

الإجهاد التأكسدي ومضادات الأكسدة للفئران المصابة بالسكري والتي تم تغذيتها على بعض
مستخلصات الأجزاء النباتية
إعداد/ أ.د / محمد الزغبى، أ.م.د / صفاء الوصيف، د / نجلاء سالم، أ / زينب عريف

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Oxidative stress and antioxidant defense systems status in diabetic rats feeding some selected plant partextract

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المستخلص:

يلعب الإجهاد التأكسدي دورا فعالا في تطوير الأمراض المختلفة بما في ذلك مرض السكري ، وينتج عنه عدم التوازن في إنتاج مضادات الأكسدة .وتهدف هذه الدراسة إلي دراسة حالة نظام التأكسد في الفئران المصابة بمرض السكريالتي تتغذي علي بعض أجزاء النبات المختارة مثل (OFWE) ، (MLWE)، (CLWE)، وخليطهم.تسببت معاملة الفئران بالأكسجينوسيد في زيادة معنوية ($p \leq 0.05$) في تركيز الجلوكوز بالدم بنسبة (136.01%) مقارنة بالمجموعة السالبة (-) وعند إضافة 2% من وزن (OFWE)، (MLWE)، (CLWE) ، وخليطهم للمكملات الغذائية للفئران انخفضت هذه القيمة مقارنة بالمجموعة الضابطة وسجلت 57.35% ، 38.69%، 27.05%، 35.48% علي التوالي كما أن استهلاك (OFWE)، (MLWE)، (CLWE)، وخليطهم تسبب في تحسينات كبيرة فى تركيز الجلوتاثيون فى السيرم من خلال زيادة (GSSG ،GSH) مقارنة مع الضوابط العادية بنسبة -15.31، -11.22، -14.29، -11.22، -7.14، -14.29 علي التوالي ،وقد لوحظ الاتجاه المعاكس مع المواد المؤكسدة البيولوجية (NO₂، NO₃) كما تدعم الدراسة الحالية فوائد التعديل الغذائي لتخفيف الإجهاد التأكسدي المرتبط بالسكري .

الكلمات المفتاحية: ثمار البامية، ورق التوت، ورق الكرفس، مرض السكري، الجلوتاثيون المختزل، الجلوتاثيون المؤكسد، الثيوباربيتوريك، أكيد النتروز

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Oxidative stress and antioxidant defense systems status in diabetic rats feeding some selected plant partextract

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Abstract:

Oxidative stress plays a pathological role in the development of various diseases including diabetes. Systemic oxidative stress results from an imbalance between oxidants derivatives production and antioxidants defenses. The present study aims to investigate the oxidative stress and antioxidant defense system status in diabetic rats feeding some selected plant parts such okra fruits water extract (OFWE), white mulberry leaves water extract (MLWE), celery leaves water extract (CLWE) and their Mixture. Treatment of animals with alloxan caused a significant increased ($p \leq 0.05$) in serum glucose concentration by the ratio 136.51% compared to positive animals (negative control group). Supplementation of the rat diets with 2% w/w by OFWE, MLWE, CLWE and their mixture decreased this elevation value compared to normal controls and recorded 57.35, 38.69, 52.27 and 35.48%, respectively. Also, consumption of OFWE, MLWE, CLWE and their mixture induced significant improvements on serum glutathione fractions concentration through increasing the reduced glutathione (GSH) and oxidized glutathione compared to positive controls by the ratio of -15.31; -11.22, -14.29 and -7.14%; and -15.31, -11.22, -14.29 and -7.14%, respectively. The opposite direction was observed with the biological oxidants including thiobarbituric acid reactive substances (TBARS) and nitric oxides (NO_2 and NO_3). In conclusion, the present data support the benefits of dietary modification, including bioactive compounds and antioxidant vitamins supplementation, in alleviating oxidative stress associated diabetes.

Keywords: okra fruits, white mulberry leaves, celery leaves, GSH, GSSG, TBARS, NO_2 .

Introduction

Oxidative stress was initially defined by Sies (1985, 1986) as a serious imbalance between oxidation and antioxidants, “a disturbance in the prooxidant–antioxidant balance in favor of the former, leading to potential damage”. So, it reflects an imbalance between the systemic manifestation of reactive oxygen species (ROS) and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA. Oxidative stress from oxidative metabolism causes base damage, as well as strand breaks in DNA (Toshniwal and Zarling, 1992). Base damage is mostly indirect and caused by reactive oxygen species (ROS) generated, e.g. O_2^- (superoxide radical), OH (hydroxyl radical) and H_2O_2 (hydrogen peroxide) (Toshniwal and Zarling, 1992). Further, some reactive oxidative species act as cellular messengers in redox signaling. Thus, oxidative stress can cause disruptions in normal mechanisms of cellular signaling (Evans and Davis, 2005).

In humans, oxidative stress is thought to be involved in the development of in several diseases including cancer, atherosclerosis, malaria, chronic fatigue syndrome, rheumatoid arthritis and neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, and Huntington's disease (Halliwell, 1991 and Chaitanya *et al.*, 2010). 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. Infection by *Helicobacter pylori* which increases the production of reactive oxygen and nitrogen species in human stomach is also thought to be important in the development of gastric cancer (Vasavidevi and Raghavendra, 2006). Furthermore, associations between obesity and markers of oxidative stress and the susceptibility of lipid to oxidative modification have been observed in humans (Van Gaal *et al.*, 1998).

Diabetes mellitus has been found to be one of the most crippling diseases that man has ever seen, and its prevalence has risen dramatically over the past two decades. It causes many problems ranging from amputation to death. Therefore, it's a critical need for its management Cantrill (1999) data compiled by World Health Organization (1999) showed that about 150 million peoples suffer from diabetes in world wide, and this number may be doubled by the year 2025. Much of this increase may occur in developing countries due to aging, unhealthy diet, obesity and a sedentary pattern of living. Agency for Health Care Research and Quality (2005) revealed that diabetes, as an increasingly common

chronic disease, currently affects 18 million Americans. The Complications of this disease that may require hospitalization care include heart diseases, stroke, kidney failure, blindness, as well as nerve and blood circulation problems that can lead to lower limb amputations. Data established by Melody, (2008) estimates that diabetes causes about 5 percent of all deaths globally each year, affects 246 million people worldwide. It likely will affect 380 million by 2025, says the International Diabetes Federation (IDF), a worldwide alliance of diabetes associations in more than 160 countries. In the United States, 20.8 million people have diabetes, 6.2 million of them not yet diagnosed. In 2007 the United States joined India, China, Russia and Germany as one of the nations with the largest numbers of diabetics.

