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Phyto-extracts applied in beef meatballs ameliorates alterations in free radical defense mechanisms in alloxan-induced diabetes in rats

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Abstract: Diabetes mellitus (T2DM) is one of the world's most common chronic diseases as changing life styles lead to reduced physical activity and increased obesity. Many stratigies to improve diabetic complecations have been proposed, because early treatment and prevention play a pivotal role in reducing the population burden of diabetes. The present study aims to investigate the effectiveness of three phyto methanolic extracts coming from prickly pear peel (PPP), red onion skin (ROS) and potato peel (PP) and blending in beef meatballs in modulating hyperglycemia using alloxane-induced diabetic rats. The selected phyto extract showed high antioxidant activity ranged 70.62 to 89.78 % and rich in different bioactive compounds including phenolics and carotenoids. Treatment of animals with aloxane caused a significant increased ($p \leq 0.05$) in serum glucose concentration by the ratio 118.09% compared to normal controls. Supplementation of the rat diets with meatballs (20%) decreased the value which recorded 93.63%. The decreasing rate was elevated with the blending of the meatballs with 0.25% w/w by PPP, ROS and PP and their mixture which recorded 61.59, 41.28, 52.10 and 37.15%, respectively. The same behavior was recorded for thiobarbituric acid reactive substances and nitrogen reactive oxides (NO2 and NO3) levels in plasma, the biomarkers of oxidative stress. On the same time, improving in the antioxidant enzymatic and nonenzymatic defense systems in both serum and RBC's were recorded. In conclusion, the tested phyto extracts ameliorates the alterations in free radical defense mechanisms of diabetic rats through the high antioxidant activities as the result of high levels of many bioactive compounds found in such extracts. Also, these findings provide a basis for the use of phyto extracts for the prevention and/or treatment of type-2 Diabetes mellitus.

Keywords: Prickly pear peel, red onion skin, potato peel, antioxidant activity, oxidative stress, antioxidant enzymes, antioxidant vitamins, RBC's

Introduction

A free radical is an atom, molecule, or compound that is highly unstable because of its atomic or molecular structure (i.e., the distribution of electrons within the molecule). As a result, free radicals are very reactive as they attempt to pair up with other molecules, atoms, or even individual electrons to create a stable compound. To achieve a more stable state, free radicals can "steal" a hydrogen atom from another molecule, bind to another molecule, or interact in various ways with other free radicals (Wu and Arthur, 2003). Oxygen, nitrogen, and chlorine species (ROS/RNS/RCS) are represent the most free radicals producing by living organisms as a result of normal cellular metabolis (Esra *et al.,* 2012). At low to moderate concentrations, they function in physiological cell processes, but at high concentrations, they produce adverse modifications to cell components, such as lipids, proteins, and DNA (Halliwell and Gutteridge, 1985 and Stadtman, 2004). The shift in balance between oxidant/antioxidant in favor of oxidants is termed "oxidative stress." It was initially defined by Sies (1985) as a serious imbalance between oxidation and antioxidants, "a disturbance in the prooxidant–antioxidant balance in favor of the former, leading to potential damage".

Oxidative stress is thought to be involved in the development of in several diseases including cancer, neurological disorders, atherosclerosis, hypertension, ischemia/perfusion, diabetes, acute respiratory distress syndrome, idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease, and asthma (Halliwell, 1991, Wu and Arthur, 2003 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 (Rahman *et al.,* 2012). Furthermore, associations between diabetes and markers of oxidative stress including lipid oxidative modifycation have been observed in humans (Van Gaal *et al.,* 1998). Lipid peroxidation is important *in vivo* for several reasons, in particular because it contributes to the development of atherosclerosis (Steinberg et al., 1989 and Esterbauern *et al.,* 1992), a process known to be accelerated in diabetic patients (Tesfamariam, 1994). Lipid peroxides and other end

products of the peroxidation process may be toxic to vascular endothelium in diabetics (Pieper and Siebeneich, 1997). Also, free radical attack can generate protein peroxides in diabetes, which may decompose to generate free radicals (Davies and Dean,1997). Glycoxidation seems to play an important role in the vascular endothelial dysfunction detected in diabetic patients and perhaps in the nephropathy (Mayhan, 1997). Elevated glucose may cause increased generation of *0;-* by endothelium, antagonizing the action of NO and perhaps forming peroxynitrite, ONOO- (Cosentino *et al.,* 1997). All these effects should be amenable to treatment by appropriate antioxidants (Mayhan, 1997).

Many studies indicated that all these effects should be amenable to treatment by appropriate antioxidants (Low et al., 1997 and Mayhan, 1997 and Esra et al., 2012). In general, there are two basic categories of antioxidants, namely, synthetic and natural. Synthetic antioxidants are compounds with phenolic structures of various degrees of alkyl substitution, whereas natural antioxidants can be vitamins (C and E), minerals (selenium and copper) and phytonutrients (phenolic compounds, nitrogen compounds and carotenoids) (Jacob and Burri, 1996). Science the beginning of 20th century, synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been used as antioxidants. Restrictions on the use of these compounds, however, are being imposed because of their carcinogenicity (Ito *et al.,* 1983). Thus, the interest in natural antioxidants has increased considerably.

