetic *Momordica charantia* fruit extract in streptozotocin induced diabetic rats. Diabetes Res. Clin. Pract, 2010; 51(3): 155-161.

[65] Vega-Avila E, Cano-Velasco J L, Alarcon-Aguilar FJ, Ortiz, MF, Almanza-Perez JC, Roman-Ramos R, Hypoglycemic Activity of Aqueous Extracts from *Catharanthus roseus.* Journal ListEvid Based Complement Alternat Med 2012; 2012: 934258.

[66] Islam MA, Akhtar MA, Khan MR, Hossain MS, Alam AH, Ibne-Wahed MI, "Oral glucose tolerance test (OGTT) in normal control and glucose induced hyperglycemic rats with Coccinia cordifolia L. and Catharanthus roseus L.," Pakistan Journal of Pharmaceutical Sciences, 2009; 22: 402-404.

[67] Rasineni K, Bellamkonda R, Reddy SS, Desireddy S, Antihyperglycemic activity of Catharanthus roseus leaf powder in streptozotocin – induced diabetic rats. Pharmacogen Res., 2010; 3: 195-200.

[68] Singh R, Kharb P, Rani K, Rapid micropropagation and callus induction of Catharanthus roseus in vitro using different explants. World J Agric Sci, 2011; 7(6):699-704.

[69] Das S, Sharangi A, Madagascar Vinca (Catharanthus roseus L.): Diverse medicinal and therapeutic benefits to humankind, Journal of Pharmacognosy and Phytochemistry, 2017; 6(5): 1695-1701.

[70] Riaz K, Fatima Z, Hyder S, Rashed R, Antibacterial, Antitumor Activity and Phytochemical Studies of Methanolic Extract of *(Catharnthus Roseus)* (linn.) g. don. Pak. J., 2021; 53 (6).

[71] Jung EJ, Bae MS, Jo EK, Jo YH, Lee SC, Antioxidant activity of different parts of eggplant. J Med Plant Res, 2011; 5 (18): 4610-4615.

[72] Elhassaneen Y, Ragab S, Saleh A, Effect of selected plant parts extracts on liver injuries induced by CCl_4 *in vitro*. Pyrex Journal of Medicinal Plant Research, 2015; 2 (2): 8-20.

[73] Zheng W, Wang W, Antioxidant activity and phenolic compounds in selected herbs. J. Agric. Food. Chem. 2001; 49: 5162-5270.

[74] Rzaeizadeh A, Zuki ABZ, Abdollahi M, Goh YM, Noordin MM, Azmi TI, Determination of antioxidant activity in methanolic a chloroformic extracts of Momordica charantia. Afr. J. Biotechnology, 2011; 10: 4932-4940.

[75] Shamberger RJ, Andreone TL, Willis CE, Antioxidants and cancer. IV. Malonaldehyde has initiating activity as a carcinogen. J. Natr. Cancer Inst, 1974; 53: 1771.

[76] Larsson A, Mattsson B, Hagenfeldt L, Moldeeus P, Glutathione synthetase-de¢cienthuman ¢broblasts in culture.Clin Chim Acta, 1983; 135: 57-64.

[77] Lina M, Chantal E, Pierre S, Francine D, Marc B, Resistance to Fas-induced apoptosis in hepatocytes: role of GSH depletion by cell isolation and culture. *Am J Physiol Gastrointest Liver Physiol*, 2002*;* 283: G709-G718.

[78] Moussa, S. A. (2008): Oxidative stress in diabetes mellitus. *Rom J Biophys.* 2008; 18: 225-236.

[79] Lee AY, Chung SS, Contributions of polyol pathway to oxidative stress in diabetic cataract. *FASEB J*, 1999; 13: 23-30.

[80] Di Giulio RT, Indices of oxidative stress as biomarkers for environmental contamination. Aquatic toxicology and risk assessment: 14th volume, ASTM STP 1124, M.A. Mayes and M.G. Barron, Eds., American Society for Testing and maerials, Philadelphia, 1991; pp. 15-31.

[81] Elhassaneen YA, The effect of charcoal broiled meat consumption on antioxidant defense system of erythrocytes and antioxidant vitamins in plasma. Nutrition Research, 2004; 24 (6): 435-446.

[82] Champe CP, Harvey AR, Biochemistry. 1994; 2nd edition, J.B. Lippincott Company, Philadelphia, USA.

[83] Bedard K, Krause KH. The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. Physiol Rev. 2007; 87(1): 245-313.

[84] Thomas JP, Afaiorino M, Uesini F, Protective action of phospholipid hydroperoxide. Glutathione peroxidase against membrane damaging lipid peroxidation. J. Biol. Chem, 1990; 265: 454-461.

[85] Galinier A, Carriere A, Fernandez Y, Adipose tissue proadipogenic redox changes in obesity, The Journal of Biological Chemistry, 2004; 281 (18): 12682-12687.

[86] Cao H, Adipocytokines in obesity and metabolic disease, Journal of Endocrinology, 2014; 220 (2): T47-T59.

[87] Curtis JM, Grimsrud PA, Wright WS, Down regulation of adipose glutathione-S-transferase A4 leads to increased protein carbonylation, oxidative stress, and mitochondrial dysfunctio. Diabetes. 2010; 59 (5): 1132-1142.

[88] Alba BM, Bhise S B. Comparative Studies on Antioxidant Properties of *Catharanthus Rosea* and Catharanthus. International Journal of Pharmaceutical Techniques. 2011; 3(3): 1551-1556.

[89] Eman GE, Abd-Elwahab SA, Atef MM, Anwaar A, Physiological Effect of Peri winkle (*C.roseus*) On Diabetic Albino Rat, The Egyptian Journal of Hospital Medicine, 2012; 49: 896-910.

[90] Eman HA, Abdulkarim J, Mohammed MD, Assessment of Anti-Diabetic Activity of Vinca Rosea Extract on Induced Diabetic Mice, Indian Journal of Forensic Medicine & Toxicology, 2020; 14 (4): 2311-2318.