Extensively studied sources of such natural compounds are fruits and vegetables, seeds, cereals, berries, wine, tea, onion bulbs, olive oil and aromatic plants. Attempts are also made to identify and evaluate these bioactive compounds in agricultural by-products, ethnic and traditional products, herbal teas, cold pressed seed oils, exudates resins, hydrolysis products, not evaluate fruits and edible leaves and other raw materials rich in antioxidant phenols that have nutritional importance and/or the potential for applications in the promotion of health and prevention against damages/complications caused by many diseases including diabetes mellitus.

Mulberry (*Morus alba L.*), belongs to the family of *moraceae* and genus *morus* usually cultivated to feed silkworm for manufacturing of silk. *Morus alba* (white mulberry) and *Morus indica* (Indian mulberry) are the most popular species of mulberry. According to Zou and Chen (2003), mulberry leaves contain N-containing sugars, rutin, quercetin, volatile oil, amino acids, vitamins and microelements, which have so many pharmacological activities such as reducing blood glucose, antihyperlipidemia, hypertensive, bacteriostasis and antivirus. Mulberry leaves, a traditional Chinese herb, has been used to treat diabetes mellitus and to alleviate thirst. From mulberry leaf extract, 6 N-containing sugars, such as N-methyl-1-deoxynojirimycin, 2-O- α -D-galactopyranosyl- DNJ, and fagomine, have recently been identified and may have antihyperglycemic effects (Yoshikuni *et al.*, 1988). Furthermore, Li *et al.*, (2013) recorded that mulberry leaf can be used to treat wasting and thirsting (xiaoke) syndrome, which is now recognized as typical of diabetes. There is increasing evidence that mulberry leaf contains active constituents that may be of benefit at reducing blood glucose levels. Pharmacologic experiments have shown that mulberry leaf contains alkaloids, flavonoids, polysaccharides, amino acids, simple phenylpropanoids, phenols, and other hypoglycemic components (Asano *et al.*,

2001). On the other side, Kimura *et al.*, (2007) showed that food-grade mulberry powder suppressed the elevation of postprandial blood glucose in humans. Some alkaloids in mulberry leaf are effective inhibitors of digestive enzymes in mammals (Asano *et al.*, 2001). These alkaloids and especially 1-deoxynojirimycin (DNJ) can reduce the activity of α -glucosidase by competitively inhibiting the binding of natural substrates to the active site of the enzyme (Toshiyuki, 2011 and Gao *et al.*, 2016). In addition, DNJ has been reported to inhibit intestinal glucose absorption and accelerate hepatic glucose metabolism by directly regulating the expressions of several proteins involved in glucose transport, glycolysis, and gluconeogenesis (Li *et al.*, 2013).

Celery (*Apium graveolens*, Family: *Umbelliferae*) is one of the most well known plants used in the history of mankind as a medicament or spice. The whole plant has a specific taste and aromatic smell, especially the leaves and roots. Medicinal and aromatic substances are present in the roots, stem and leaves. The healing properties of celery are due to the essential oils and flavonoids, mostly pain and apigenin. Essential oils are present in all parts of the plant: in roots and leaves up to 1%, while their content in the seed can amount even to 7%. Two main components of essential oils in celery are d-limonene and selinene, the latter being present both in the α - and β -form, santalol, α - and β -eudesmol, apiol and myristicin (Wichtl and Bisset, 1994; Barnes *et al.*, 2002). Celery can lower blood pressure, regulate heart function, as well as the blood glucose level by stimulating the pancreas to insulin secretion, so that it can be used to slow down and treat complications caused by diabetes. From old times celery has been known as an aphrodisiac and the investigations showed that celery contains the male sex hormone and rooster one (Teng *et al.*, 1985). Apigenin from celery seed exhibits an anti aggregation effect *in vitro* (Teng *et al.*, 1985). It also inhibits contractions of the isolated smooth muscle of the thoracic aorta (Ko *et al.*, 1991).

Okra (*Abelmoschus esculentus* Linn) is the only fruit crop of significance in the *Malvaceae* family and is very popular in the Indo-Pak subcontinent. The term "okra" most commonly refers to the edible seedpods of the plant. Okra contains potassium, vitamin B, vitamin C, folic acid, and calcium. It's low in calories and has a high dietary fiber content. Popular forms of okra for medicinal purposes include okra water, okra peels, and powdered seeds. In traditional medicine Okra seeds are reported to have ability in managing increased blood glucose concentration. Modern research has correlated this traditional claim with Tomoda *et al.*, (1989) reported that okra polysaccharide possesses anticomplementary and hypoglycemic activity in normal mice. Okra was found to

have hypolipidemic activity in *in vivo* tested rat model (Trinh *et al.*, 2008) and in mice (Ngoc *et al.*, 2008). Okra polysaccharide lowers the cholesterol level in blood and may prevent cancer by its ability to bind bile acids (Kahlon *et al.*, 2007). Cholesterol levels decreased 56.45%, 55.65%, 41.13%, 40.50% and 53.63% respectively in mice groups orally administered with dichloromethane okra plant extract, methanol okra plant extract, dichloromethane okra fruit extract, methanol okra fruit extract and simvastatin as compared to the tyloxapol injected group (Ngoc *et al.*, 2008). The effects of crude extracts of *A. esculentus* on albumin and total bilirubin levels of diabetic albino rats were reported to have a significant ($P < 0.05$) increase (82%) in total bilirubin levels in diabetic control group over the normal control (Urakuet *et al.*, 2010). Administration of peel and seed powder at 100 and 200 mg/kg dose in diabetic rats showed significant ($P < 0.001$) reduction in blood glucose level and increase in body weight than diabetic control rats. Water-soluble fraction of the fruits of Okra was studied to check the absorption of oral glucose as well as metformin from the gastrointestinal tract in the Long Evans rats. It showed significant reduction in absorption of glucose as studied in the 24 hr fasting rats (Khatun *et al.*, 2011). Also, Thanakosai and Phuwapraisirisan, (2013) has reported, the presence of two major flavonolglucosides named isoquercetin (2) and quercetin-3-O-beta-glucopyranosyl- (1"→6")-glucoside (3) in okra seeds which are α -glucosidase inhibitors. These two compounds selectively inhibited rat intestinal maltase and sucrase, in which isoquercetin (2) was 6 - 10 times more potent than its related diglucoside 3. Furthermore, Fan *et al.*, (2014) reported that the extract of okra lowers blood glucose and serum lipids in high-fat diet-induced obese C57BL/6 mice. Ethanol extract of okra (EO) and its major flavonoids isoquercitrin and quercetin 3-O-gentiobioside reduced blood glucose and serum insulin levels and improved glucose tolerance in obese mice.

The present study aims to observe the oxidative stress and antioxidant defense status in diabetes, which represents one of the leading preventable causes of death worldwide. Also, the effect of feeding some selected food processing by-products rich in bioactive compounds (acts as antioxidants) on that status in diabetes rats will be in the scope of this investigation.