Plant parts of vegetables, fruits and medicinal plants are the primary source of natural antioxidants for human being. Many phytochemicals naturally occurring in such plant parts would be desirable options. Amongest all of these bioactive compounds flavonoids, phenolic compounds, organosulfur compounds and anthocyanins are represent the central position. Such compounds has been reported to improve diabetic status by decreasing oxidative stress (Dias *et al.,* 2005 and Coskun *et al.,* 2005) or by reducing the disturbance of hepatic gene expressions (Kobori *et al.,* 2009). 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/complecations caused by many dieseases including diabetes mellitus. Amongst of these agricultural by-products, prickly pear fruits (*Opuntia ficus*-indica) peel, red onion (*Allium cepa* L.) skin and Potato (*Solanum tuberosum* L.) peel are producing in large quantities in food-processing plants.

Potato (*Solanum tuberosum* L.) is the largest vegetable crop worldwide, amounting to approximately 320 million metric tons annually. Processing of potatoes (mainly for the production of chips, French fries, and dehydrated products) has presented a steady increase during the last decades, exceeding considerably the amount of the vegetable consumed as fresh. Solid waste generated during processing consists mostly of potato peels but also contains green, immature, and cull potatoes and amounts to 15–45% depending on the procedure applied. It is used as animal feed, though fermentation for the production of single cell or alcohol has been considered. Water from potato processing is used for the recovery of proteins by heat coagulation. Recent investigations suggested the use of water extracts from potato processing waste for the recovery of antioxidants (Rodriguez de Sotillo *et al.,* 1994).

Prickly pear (*Opuntia ficus-indica*), commonly known as prickly pear, belongs to the family *Cactaceae*. Being so water-use efficient, they are highly useful in arid and semiarid environments, particularly during prolonged dry spells or failure of the monsoon. So, it is widely distributed inLatin America, South Africa and the Mediterranean area including Egypt, (Hassan, 2011). It has been used in traditional folk medicine because of its role in treating a number of diseases and conditions, including anti-inflammatory effects, hypoglycemic effects, inhibition of stomach ulceration, neuroprotective effects. Through antioxidant actions and also used for treating diabetes, hypertension, burns,bronchial, asthma edema, and indigestion in many countries over the world. One of the most frequently utilized fruit and vegetable technologies is juice production. Juices, in general, are a good source of sugars, vitamins and minerals; all valuable components to human

health. In addition to their nutritional and medicinal properties, these plants contain compounds which have several commercial applications.

The major by-products resulting from industrial peeling of onion bulbs are brown skin, the outer two fleshy leaves and the top and bottom bulbs. The outer dry layers of onion bulbs (Onion skin, OS), which are not edible and removed before processing, have been shown to contain a wide spectrum of polyphenolic components (Singh *et al*., 2009). Also, it is a source of flavour components and fiber compounds and particularly rich in flavonoids including quercetin glycosides (Hertog *et al*., 1992 and Waldron, 2001). Since quercetin from onions and their skins is rapidly absorbed and slowly eliminated, it could contribute significantly to antioxidant defense (Hollman *et al*., 1997). For this reason and others, OS extracts used suceesfully in many different technological and therapeutic applications. For example, Elhassaneen et al., (2016-a) improved the bioactive compounds content and antioxidant properties in crackers with the incorporation of OS poweder. Also, OS extracts could effectively protect against CCl_4 and benzo(*a*)pyrene induced hepatotoxicity (Elhassaneen *et al.,* 2016-b).

All of the previous studies conferemd the use of plants for the treatment of common diseases such as diabetes are very common. In line with the WHO (1999) 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 these phyto-products, this present study was designed to determine the effects of three phyto-extracts and their mixture bended in beef meatballs on alloxane-induced diabetic rats. Also, their possible mechanisms of action regarding alterations in free radical defense mechanisms, for possible use in the control of hyperglycaemia characteristic of diabetes mellitus will be in the scope of this investigation.

Materials and methods

Materals

Plant parts: Pomegranate and eggplant fruits used for their peels preparation from Zagazig local markets, Zagazig City, Egypt. Red onion skin (ROS) was obtained with special arrangements from the New Beni Suef company for Preservation, dehydration and

Industrialization of Vegetables, Beni Suef Elgudida City, Nile East, Beni Suef, Egypt;

Meat samples: Rose meat samples were obtained from the Egyptian local markets, transported to the lab, cutted into small pieces using sharp knife and mincing using electrical mixer (Moulinex Egypt, Al-Araby Co., Egypt) and used for meatballs processing.

Experimental animals: Normal male albino rats (140±10g) were obtained from Research Institute of Ophthalmology, Medical Analysis Department, Giza, Egypt.

Chemicals: Alloxan, used for induction of diabetes mellitus among rats, Folin-Ciocalteu reagent, *o*-phosphoric acid, serine borate buffer (SBB), N-1-(pyrenyl) maleimide (NPM), dithiothreitol (DTT) and reduced glutathione (GSH) were obtained from Sigma Chemical Co., St. Louis, Mo. Casein, as main source of protein from Morgan Company for Chemicals. Cairo, Egypt and Vitamins and salts mixtures, all organic solvents and other chemicals were of analytical grade were purchased from El-Ghomhorya Company for Drugs, Chemicals and Medical instruments Trading, Cairo, Egypt.