Materials and methods

Materials

Plant parts

Okra fruits, white mulberry leaves and celery leaves were purchased from Port Said local markets, Port Said City, Egypt.

Experimental animals

Normal male albino rats (150-170 g) were obtained from Research Institute of Ophthalmology, Medical Analysis Department, Giza, Egypt.

Chemicals

Thiols compounds (GSH and GSSG) were purchased from Sigma Chemical Co., St. Louis, MO, USA. All reagents and solvent required for HPLC were purchased from (Fisher, UK). De-ionized water (Milli-Q 18.2 MΩ) was used in the preparation of the mobile phases, reagent solutions and standards. Alloxan, used for induction of diabetes mellitus among rats, was obtained from Sigma Chemical Co., St. Louis, CA); Casein, as main source of protein from Morgan Co for Chemicals. Cairo, Egypt and Vitamin and salt mixtures, from El- Gomhoriya Company for Chemical, Drugs and Medical Instruments, Cairo, Egypt. All solvents, buffers and specific kits were high analytical grade and purchased from El- Gomhoriya Company for Chemical, Drugs and Medical Instruments, Cairo, Egypt.

Equipments

Throughout this study a SP Thermo Separation Products Liquid Chromatograph (Thermo Separation products, San Jose, CA, USA) was used with a ConstaMetvic 4100 pump, a Spectra Series AS100, Spectra System UV 1000 UV/Visible Spectrophotometer Detector, Spectra System FL 3000 and a PC 1000 system software. The columns used (Alltech, Deerfield, IL, USA) were a Spherosorb ODC-2 (5 µm, 150 x 4.6 mm I.d.) for glutathione fractions Also, absorbance for different assays were measured using Labo-med. Inc., spectrophotometer, CA and Schematzu fluorescence apparatus, Japan, respectively.

Methods

Plant parts extract preparation

Plant parts extracts were prepared according to the methods of Matazu *et al.*, (2018). Okra fruits, white mulberry leaves and celery leaves were cleaned and cut into pieces, then mashed using a blender (Moulinex Egypt, Al-Araby Co., Egypt) with enough water. The mixture was filtered using a filter paper and the filtrate was concentrated using freeze drier.

Antioxidant activity (AA)

Antioxidant activity (AA) of selected vegetables processing by-product extracts and standards (α -tocopherol and BHT) was determined according to the BCB assay following a modification of the procedure described by Marco, (1968).

β -carotene bleaching (BCB) assay

For β -carotene bleaching (BCB) assay, antioxidant activity (AA) against time (every 10 min thereafter for 120 min) for the all tested vegetables processing by-product extracts was measured/constructed according to Marco, (1968). The AA was all calculated as percent inhibition (bleaching rates of β -carotene in reactant mixture of plant part extracts) relative to control (bleaching rates of β -carotene in reactant mixture of without plant part extracts) such as described by Al-Saikhanet al., (1995).

Determination of total phenolics

Total phenolics in selected plant parts extracts were analyzed according to the method of Al-Saikhanet al., (1995). Total phenolics were determined using Folin-Ciocalteu reagent. Two hundred milligrams of sample was extracted for 2 h with 2 mL of 80% MeOH containing 1% hydrochloric acid at room temperature on an orbital shaker set at 200 rpm. The mixture was centrifuged at 1000g for 15 min and the supernatant decanted into 4 mL vials. The pellets were combined and used for total phenolics assay. One hundred microliters of extract was mixed with 0.75 mL of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand at 22 0C for 5 min; 0.75 ml of sodium bicarbonate (60g/L) solution was added to the mixture after 90 min at 22 0C, absorbance was measured at 725 nm. Results are expressed as gallic acid equivalents (GAE).

Biological experimental

Animals

Animals used in this study, adult male albino rats (150-170 g per each) were obtained from Helwan Station, Ministry of Health and Population, Helwan, Cairo, Egypt.

Basal Diet:

The basic diet prepared according to the following formula as mentioned by (AIN, 1993) as follow: protein (10%), corn oil (10%), vitamin mixture (1%), mineral mixture (4%), choline chloride (0.2%), methionine (0.3%), cellulose (5%), and the remained is corn starch (69.5%). The used vitamin mixture component was that recommended by (Campbell, 1963) while the salt mixture used was formulated according to (Hegsted, 1941).

Induction of diabetes:

Diabetes was induced in forty nine normal healthy rats by injection into operationally with freshly prepared alloxan monohydrate in saline at a dose level of 150 mg/ kg body weight (Lazarow and palay, 1954). Immediately after injection animals were received 5% glucose solution over night to overcome drug induced

hypoglycemia (WohaiebandGodin, 1987 andKakkar et al., 1998). After five days blood glucose was analyzed by a drop of blood was obtained from tail vein and subjected to a strip of haemogluco test. All rats with fasting blood sugar > 126 mg/dl were considered to be diabetics and included in the experiment. Their initial weights were recorded.

Experimental design

All biological experiments performed complied with the rulings of the Institute of Laboratory Animal Resources, Commission on life Sciences, National Research Council (NRC, 1996). Rats (n=42 rats), 130-150g per each, were housed individually in wire cages in a room maintained at $25 \pm 2^{\circ}\text{C}$ and kept under normal healthy conditions. Concentrations of the tested parts extracts were decided according to many different studies carried out by Elmaadawy, (2016),Hassan, (2014), Salamaet al., (2017).All rats were fed on basal diet for one-week before starting the experiment for acclimatization. After one week period, the rats were divided into two main groups, the first group (Group 1, 6 rats) still fed on basal diet and the other main group (43 rats) was injected subcutaneous by alloxan monohydrate to induce diabetic rats then classified into sex sub groups as follow:

- Group (2): Fed on standard diet only as a positive control (rats with diabetes).
- Group (3): Fed on standard diet containing 2% (w/w) of okra fruits water extract (OFWE).
- Group (4): Fed on standard diet containing 2% (w/w) of mulberry leaves water extract (MLWE)
- Group (5): Fed on standard diet containing 2% (w/w) of Celery leaves water extract (CLWE).
- Group (6): Fed on standard diet containing 2% (w/w) mixture OFWE+ MLWE+ CLWE by equal parts.

Blood sampling

At the end of experiment period, 28 days, blood samples were collected after 12 hours fasting using the abdominal aorta and rats were scarified under ether anesthetized. Blood samples were received into clean dry centrifuge tubes and left to clot at room temperature, then centrifuged for 10 minutes at 3000 rpm to separate the serum according to Drury and Wallington, (1980). Serum was carefully aspirate, transferred into clean covet tubes and stored frozen at -20°C until analysis.