Equipments: Throughout this study a SP Thermo Separation Products Liquid Chromatograph (Thermo Separation products, San Jose, CA, USA) was used with a Consta Metvic 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.

Methods

Preparation of plant parts powder and extracts

Unripe prickly pear peel were soaked in 0.1% sodium metabisulphite solution for 30 min, washed, sliced and dried at 60°C for 24 hours in hot air oven (AFOS Mini Smoker, England). This is followed by milling with grinder (Retsch Micro Universal Bench Top Grinder, Germany) to produce the respective flour types.

Potato peel and red onion skin were washed and then dried in a hot air oven (Horizontal Forced Air Drier, Proctor and Schwartz Inc., Philadelphia, PA) at 55 ^{0}C until arriving by the moisture in the final product to about 8%. The dried peels 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.

Powders of the selected plant parts were used for their different types extracts as follow: A 20 g from dried plant powder plus 180 ml methanol (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. KG, 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. KG, Germany) and the extract could be ready for the meatballs blending purpose.

Meatball manufacture
Meatballs formulation

Beef meatballs, Egyptian-style, were formulated as follow: 79.5% minced beef (~20% fat content), 15% corn powder (Freek), 5% water and 0.5% salt. A set of 5 treatment samples differing only by the plant parts methanol extract added were prepared as follow: beef meatballs formula (control samples), beef meatballs formula + 0.25 % (w/w) prickly pear peel methanol extract (PPP), beef meatballs + 0.25 % (w/w) red onion skin methanol extract (ROS), beef meatballs + 0.25 % (w/w) potato powder methanol extract (PP) and beef meatballs + 0.25 % (w/w) mixture, PPP+ ROS+ PP by equal parts. Plant parts were used at the recommended concentrations as previous mentioned by the studies of Elhassaneen *et al.,* (2016-a).

Beef meatballs processing

Beef meatballs were prepared in a pilot plant resembling to commercial processing conditions. All ingredients were homogenized in a bowl mixer with a spiral dough hook during 5 min. For each treatment, the corresponding plant part extract was added at the concentrations suggested, and then mixed again for 5 min. Meatballs

were formed by hand (20 g, balls with ~3 cm in diameter) and then subjected to a cooking process as follow: the meatballs were flash fried into corn oil at 185 ^{0}C for 0ne minute to seal the surface of the ball and produce the characteristic browned look. They were then thoroughly cooked in a forced draught oven (Zanussi, Egypt) at 250 ^{0}C during 4 min to reach an internal temperature of 75 ^{0}C in the center of the meatball. When the endpoint temperature was achieved, the samples were immediately placed in a refrigerator (4^{0}C) to reach a product temperature below 10 ^{0}C.

Chemical analysis

Determination of total phenolics and carotenoids

Total phenolics and carotenoids in selected phyto extracts were analyzed . 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 and equivalents. The total carotenoids in 80% acetone extract were determined by using the method reported by Lima *et al*., (2005).

Antioxidant activity

Antioxidant activity of the phyto extracts was determined according to the β-carotene bleaching method following a modification of the procedure described by Al-Saikhan *et al.,* (1995). For a typical assay, 1mL of β-carotene (Sigma) solution, 0.2 mg/mL in chloroform, was added to round-bottom flasks (50 mL) containing 0.02 mL of linoleic acid (J.T. Baker Chemical Co., Phillipsburg, NJ) and 0.2 mL of Tween 20 (BDH Chemical Co., Toronto, On). Each mixture was then dosed with 0.2 mL of 80% MeOH (as control) or corresponding plant extract or standard. After evaporation to dryness under vacuum at room temperature, oxygenated distilled water (50 ml) was added and the

mixture was shaken to form a liposome solution. The samples were then subjected to thermal autooxidation at 50 ^{0}C for 2 h. The absorbance of the solution at 470 nm was monitored on a spectrophotometer (beckman DU-50) 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. All samples were assayed in triplicate. A 50 g per letter of α-tocopherol in 80% methanol was used as the control. Antioxidant activity was expressed as antioxidant activity (AA) and calculated as percent inhibition relative to control using the equation [AA= $(R_{control} - R_{sample}) / R_{control} \times 100$, where $R_{control}$ and R_{sample} were the bleaching rates of beta-carotene in reactant mixture without antioxidant and with plant extract, respectively].

Biological experimental

Animals

Animals used in this study, adult male albino rats (150±10 g per each) were obtained by special arrangement from Research Institute of Ophthalmology, Medical Analysis Department, Giza, 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 and salts mixtures were formulated according to Campbell, (1963) and Hegsted, (1941), respectively.

Induction of diabetes

Diabetes was induced in 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 (Wohaieb and Godin, 1987and Kakkar *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.