Analytical methods

Serum glucose

Enzymatic determination of serum glucose was carried out colorimetrically according to Yound, (1975).

Antioxidant status

Antioxidant: Glutathione fractions

GSH and GSSG were determined by HPLC according to the method of Mc-Farris and Reed (1987).

Oxidant status

Nitrite determination

Nitrite was determined flourometric such as described by Miskoet *al.*, (1993).

Nitrite/nitrate detection

Plasma is filtered through an ultrafreemicrocentrifuge filter unit (14000 rpm for 15 min) to remove the hemoglobin resulting from cell lysis. The filtrate should contain mostly nitrate (recovery greater than 90%) due to the reaction of NO with the iron-heme center of the protein. Nitrate is converted to nitrite by the action of nitrate reductase (from *Aspergillusniger*, Sigma Chemical Co., St. Louis, MO, USA) such as follow: the sample is incubated with 40 µM NADPH (to initiate the reaction) and 14 mU of enzyme in a final volume of 50 µl of 20 mMTris buffer (pH, 7.6). The reaction is terminated after 5 min at 20 °C by dilution with 50 µl of water followed by addition of the DNA reagent for determination of nitrite. Nitrite levels in samples are then calculated by first subtracting the value of the enzyme blank (i.e., nitrate reductase plus NADPH) from the experimental and then calculating the value using a standard curve for nitrite to which NADPH has been added.

Thiobarbituric acid reactive substances (TBARS) content

TBARS were measured as described by Buege and Aust, (1978). 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% butylatedhydroxytoluene in 0.25 N HCl. Twenty-five microliters of 0.1 M FeSO₄.7H₂O 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 malonicdialdehyde.

Statistical Analysis

All measurements were done in triplicate and recorded as mean±SD. Statistical analysis was performed with the Student *t*-test and MINITAB 12 computer program (Minitab Inc., State College, PA).

Results and Discussion

Antioxidant activities and total phenolics in selected plant parts

Antioxidant activities of selected plant parts

The antioxidant activities of the selected plant parts and their mixture are shown in Table (1). From such data it could be noticed that the selected plant parts extracts showed considerable differences in antioxidant activity (AA= 62.97-78.41%) when it was calculated by different methods used in this study. White mulberry leaves water extract (MLWE) showed strong activity because of its high phenolic content (621.82(mg GAE.100 g⁻¹) while Celery leaves water extract (CLWE), Okra fruits water extract (OFWE) and Mix (OFWE+MLWE+CLWE) showed relatively medium content in both antioxidant activity and the total phenolics.

Many similar studies indicated that big differentiations have been recorded amongst different vegetables parts. For example, pharmacologic experiments have shown that mulberry leaf contains alkaloids, flavonoids, polysaccharides, amino acids, simple phenylpropanoids, phenols, and other hypoglycemic components (Asano *et al.*, 2001). Active components of celery extraction exhibit antioxidant actions (Hoffmann, 1990; Momin and Nair, 2002). Fan *et al.*, (2014) reported that the antioxidant activity recorded by okra fruits attributed to its high content of phenolic compounds particularly flavonoids, isoquercitrin and quercetin 3-O-gentiobioside. Comparing with the other plant parts, Kumar *et al.*, (1991) and Onyeneho and Hettiarachchy (1993) reported that the peels from the red potatoes contained more polyphenols than those from the brown-skinned varieties. Furthermore it was shown that the peel and the pulp of the tubers contain nine phenolic acids differed in their concentrations and appeared to be mainly responsible for the strong antioxidant activities of the peel extracts. Also, El-Saadany, (2001) found that the mean of phenolic acids content was 2439.21± 113.8 mg/100 g extract of potato peel. Furthermore, Elhassaneen *et al.*, (2013) indicated that the antioxidant activities of control and enriched mango peel powder biscuits. The antioxidant activity (AA) in control biscuits was 31.34% which increased to 37.92 and 45.12% with the incorporation of mango peel powder by 5 and 10%, respectively. Mango peel powder enriched biscuits showed strong activity probably due to its high bioactive compounds (carotenoids and phenolics) content. The same date and antioxidant behavior for

onion and potato parts (skin and peel powders) are recorded by Sayed Ahmed, (2016) and Elhassaneen *et al.*, (2016).

β -Carotene Bleaching (BCB)

β -Carotene Bleaching (BCB) assay based on measured the ability of an antioxidant to inhibit lipid peroxidation (LP). In the BCB method, a model system made of β -carotene and linoleic acid undergoes a rapid discoloration in the absence of an antioxidant. The free linoleic acid radical formed upon the abstraction of a hydrogen atom (H^+) from one of its methylene groups ($-HC=CH-$) attacked the β -

Table 1. Antioxidant activity and total phenolics of selected plant parts extracts

Samples	Antioxidant value ^a AOX (A/h)	Antioxidant activity ^b AA (%)	Oxidation rate ratio ^c (ORR)	Antioxidant activity coefficient ^d (AAC)	Total phenolics (mgGAE.100 g ⁻¹)
Okra fruits water extract (OFWE)	0.209 ± 0.012	62.97 ± 5.66	0.369 ± 0.012	267.02 ± 49.68	291.01 ± 37.79
White mulberry leaves water extract (MLWE)	0.122 ± 0.020	78.41 ± 9.66	0.215 ± 0.031	535.44 ± 24.90	621.82 ± 31.76
Celery leaves water extract (CLWE)	0.179 ± 0.036	68.39 ± 2.79	0.315 ± 0.106	361.24 ± 21.68	321.76 ± 59.00
Mix (OFWE+MLWE+CLWE)	0.190 ± 0.028	66.34 ± 4.06	0.336 ± 0.022	325.60 ± 14.03	390.45 ± 56.62
Control	0.581 ± 0.062	0.00 ± 0.00	1.000 ± 0.218	0.00 ± 0.00	
BHT, 50 mg/L	0.090 ± 0.011	84.11 ± 6.65	0.158 ± 0.013	634.53 ± 50.89	
BHT, 200 mg/L	0.023 ± 0.006	95.98 ± 4.91	0.040 ± 0.005	840.88 ± 40.14	
α-tocopherol, 50 mg/L	0.017 ± 0.001	96.91 ± 4.91	0.031 ± 0.003	857.05 ± 32.46	

^a Antioxidant value (AOX, A/h) = The absolute value of slope (Abs was plotted against time).

^b Antioxidant activity (AA, %) = (R control - R sample) / R control x 100 where: R control and R sample were the bleaching rates of beta-carotene in reactant mixture without antioxidant and with plant extract, respectively

^c Oxidation rate ratio (ORR) = R sample / R control

^d Antioxidant activity coefficient (AAC) = (Abs S 120 - Abs C 120) / Abs C 0 - Abs C 120) x 1000 where: Abs S 120 was the absorbance of the antioxidant mixture at time 120 min, Abs C 120 was the absorbance of the control at time 120 min, Abs C 0 was the absorbance of the control at zero time.