Experimental design

Animals in Group (1) 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). Animals in Group (2) were given alloxan to induce diabetes. Groups (3-7) rats were classified and feeding as follow:

- Group (3): Fed on BD containing 20% beef meatballs.
- Group (4): Fed on BD containing 20% beef meatballs blending with 0.25 % (w/w) PPP methanol extract.
- Group (5): Fed on BD containing 20% beef meatballs blending with 0.25 % (w/w) ROS methanol extract.
- Group (6): Fed on BD containing 20% beef meatballs blending with 0.25 % (w/w) PP methanol extract.
- Group (7): Fed on BD containing 20% beef meatballs' blending with 0.25 % (w/w) mixture, PPP+ ROS+ PP methanol extracts by equal parts.

The treatment with phyto extracts to the animal belonging to groups (3) to (7) 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.

Blood sampling

At the end of experiment period, 8 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., plasma was with drown and used for the analysis of blood lipid parameters and vitamins. The erythrocyte residue was washed with three successive portions of sodium chloride solution (0.9 %) and then haemolysed with deionised 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 (Stroev and Makarova, 1989).

Hematological analysis

Glutathione fractions

GSH and GSSG were determined by HPLC according to the method of McFarris and Reed (1987). In brief, 100 µl of aliquot were placed in 2 ml of 10% perchloric acid containing 1 mM bathophenanthroline disulfonic acid and homogenized. The homogenate was cold centrifuged at 10000 rpm for 5 min and the internal standard (γ-glutamyl glutamate) was added to the supernatant. A 250 µl aliquot of acidic extract was mixed with 100 µl of 100 mM iodoacetic acid in 0.2 mM cresol purple solution. The acid solution was brought to pH 8.9 by the addition of 0.4 ml of KOH (2 M) – $KHCO_3$ (2.4 M) and allowed to incubate in the dark at room temperature for 1 hr to obtain S-carbooxymethyl derivatives. The N-nitrophenol derivatization of the samples were taken overnight at 4 ^{0}C in the presence of 0.2 ml of 1% 1-fluoro-2,4-dinitrobenzene and injected onto the HPLC system.

Antioxidant enzymes

GSH-Px and CAT activities were measured as described by Splittgerber and Tappel, (1979) and Aebi, 1974, respectively. SOD activity was measured by Ransod kit (Randox laboratories mmited, Germany). GSH-Rd activity was determined according to the method recommended by the International Committee for Standardization in Haematology (ICSH, 1979). Activities of SOD and GSH-Px enzymes were expressed in international unit per milliliter erythrocyte sediment and one unit of SOD was expressed as the enzyme protein amount causing 50% inhibition in 2- (4-iodophenyl)-3 (4-nitrophenol) 5-phenyltetrazolium chloride ($INTH_2$) reduction rate.

Antioxidant vitamins

All vitamins (A, C, and E) were extracted and analyzed by HPLC techniques as follow: Vitamin A was extracted by adaptation the method of **Epler *et al.*, (1993).** A 0.3 ml of serum were saponified by 0.1 ml of sodium hydroxide solution (60%) and 1- 2 ml ethyl alcohol; heated on a water bath at 85 - 90 ^{0}C under reflux for two hrs until the serum components were completely dissolved; 1 - 2 ml of ethyl alcohol and about 2 to 4 ml of distilled water were added. The unsuponified portion was extracted three times by ether using 5 ml in

the first and second extraction and 2.5 ml in the third one. The ether extraction was washed 3 to 4 times with water until the washed water became neutral, 0.6 – 0.8 grams sodium sulphate were added and the mixture was left for 90 min and filtrated. After removing the ether from the solution, the residual matter was dissolved in one ml chloroform and diluted to 2.5 ml with the same solvent, 0.1 ml of the chloroform extract was transferred to a small screw-captest tube. The chloroform solution was dried under a stream of nitrogen, re-dissolved in exactly 0.1 or 0.2 ml isopropanol -hexane (1: 99, v/v), and used for HPLC injection.

Vitamin E (α-tocopherol) was extracted by adaptation the method of Hung *et al*., (1980). Approximately 50 µl of serum were homogenized in 1.5 ml dioxane-isooctane (20 + 80, v/v) for 1 min, using a Polytron homogenizer (Beckman, Toronto). The homogenate was centrifuged at 10000 rpm for 5 min, and 0.5 ml supernate was placed in a 25 ml round-bottom flask. Remainder of the supernate was discarded. The residue was homogenized with another 1.5 ml dioxane-isooctane solution, centrifuged as before, and 0.5 ml supernate was pooled with the previous supernate and dried under vacuum in a rotary evaporator to near dryness. The residue was extracted 3 times with 0.5 ml acetonitrile and the pooled acetonitrile extracts were filtered through glass wool into a 5 ml screw-cap test tube. The filtrate then was extracted with 1.5, 1, and 0.5 ml isooctane. The isooctane extracts were pooled in a 25 ml round-bottom flask and dried under vacuum in a rotary evaporator. The residue was dissolved in 0.2 - 0.4 ml petroleum ether (bp 30 - 60 ^{0}C) and transferred to a small screw-captest tube. The petroleum ether solution was dried under a stream of nitrogen, re-dissolved in exactly 0.1 or 0.2 ml isopropanol -hexane (1: 99, v/v), and used for HPLC injection.

Vitamin C (ascorbic acid) was extracted according to the method of Moeslinger *et al*., (1994). One hundred µl of plasma were deproteinized by 400 µl ice-cold 8% perchloric acid which was described as stabilizing ascorbic acid in biological samples, centrifuged at 10000 g for 10 min at 4 ^{0}C, and neutralized by the addition of 4 M potassium hydroxide. The residues was dried under a stream of nitrogen, re-dissolved in exactly 0.1 or 0.2 ml methanol and used for HPLC injection.

The chromatographic conditions for vitamins A and E were flow rate, 1.5 ml/min; detection, UV absorption at 265 nm, volume of injection, 20 µl; temperature, room temperature; and the mobile phase composition was an isocratic system of isopropanol : hexane (1:99) while in vitamin C were flow rate, 1 ml/min; detection, UV absorption at 254 nm, volume of injection, 20 µl; temperature, room temperature, and mobile phase composition was an isocratic system of 100 % methanol. Retention times and absorbance ratio against those of standards were used to identify the separated vitamins. Quantitative determination of each vitamin was determined from its respective peak area and corresponding response factor. The percent recoveries of vitamins were also studied by adding each vitamin to serum after sample preparation and HPLC determination. Under such chromatographic conditions, the Mean ±SD values of vitamins A, C and E recoveries were 92.78 ± 4.0, 87.32 ± 1.98 and 86.21 ± 3.4, respectively.

Nitrite determination

Nitrite was determined flourometric such as described by Misko *et al.*, (1993). Ten µl of freshly prepared 2, 3-diaminonaphthalene (DAN, 0.05 mg/ml in 0.62 M HCl, protected from light) is added to100 µl of sample and mixed immediately. Nitrate standards (> 98% pure, Sigma) are routinely made fresh, dissolved in DI H_2O, and kept on ice prior to use. After 10 min incubation at 20 ^{0}C, the reaction was terminated with 5 µl of 2.8 N NaOH. The intensity of the fluorescent signal produced by the product is maximized by the addition of base. Formation of the 2,3-diaminonaphthtriazole was measured using a Schematzu fluorescence apparatus with excitation at 365 nm and emission read at 450 nm with a gain setting at 100%.

Nitrite/nitrate detection

Plasma is filtered through an ultrafree microcentrifuge 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 *Aspergillus niger*, 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 mM Tris buffer (pH, 7.6). The reaction is terminated after 5 min at 20 ^{0}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.

Thiobarpituric 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% butylated hydroxytoluene 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 discussions

Antioxidant activity, total phenolics and carotenoids of selected plant parts methanolic extracts

The antioxidant activities, total phenolics and total carotenoids of three selected plant parts methanolic extracts are shown in Table (1) From such data it could be noticed that the selected plant parts methanolic extracts showed considerable differences in antioxidant activity (AA= 70.62 to 89.78 %). Red onion skin methanolic extract (ROS) and potato peel methanolic extract (PP) showed strong activity because of its probaply high phenolics content (198.44 and 102.54 GAE. g^{-1}, respectively) while brickly pear peel methanolic extract

(PPP) showed relatively medium content in both antioxidant activity and the total phenolics (70.62 % and 35.98 mg GAE. g^{-1}, respectively). The opposite direction was recorded for the carotenoids content, the highst content was observed in PPP (19.76 mg. g^{-1} extract) while the lowest content for ROS (3.78 mg. g^{-1} extract). Such data meaning that phenolic compounds constitute the high effect in antioxidant activity of the tselected extracts and the carotenoids comes late. By other meaning, phenolic compounds probably play a major role in the antioxidant activity of the selected plant parts methanolic extracts and the rest/some roles were depended on the occurrence of other bioactive compounds beside the phenlics such caroteioids, vitamins (ascorbic acid and tocopherols), sterols, pigments and minerals (Mashal, 2016 and Sayed Ahmed, 2016). The present data are similar to that obtained by many authors (Elhassaneen *et al.,* 2016-b; Mashal, 2016 and Sayed Ahmed, 2016) who found that several food by-products/plant parts extracts including onion skin, potato peel and pricly pear peels recorded highly antioxidant activity, total phenolics and total carotenoids content as well as exhibited high antioxidant stability when comparing with the α-tocopherol as the standard antioxidant. Data of the present study with the others proved the importance of using all selected plant parts methanolic extracts as natural antioxidants/additives in different therapieutic applications.

Table 2. Antioxidant activity and total phenolics of selected phyto by-products methanolic extracts

Extract	Antioxidant activity AA (%)	Total phenolics (mg GAE. g^{-1} extract)	Total carotenoids (mg. g^{-1} extract)
Prickly pear peel methanolic extract (PPP)	70.62 ± 4.87 [c]	35.98 ± 7.98 [c]	19.76 ± 3.72 [a]
Red onion skin methanolic extract (ROS)	89.78 ± 7.15[a]	198.44 ± 20.11 [a]	3.78 ± 1.03 [c]
Potato peel methanolic extract (PP)	81.30 ± 6.21 [b]	102.54± 23.47 [b]	11.43 ± 4.10 [b]

[*]Each value represents mean ±SD. Means in the same column with different superscript letters are significantly different at $p < 0.05$.