Okra fruits water extract (OFWE), White mulberry leaves extract (MLWE), Celery leaves water extract (CLWE) and Mix means OFWE+ MLWE+ CLWE by equal parts.

Each value represents mean ±SD.

carotene molecules, which lost the double bonds and therefore, its characteristic orange color. The absorbance of the solution at 470 nm was monitored on aspectro

photometer by taking measurements at 10 min intervals, and the rate of bleaching of β -carotene was calculated by fitting linear regression to data over time according to Marco (1968). The decrease in absorbance of β -carotene in the presence of selected plant parts (and well-known antioxidants used as standards) with the oxidation of β -carotene and linoleic acid is shown in Figure (1). Such data indicated that Mix (OFWE+MLWE+CLWE) recorded the lowest decreasing followed by White mulberry leaves water extract (MLWE), Celery leaves water extract (CLWE) and Okra fruits water extract (OFWE), respectively. Comparing with the antioxidants standard used, the values of Mix and MLWE extracts absorbance's through 120 min are coming well i.e. closing the line of 50 mg/L of α -tocopherol and 200 mg/L of BHT up to the line of 50 mg/L of BHT standards followed by the rest of tested plant parts. These data proved the very high stability of the Mix and MLWE relatively high stability of the rest tested plant parts when comparing with that more common standards, α -tocopherol and BHT. The present data are in accordance with the obtained by Ghaly, (2004), and Elhassaneen and Abd Elhady, (2014) who studied AA stability of many plant parts extracts commonly distributed in the Egyptian local markets.

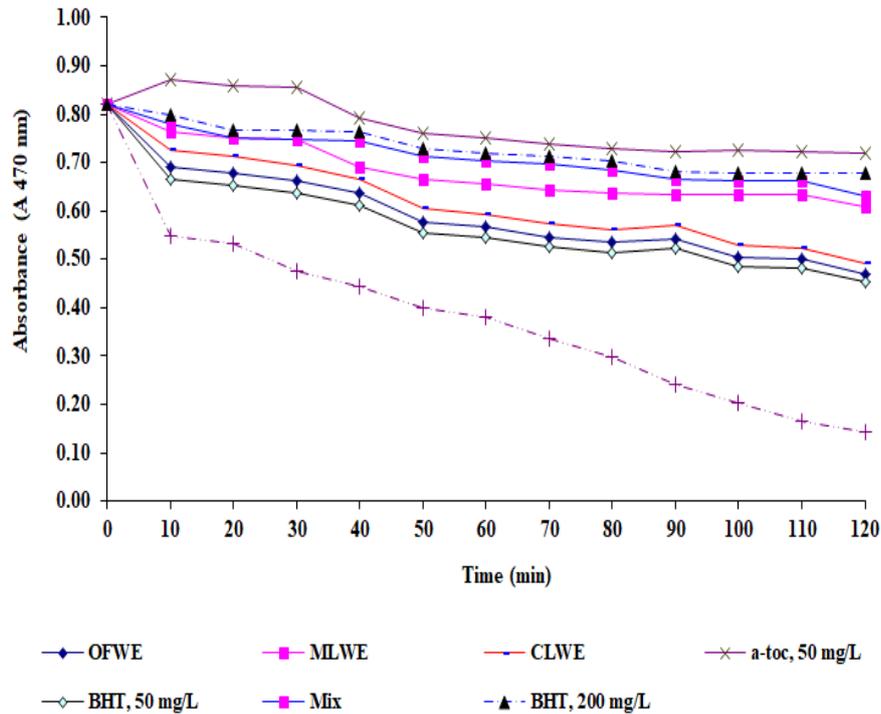


Figure 1. Activity of selected plant parts extracts assayed by the β -carotene bleaching method (BHT α -tocopherol at 50 mg/L concentration was used as a reference).

* OFWE, Okra fruits water extract; MLWE, White mulberry leaves water extract; CLWE, Celery leaves water extract, Mix, mixture extract of OFWE, MLWE and CLWE by equal parts, BHT, butylhydroxytoluene, a-toc, alpha-tocopherol.

Relationship between phenolic contents and antioxidant activity in the selected plant parts

The total phenolics content of the tested plant parts extracts investigated in this study varied and recorded 291.01, 621.82, 321.76 and 390.45 mg GAE/100g for Okra fruits water extract (OFWE), White mulberry leaves water extract (MLWE), Celery leaves water extract (CLWE) and Mix (OFWE+MLWE+CLWE) (Figure 6). The relationship between total phenolics content and antioxidant activity of tested plant parts is shown in Table (2). The results indicated that Mix, MLWE and CLWE showed positive and highly significant ($r^2= 0.9011$, $r^2= 0.8577$ and $r^2= 0.8261$ and $P \leq 0.01$) relationships between total phenolics and antioxidant activity, respectively. While OFWE showed positive and medium significant ($r^2= 0.6412$ and $P \leq 0.01$) relationships between total phenolics and antioxidant activity, respectively. This indicates that phenolics can play a major role in the antioxidant activity of the tested plant parts. In similar study, Velioglu *et al.*, (1998) reported that the correlation coefficient between total phenolics and antioxidative activities of 28 plant products, including plant by-products was statistically significant. Also, many studies indicated that there was a positive and significant ($p < 0.01$) relationship between total phenolics and antioxidant activity in different plant parts (El-Mokadem, 2010; Hegazy, 2009, Elhassaneen *et al.*, 2016 and Sayed Ahmed, 2016).

The data of this study with the others proved the importance of using selected plant parts extracts as natural antioxidants in nutritional therapy applications. For examples, Majid *et al.*, (1991) found feeding of phenolic acid (ellagic, found in all selected plant parts) significantly increased the levels of reduced glutathione and glutathione reductase in liver and lungs of male and female mice as well as increase in inhibition of NADPH-dependent lipid peroxidation. The antioxidant activity of four phenolic acids, representative of three chemical groups, present in the all tested plant by-products, upon low density lipoprotein peroxidation was studied *in vitro* in a low density lipoprotein (LDL) oxidation model by Laranjinha *et al.*, (1994). Antioxidants help protect cells from the potentially damaging physiological process known as "oxidative stress" (damage to healthy cells or DNA by unpaired electrons known as free radicals). Oxidative stress is thought to be associated with the development of chronic diseases including cancer, heart disease, conditions of aging including neurodegenerative diseases such as Parkinson's and Alzheimer's disease. Serum glucose concentration of alloxan-induced diabetic rats consumed the tested plant by-product powder were studied by Shalaby (2015). It was noticed that treatment of animals with aloxane caused a significant increased ($p \leq 0.05$) in serum glucose concentration (41.49%) compared to normal controls. Supplementation of the rat diets with meatballs (20%) decreased the rise of mean serum glucose by the ratio 9.64%. The rate of decreasing was increased with the supplementation of the meatballs with 0.25% w/w/ by (OFWE), (MLWE) and (CLWE) powders and their mixture by 23, 28.6, 26.25 and 35.55%, respectively. The mixture treatment gave maximum hypoglycemic yield when compared with the tested plant parts separated. It could be mean that a combination of different plant parts may be more efficient for reducing the serum glucose level because the interactive effects occurred by different categories of bioactive compounds of plant parts used. Data of the present work with that carried out by the others could be represent the mile stone towards