Effect of the selected phyto extracts applied in beef meatballs on serum glucose of the diabetic rats

Data in Table (2) were shown the serum glucose concentration of alloxane-induced diabetic rats consumed the selected phyto extracts applied in beef meatballs. From such data it could be noticed that treatment of animals with aloxane caused a significant increased (p≤0.05) in serum glucose concentration by the ratio 118.09% compared to normal animals (negative control group). Supplementation of the rat diets with meatballs (20%) decreased this value which recorded 93.63%. The decreasing rate in serum glucose was elevated with the blending of the meatballs with 0.25% w/w by PPP, ROS, PP and their mixture which recorded 61.59, 41.28, 52.10 and 37.15%, respectively. So, the maximum hypoglycemic yield was recorded for the extracts mixture treatment when compared with the tested phyto by-products extracts individually. Such data probably mean that a combination of different phyto by-products extracts may be more efficient for reducing the serum glucose level due to the interactive effects occurred by different catagories of bioactive compounds of phyto by-products extracts used. Such behavior was recorded in several previous studies (Elhassaneen et al., 2016-a and Sayed Ahmed, 2006).

Table 2. The effect of phyto by-products methanolic extracts applied in beef meatballs on serum glucose concentration (mg/dL) of diabetic rats*

Value	Control (ve-) Std diet	Control (ve+) Diabetic	Control (ve+) + 20% meatballs	Meatballs + Phyto by-product methanolic extracts (0.25%, w/w)			
				PPP	ROS	PP	Mix
Mean	92.71 [f]	202.19 [a]	179.51 [b]	149.81 [c]	130.98[e]	141.01 [d]	127.15 [e]
SD	9.21	10.65	11.1	6.88	10.9	9.65	6.19
% of Change	0.00	118.09	93.63	61.59	41.28	52.10	37.15

*PPP, prickly pear peel methanolic extract; ROSME, red onion skin methanolic extract; PP, potato peel methanolic extract and Mix, mixture of PPP+ ROS+ PP by equal parts. Means in the same row with different superscript letters are significantly different at $p < 0.05$.

In line with the results of the present study, several researches have been done on the effect of prickly peer, onion and potato consumption on diabetic conditions. Therapeutic potential of prickly

peer parts has been suggested for metabolic syndrome including diabetes type 2 (Kaur et al., 2012). Such effect could be attributed to the hypoglycemic effect observed by its bioactive compounds content including phenolic acids, flavonoids, kaempferol, quercetin and isorhamnetin (Kuti, 2004; Moussa-Ayoub et al., 2011 and Jorge et al., 2013). Also, PP methanolic extract display potent hypoglycemic action in alloxane-induced diabetic rats. Such activity may be related to diverse phenolic compounds present in pomegranate and potato peel including punicalagin isomers, ellagic acid derivatives and anthocyanins (delphinidin, cyanidin and pelargonidin 3-glucosides and 3,5-diglucosides) chlorogenic, gallic, protocatechuic and caffeic acids (Onyeneho and Hettiarachchy, 1993 and Rodriguez *et al.*, 1994). These compounds are known for their properties in scavenging free radicals, inhibiting lipid oxidation *in vitro* and improve glucose response and insulin resistance associated with type 2 diabetes (Noda *et al.,* 2002 and Jung *et al.,* 2011 and Elhassaneen *et al.,* 2015). Furthermore, the organosulfur compounds *S*-methylcysteine sulfoxide and *S*-allylcysteine sulfoxide in onion skin were linked to significant amelioration of weight loss, hyperglycemia, low liver protein and glycogen, and other characteristics of diabetes mellitus in rats. They found that the use of such compounds in a concentration 200 $mg.kg^{-1}.day^{-1}$ gave results comparable to treatment with insulin or glibenclamide but without the negative side effect of cholesterol synthesis stimulation. Also, Jung *et al.,* (2011) reported that onion peel extract might improve glucose response and insulin resistance associated with type- 2 diabetes 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.

Effect of the selected phyto extracts applied in beef meatballs on oxidative stress status of diabetic rats

Oxidants concentration (i.e. oxidative stress) in diabetic rats plasma feeding some selected phyto extracts applied in beef meatballs was assessed by measuring thiobarbituric acid reactive substances (TBARS) and nitric oxides (nitrite, NO_2 and nitrate, NO_3) (Table 3). From such data it could be noticed that diabetes induced a significant increased ($p \leq 0.05$) in TBARS, NO_2 and NO2/NO3concentration in

plasma by 47.29, 31.02 and 36.91% compared to normal controls, respectively. Supplementation of the rat diets with meatballs (20%) decreased these values which recorded 31.34, 22.91 and 22.44%, respectively. The decreasing rate in plasma oxidant concentration were elevated with the blending of the meatballs with 0.25% w/w by PPP, ROS, PP and their mixture which recorded 19.09, 17.61 and 19.95%; 14.25, 14.67 and 13.47%; 17.09, 16.73 and 15.71; and 11.40, 9.56 and 11.97% for TBARS, NO_2 and NO_2 + NO_3, respectively. The highst suppression was recorded with the mixture treatment. It could be mean that a combination of different phyto extracts may be more efficient for reducing liver tissue TABRS and nitrous oxide levels, the biomarkers of oxidative stress and inflammation in liver, because the interactive