Table 2. Relationship between antioxidant activities (AA) and total phenolic contents of selected plant parts extracts (n=15)

Relationship between antioxidant activities and total phenolic contents		R ²
Okra fruits water extract (OFWE)	Total phenolics (mg.100g ⁻¹ , d.b.) = 20.891 (Antioxidant activity, %) – 1065.9	0.6412*
White mulberry leaves water extract (MLWE)	Total phenolics (mg.100g ⁻¹ , d.b.) = 23.233 (Antioxidant activity, %) - 1158.1	0.8577
Celery leaves water extract (CLWE)	Total phenolics (mg.100g ⁻¹ , d.b.) = 8.7018x (Antioxidant activity, %) - 301.32	0.8261
Mix (OFWE+MLWE+CLWE)	Total phenolics (mg.100g ⁻¹ , d.b.) = 11.107 (Antioxidant activity, %) - 398.15	0.9011

P ≥ 0.05

the extension of using the selected plant extracts such OFWE, MLWE, CLWE and their mixture, as natural antioxidants in many different nutritional/therapeutic applications. Synthetic antioxidants such as butylatedhydroxyanisole (BAH) and butylatedhydroxytoluene (BHT) have been used as antioxidants since the beginning of the last century. Restrictions on the use of these compounds, however, are being imposed because of their carcinogenicity. Thus, the interest in natural antioxidants has increased considerably (Elhassaneen *et al.*, 2016 and Sayed Ahmed, 2016).

The effect of selected plant parts extracts on serum glucose concentration of obese rats

Serum glucose concentration of alloxan-induced diabetic rats consumed the selected plant parts extractswere shown in Table (3). Treatment of animals with alloxan caused a significant increased ($p \leq 0.05$) in serum glucose concentration by the ratio 136.51% compared to normal animals (negative control group). Supplementation of the rat diets with 2% w/w by OFWE, MLWE, CLWE and their mixture decreased this elevation value compared to normal controls and recorded 57.35, 38.69, 52.27 and 35.48%, respectively. So, the maximum hypoglycemic yield was recorded for the extracts mixture treatment when compared with the rest selected plant parts extracts individually. Such data probably mean that a combination of different selected plant parts extracts may be more efficient for reducing the serum glucose level due to the interactive effects occurred by their different categories of bioactive compounds content. Such behavior was recorded in several previous studies on different plant parts carried out by Sayed Ahmed, (2016), Elmaadawy, (2016) and Elhassaneen *et al.*, (2016-a).

Table 3. Effect of plant parts extracts on serum glucose concentration (mg/dL) of diabetic rats*

Value	Control (-) Std diet	Control (+) Diabetic	Plant parts extracts (2%, w/w)			
			OFWE	MLWE	CLWE	Mix
Mean	90.53 ^c	214.11 ^a	142.45 ^b	125.56 ^c	137.85 ^b	122.65 ^d
SD	4.21	11.45	7.84	10.56	11.37	6.67
% of Change	0.00	136.51	57.35	38.69	52.27	35.48

* OFWE, Okra fruits water extract; MLWE, White mulberry leaves water extract; CLWE, Celery leaves water extract and Mix, mixture extract of OFWE, MLWE and CLWE by equal parts. Means in the same row with different superscript letters are significantly different at $p \leq 0.05$.

The antioxidant or free radical scavenging property in plants such as Okra fruit may inhibit oxidative reactions associated with glycation. The observed increase in the level of glycated hemoglobin (HbA1c) in the diabetic untreated group (Table 1) could be due to the persistent hyperglycemia in diabetic condition; because in diabetes, the persistent and excess amount of glucose present in the blood reacts with hemoglobin to form glycated hemoglobin which may also induce the generation of oxygen derived free radicals and other diabetes-associated complications in prolonged diabetic condition (Alyassin *et al.*, 1981). Anthocyanins are the main phenolic compounds in some okra species which provide a myriad of health benefits including lower blood glucose level, management or control of postprandial

hyperglycemia associated with T2DM and reduce free radicals and thereby improve memory deficits caused by T2DM (Nanda *et al.*, 2013; Esther *et al.*, 2013 and Sepidehet *et al.*, 2016). On the other side, mulberry leaves contain N-containing sugars, rutin, quercetin, volatile oil, amino acids, vitamins and microelements, which have so many pharmacological activities such as reducing blood glucose, antihyperlipidemia, hypertensive, bacteriostasis and antiviral (Zou and Chen, 2003). Also, mulberry leaves, a traditional Chinese herb, has been used to treat diabetes mellitus and to alleviate thirst. From mulberry leaf extract, 6 N-containing sugars, such as N-methyl-1-deoxynojirimycin, 2-O- α -D-galactopyranosyl- DNJ, and fagomine, have recently been identified and may have antihyperglycemic effects (Yoshikuni *et al.*, 1988). Furthermore, Li *et al.*, (2013) recorded that mulberry leaf can be used to treat wasting and thirsting (xiaohe) syndrome, which is now recognized as typical of diabetes. Regarding celery leaves. Celery can lower blood pressure, regulate heart function, as well as the blood glucose level by stimulating the pancreas to insulin secretion, so that it can be used to slow down and treat complications caused by DM. *In vivo* analysis of the effects of quercetin (found in all selected plant parts) on human diabetic lymphocytes showed a significant increase in the protection against DNA damage from hydrogen peroxide at the tissue level. Jung *et al.*, (2011) reported that quercetin might improve glucose response and insulin resistance associated with T2DM by alleviating metabolic dysregulation of free fatty acids, suppressing oxidative stress, up-regulating glucose uptake at peripheral tissues, and/or down-regulating inflammatory gene expression in liver.