Table 3. The effect of phyto by-products methanolic extracts applied in beef meatballs on plasma oxidants concentration of diabetic rats*

Value	Control (ve-) Std diet	Control (ve+) Diabetic	Control (ve+) + 20% meatballs	Meatballs + Phyto by-product methanolic extracts (0.25%, w/w)			
				PPP	ROS	PP	Mix
Thiobarbituric acid reactive substances (TBARS, nmol/mL)							
Mean	3.51 [c]	5.17 [a]	4.61 [a]	4.18 [a]	4.01 [ab]	4.11 [ab]	3.91 [b]
SD	0.21	0.19	0.37	0.65	0.33	0.32	0.11
% of Change	0.00	47.29	31.34	19.09	14.25	17.09	11.40
Nitrite (NO_2, nmol/L)							
Mean	2.71 [c]	3.56 [a]	3.34 [a]	3.19 [a]	3.11 [ab]	3.17 [ab]	2.97 [c]
SD	0.31	1.11	0.50	0.49	0.55	0.39	0.25
% of Change	0.00	31.02	22.91	17.61	14.67	16.73	9.56
Nitrite/Nitrate (NO_2/NO_3, nmol/L)							
Mean	4.01 [c]	5.49 [a]	4.91 [ab]	4.81 [ab]	4.55 [ab]	4.64 [ab]	4.49 [ab]
SD	1.11	1.06	0.99	1.14	1.06	1.20	0.77
% of Change	0.00	36.91	22.44	19.95	13.47	15.71	11.97

*PPP, prickly pear peel methanolic extract; ROSME, red onion skin methanolic extract; PP, potato peel methanolic extract and Mix, mixture of PPP+ ROS+ PP by equal parts. Means in the same row with different superscript letters are significantly different at $p < 0.05$.

effects occurred by different catagories of bioactive compounds of extracts applied.

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 (Sayed Ahmed, 2016). 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 *et al.,* 2014-a). 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 (OPE) administration. Also, hepatic oxidant stress was reduced by 1% OPE, as assessed by increasing superoxide dismutase activity and blocking TBARS formation. Moreover, hepatic expressions of TNF-a and IL-6 were suppressed by either 1% OPE or quercetin. Furtehrmore, Coskun *et al.,* (2005) reported that quercetin, domainant flavonoid in the selected phyto by-products extracts, had anti-oxidative and anti-inflammatory activities.

On the other side, systemic metabolic alterations associated with diabetes contribute to the increase in oxidative stress have been reported by many authors. For example, hyperglycemia as a hallmark of type II diabetes, a metabolic complication of obesity, induces oxidative stress through activation of the polyol and hexosamine pathways, production of advanced glycation end-products (AGE), and increase of diacylglycerols (DAG) synthesis (DCCTRG, 1993 and Le Lay *et al.,* 2014). Excess of circulating lipids induces ROS formation pathways, which contribute to the increase in lipid oxidation and protein carbonylation (Jensen *et al.,* 1989). Also, Elhassaneen *et al.,* (2014-b) reviewed that RNS (NO_2 and NO_3) been shown to be increased in plasma derived from alloxan- induced diabetes in rats.

Several decades ago, interest in the possible significance of TBARS on human health has been stimulated by reports that are mutagenic and carcinogenic compound (Shamberger *et al.*, 1974). 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; 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 (Jacob *et al*., 1992).

The positive effects of plant parts 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.,* (2005) found that quercetin, domainant flavonoid such as found in our selected plant parts, have anti-oxidative and anti-inflammatory activities. Such dietary phenolics found in tested plant by-products 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 when compared with the tested plant by-products plant separated. It could be mean that a combination of different phyto extracts may be more efficient for reducing plasma TBARS level, the biomarkers of oxidative stress and inflammation in the body, because the interactive effects occurred by different categories of bioactive compounds of different plant parts by-products used.

Effect of the selected phyto extracts applied in beef meatballs on antioxidative defense systems of diabetic rats

Glutathione fractions concentration in plasma

Biological antioxidant macromolecules i.e. glutathione fractions concentration in plasma of diabetic rats consumed phyto extracts applied in beef meatballs were assessed (Table 4). From such data it could be noticed that diabetes induced a significant deccreased ($p \leq 0.05$) in GSH and GSSG concentrations in plasma by -29.64 and -20.00% compared to normal controls, respectively. Supplementation of the rat diets with meatballs (20%) increased these values which recorded -

24.41 and -14.44% . The increasing rate in plasma GSH fractions concentration were elevated with the blending of the meatballs with 0.25% w/w by PPP, ROS, PP and their mixture which recorded -19.96 and -13.33; -14.13 and -11.11; -17.89 and -12.22; and -10.87 and -10.00% for GSH and GSSG, respectively. The highst suppression was recorded with the mixture treatment. The same behavior was observed for the GSH/GSSG ratio. It could be mean that a combination of different phyto extracts may be more efficient for elevating plasma GSH and GSSG levels, the biomarkers of enhancing the antioxidative status plasma, because the interactive effects occurred by different catagories of bioactive compounds of extracts applied.