The effect of selected plant parts extracts on biological antioxidant (glutathione fractions in plasma) of diabetic rats

Biological antioxidant macromolecules i.e. glutathione fractions concentration in plasma of diabetic rats consumed plant parts extracts were assessed (Table 4). From such data it could be noticed that DM induced a significant decrease ($p \leq 0.05$) in reduced glutathione (GSH) and oxidized glutathione (GSSG) concentrations in plasma by -30.29 and -27.55 % compared to normal controls, respectively. Consumption of OFWE, MLWE, CLWE and their mixture induced significant improvements on plasma glutathione fractions concentration through increasing the GSH and GSSG compared to normal controls by the ratio of -15.31; -11.22, -14.29 and -7.14%; and -15.31, -11.22, -14.29 and -7.14%, respectively. The higher effects in improving of antioxidant macromolecules i.e. glutathione fractions concentration in plasma induced by alloxan in rats were recorded for the Mix followed by MLWE, CLWE and OFWE, respectively. It could be mean that a combination of different selected plant parts extracts may be more efficient for elevation glutathione fractions concentration in plasma the biomarkers of antioxidant status in blood, because the interactive effects occurred by different categories of bioactive compounds of such selected plant parts extracts used. In general, reduced glutathione (GSH), a tripeptide (L-glutamyl-L-cysteinyl-glycine) synthesized in the cytoplasm, is the most abundant intracellular nonprotein thiol involved in antioxidant elimination (Reed and

Beatty, 1980; Larsson *et al.*, 1983). The antioxidant functions of GSH includes its role in the activities of GSH enzymes family including glutathione peroxidase (GSH-Px) and peroxiredoxins (PRXs). In addition, GSH can apparently serve as a nonenzymatic scavenger of oxyradicals (Halliwell and Gutteridge, 1985 and Elhassaneen *et al.*, 2016-a). Several oxidative injuries have been associated with glutathione depletion (Linaet al., 2002). A marked decreased level of GSH is reported in the plasma of diabetic patients/animals (Moussa, 2008; Aly et al., 2017 and Lotfy and Rdwan, 2017). GSH systems may have the ability to manage oxidative stress with adaptational changes in enzymes regulating GSH metabolism. By other meaning, the link between hyperglycemia and GSH depletion has been reported. It could be interpreted by Lee and Chung, (1999) who reported that, in hyperglycemia conditions, glucose is preferentially used in polyol pathway that consumes NADPH necessary for GSH regeneration by the GSH-reductase enzyme. Hyperglycemia is therefore indirectly the cause of GSH depletion.

Table 4. Effect of plant parts extracts on serum antioxidant concentration (glutathione fractions) of diabetic rats*

Value	Control (-) Std diet	Control (+) Diabetic	Plant parts extracts (2%, w/w)			
			OFWE	MLWE	CLWE	Mix
Reduced glutathione concentration (GSH, $\mu\text{mol/L}$)						
Mean	9.97 ^a	6.95 ^b	8.09 ^a	8.81 ^a	8.31 ^a	9.11 ^a
SD	2.56	1.98	4.77	1.67	5.11	4.08
% of Change	0.00	-30.29	-18.86	-11.63	-16.65	-8.63
Oxidized glutathione concentration (GSSG, $\mu\text{mol/L}$)						
Mean	0.98 ^a	0.71 ^b	0.83 ^a	0.87 ^a	0.84 ^a	0.91 ^a
SD	3.67	5.11	4.78	4.54	3.84	7.90
% of Change	0.00	-27.55	-15.31	-11.22	-14.29	-7.14
GSH/GSSG ratio						
Mean	10.17 ^a	9.79 ^a	9.75 ^a	10.13 ^a	9.89 ^a	10.01 ^a
SD	11.89	14.92	7.88	9.91	10.94	9.81
% of Change	0.00	-3.78	-4.19	-0.46	-2.76	-1.60

* OFWE, Okra fruits water extract; MLWE, White mulberry leaves water extract; CLWE, Celery leaves water extract and Mix, mixture extract of OFWE, MLWE and CLWE by equal parts. Means in the same row with different superscript letters are significantly different at $p \leq 0.05$.

Decreasing in GSH fractions observed in diabetic rats group generally accompanied by a concomitant decreased in the ratio of GSH/GSSG. In such direction, Di Giulio (1991) 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.*, (2014) 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 (Champe and Harvey, (1994). Also, Bedard and Krause (2007) 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. Therefore, data of the present study with the other hypotheses that the selected plant parts extracts and their mixtures feeding are rich in different bioactive compounds which exhibited 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, increasing the GSH synthesis, and stimulate GSH related antioxidant enzymes activity i.e. GSH-peroxidase and GSH-reductase.

The effect of selected plant parts extracts on oxidative stress status of diabetic rats

Oxidants concentration (i.e. oxidative stress) in diabetic rats plasma feeding some selected plant parts extracts was assessed by measuring lipid peroxidation (thiobarbituric acid reactive substances, TBARS) and reactive nitrogen species (NO_2 and NO_3) such as illustrated in Table (5). From such data it could be noticed that diabetes induced a significant increased ($p \leq 0.05$) in TBARS, NO_2 and NO_2/NO_3 concentration in plasma by 52.41, 34.23 and 41.93% compared to normal rats, respectively. Consumption of OFWE, MLWE, CLWE and their mixture induced significant improvements on plasma oxidant through decreasing the TBARS, NO_2 and NO_2/NO_3 compared to normal controls by the ratio of 25.40, 13.50, 18.01 and 11.90%; 17.45, 11.07, 14.77 and 7.72%; and 26.02, 16.14, 24.58 and 14.94%, respectively. The highest suppression was recorded with the mixture treatment followed by MLWE, CLWE and OFWE, respectively. It could be mean that a combination of different selected plant parts extracts may be more efficient for reducing plasma TABRS and nitrous oxides (NO_2 and NO_3) levels, the biomarkers of oxidative stress and inflammation in liver, because the interactive effects occurred by different categories of bioactive compounds of plant parts applied. Such as reviewed by Tiwari and Madhusudana (2002), hyperglycemia alone does not cause diabetic complications. It is rather the detrimental effect of glucose toxicity due to chronic hyperglycemia, which is mediated and complicated through oxidative stress (OS). Systemic metabolic alterations associated with diabetes contribute to the increase in OS have been reported by several authors. For example, hyperglycemia as a hallmark of type II diabetes, a metabolic complication of obesity, induces OS through activation of the polyol and hexosamine pathways, production of advanced glycation end-products (AGE), and increase of diacylglycerols (DAG) synthesis (Le Lay *et al.*, 2014). Excess of circulating lipids induces ROS formation pathways, which contribute to the increase in lipid oxidation. Also, Elhassaneen *et al.*, (2014) and Lotfy and Rdwan, (2017) reviewed that RNS (NO_2 and NO_3) been shown to be increased in plasma of alloxan- induced diabetes in rats. In similar studies, clinical evidences for diabetes-associated OS have been provided by measurement of either biomarkers or end-products of free radical-mediated oxidative processes (Elhassaneen *et al.*, 2014, Lotfy and Rdwan, 2017, and Aly, 2017). For instance, lipid peroxidation markers such as malondialdehyde (MDA), one of the most important compounds

in TBARS and 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 (Elhassaneen and Salem 2014). Such data are in accordance with that observed by Jung *et al.*, (2011) who reported that oxidative stress and metabolic dysregulation of FFAs in diabetic condition were alleviated by onion peel extract administration i.e. high content with phenolics compounds such recorded in different selected plant parts extracts of the present study. They also indicated that hepatic oxidant stress was reduced by phenolic compounds of plant parts, as assessed by increasing superoxide dismutase activity and blocking TBARS formation.

Table 5. Effect of plant parts extracts on plasma oxidants concentration of diabetic rats*

Value	Control (-) Std diet	Control (+) Diabetic	Plant parts extracts (2%, w/w)			
			OFWE	MLWE	CLWE	Mix
Thiobarbituric acid reactive substances (TBARS, nmol/mL)						
Mean	3.11 ^c	4.74 ^a	3.90 ^a	3.53 ^{ab}	3.67 ^a	3.48 ^{ab}
SD	2.56	1.98	4.77	1.67	5.11	4.08
% of Change	0.00	52.41	25.40	13.50	18.01	11.90
Nitrite (NO ₂ , nmol/L)						
Mean	2.98 ^b	4.00 ^a	3.5	3.31 ^a	3.42 ^a	3.21 ^a
SD	3.67	5.11	4.78	4.54	3.84	7.90
% of Change	0.00	34.23	17.45	11.07	14.77	7.72
Nitrate + Nitrite (NO ₃ +NO ₂ , nmol/L)						
Mean	4.15 ^b	5.89 ^a	5.23 ^a	4.82 ^a	5.17 ^a	4.77 ^a
SD	11.89	14.92	7.88	9.91	10.94	9.81
% of Change	0.00	41.93	26.02	16.14	24.58	14.94

* OFWE, Okra fruits water extract; MLWE, White mulberry leaves water extract; CLWE, Celery leaves water extract and Mix, mixture extract of OFWE, MLWE and CLWE by equal parts. Means in the same row with different superscript letters are significantly different at $p \leq 0.05$.

On the other side, interest in the possible significance of MDA, one of the most important compounds in TBARS on human health has been stimulated by reports that are mutagenic and carcinogenic compound (Shamberger *et al.*, 1974). Nitric oxide is a small molecule which plays an important role in the communication among liver cells and regulates important liver functions. Nitric oxide synthase catalyzes the conversion of L-arginine to citrulline and highly reactive free radical species, nitric oxide (NO) (Manahan, 1989). Nitric oxide, in turn, can react with molecular oxygen and water to form nitrite and nitrate; with hemoglobin to form iron-nitrosyl adducts and/or nitrate in blood, with superoxide anion to make nitrate, and with the amino and thiol groups of protein to produce nitrosylated species (Manahan, 1989 and Misko *et al.*, 1993). The excess production of nitric oxides has been implicated in the pathogenesis and tissue destruction of a growing number of immunological and inflammatory diseases including septic shock, arthritis, graft rejection and diabetes (Elhassaneen *et al.*, 2014; and Elmaadawy, 2017).

The observed positive effects of plant parts extract on oxidants formation/ concentration of diabetic rats could be attributed to several mechanisms induced by their bioactive components content. In this context, Coskuner *et al.*, (2005) found

that phenolic compounds (flavonoids) such as found in our selected plant parts extracts have anti-oxidative and anti-inflammatory activities. Such dietary phenolics found in our selected plant parts extracts are metabolized in liver, inhibiting liver injury induced by diabetes i.e. enhancing lipid metabolism, reducing oxidative stress may be particularly effective, consequently. Additionally, the mixture treatment gave maximum reduction yield of plasma TBARS and nitric oxides when compared with the individual selected plant parts extracts. It could be mean that a combination of different selected plant parts extracts may be more efficient for reducing plasma TBARS and nitric oxides levels, the biomarkers of oxidative stress and inflammation in the body, because the interactive effects occurred by different categories of bioactive compounds of different selected plant parts extracts.

Correlation studies

In the correlation analysis, important differences were found between oxidative stress parameters (TBARS and nitric oxides) and antioxidant defense systems (GSH fractions) in diabetic rats feeding selected plant parts extracts (OFWE, MLWE, CLWE and their mixture) (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 plasma ($r^2 = -0.8572$), GSSG concentration in plasma ($r^2 = -0.8391$), and TBARS concentration in plasma. While, The same behavior i.e. correlations/relationship were observed between GSH concentration in plasma ($r^2 = -0.9102$), GSSG concentration in plasma ($r^2 = -0.8267$), and NO_2 concentrations in plasma. 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 TBARS and NO_2 . In similar study, Bohmet *al.*, (1997) in some model systems noticed a combination of α -tocopherol and β -carotene interact synergistically to inhibit lipid peroxidation subsequently increased TBARS. Also, high levels of lipid peroxidation i.e. MDA in the plasma of diabetic rats were associated with rather low levels of antioxidant vitamins and enzymes. Furthermore, Elmaadawy, (2017) and Lotfy and Rdwan (2017) found that high levels of lipid peroxidation i.e. TBARS/MDA and nitric oxides (NO_2 and NO_3) in the plasma of diabetic rats were associated with rather low levels of antioxidant macromolecules (GSH and GSSG).

Table 6. Correlation between oxidative stress and antioxidant defense system parameters in diabetic rats feeding some plant parts extracts

Parameters	Correlation	r^{2*}
TBARS/GSH	GSH ($\mu\text{mol /L}$) = - 2.2933 (TBARS, nmol/mL) + 14.136	- 0.9102
TBARS/ GSSG	GSSH ($\mu\text{mol /L}$) = -0.2425 (TBARS, nmol/mL) + + 1.6923	- 0.8391
NO_2 /GSH	GSH ($\mu\text{mol /L}$) = - 2.2893x (NO_2 , nmol/mL) + 13.164	- 0.8572
NO_2 / GSSG	GSSH ($\mu\text{mol /L}$) = -0.7825 (NO_2 , nmol/mL) + 3.2318	- 0.8267

* $P \leq 0.05$

In conclusion, the therapeutic and nutritional effects of [White mulberry leaves water extract (MLWE), Celery leaves water extract (CLWE) and Okra fruits water extract (OFWE)] probably attributed to its highly content of bioactive compounds, phytochemicals, including mainly phenolic compounds. The data of the present work with that carried out by the others could be represent the mile stone towards the extension of using plant parts extracts such (MLWE, CLWE, OFWE and their mixtures) as natural antioxidants in many different medical applications such improve the serum glucose, liver and kidney functions as well as prevent the lipid oxidation on serum.

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