Table 4. The effect of phyto by-products methanolic extracts applied in beef meatballs on plasma glutathione fractions concentration of diabetic rats*

Value	Control (ve-) Std diet	Control (ve+) Diabetic	Control (ve+) 20% meatballs	Meatballs + Phyto by-product methanolic extracts (0.25%, w/w)			
				PPP	ROS	PP	Mix
Reduced glutathione concentration (GSH, µmol /L)							
Mean	10.12 [a]	7.12 [c]	7.65 [c]	8.10 [ab]	8.69 [ab]	8.31 [ab]	9.02 [ab]
SD	0.98	0.97	2.60	1.39	0.85	1.40	1.02
% of Change	0.00	-29.64	-24.41	-19.96	-14.13	-17.89	-10.87
Oxidized glutathione concentration (GSSG, µmol /L)							
Mean	0.9 [a]	0.72 [b]	0.77 [ab]	0.78 [ab]	0.8 [ab]	0.79 [ab]	0.81 [ab]
SD	0.19	0.09	0.12	0.1	0.14	0.11	0.17
% of Change	0.00	-20.00	-14.44	-13.33	-11.11	-12.22	-10.00
GSH/GSSG ratio							
Mean	11.24 [a]	9.89 [ab]	9.94 [ab]	10.38 [a]	10.86 [a]	10.52 [a]	11.14 [a]
SD	1.22	1.11	1.09	1.21	0.98	1.1	1.43
% of Change	0.00	-12.06	-11.64	-7.65	-3.40	-6.45	-0.97

*PPP, prickly pear peel methanolic extract; ROSME, red onion skin methanolic extract; PP, potato peel methanolic extract and Mix, mixture of PPP+ ROS+ PP by equal parts. Means in the same row with different superscript letters are significantly different at $p < 0.05$.

In general, GSH is a tripeptide-thiol (γ-glutamyl cysteinyl-glycine) that has received considerable attention in terms of its biosynthesis, regulation, and various intracellular functions (Reed and Beatty, 1980; Larsson *et al*., 1983). Among these function are two constructing roles in detoxifications: (1) as a key conjugate of electrophilic intermediates, principally via glutathione-*s*-transferase activities in phase II metabolism, and (2) as an important antioxidant. 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). A marked decreased level of GSH is reported in the plasma of diabetic patients (Ceriello, 2000; Seghrouchni*et al.,* 2002 and Moussa, 2008). 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.

Decreasing in GSH fractions observed in diabetes rats group generally accompanied by a concomitant decreased in the ratio of GSH/GSSG. Di Giulio (1991) mentioned that a more fundamental effect of oxyradical-generating compounds as the diabets 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. Elhassaneen *et al*., (2004) mentioned that increased fluxes of oxyradicals 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. In this context, 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.

The selected phyto extracts in the present study and their mixtures feeding are rich in bioactive compounds which exhibited antioxidant effects against ROS formation as the diabets development through several mechanism of action including: 1)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.

Antioxidant enzymes activities in red blood cells (RBCs)

Antioxidant defense system in RBCs in diabetic rats feeding some selected phyto extracts applied in beef meatballs was assessed by measuring antioxidant enzymes activities including glutathione peroxidase (GSH-Px), glutathione reductase (GSH-Rd), catalase (CAT) and superoxide dismutase (SOD) (Table 5). From such data it could be noticed that diabetes induced a significant deccreased ($p \leq 0.05$) in GSH-Px, GSH-Rd, CAT and SOD activities in RBC's by -34.17, -27.24, -23.87 and -30.65 compared to normal controls, respectively. Supplementation of the rat diets with meatballs (20%) increased these values which recorded -28.01, -22.72, -19.13 and -21.86%, respectively. The increasing rate in RBC's antioxidant enxymes activity were elevated with the blending of the meatballs with 0.25% w/w by PPP, ROS, PP and their mixture which recorded -20.71, -21.01, -14.79 and -18.84; -16.73, -16.23, -11.47 and -16.83; -19.95, -17.34, -12.77 and -17.84; and -12.48, -8.88, -9.30 and -13.07%, respectively. The highst activation was recorded with the mixture treatment. It could be mean that a combination of different phyto extracts may be more efficient for elevating the RBC's activities, the biomarkers of enhancing the antioxidative status RTBC's, because the interactive effects occurred by different catagories of bioactive compounds of extracts applied.

Generally, to prevent free radical damages (oxidative stress activities), the organism has developed antioxidant defenses largely based on antioxidant enzymes able to scavenge ROS. SODs 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 reduces the

Table 5. The effect of phyto by-products methanolic extracts applied in beef meatballs on erythrocytes antioxidant enzymes activities of diabetic rats*

Value	Control (ve-) Std diet	Control (ve+) Diabetic	Control (ve+) 20% meatballs	Meatballs + Phyto by-product methanolic extracts (0.25%, w/w)			
				PPP	ROS	PP	Mix
Glutathione peroxidase (GSH-Px, U/g Hb)							
Mean	18.35 [a]	12.08 [b]	13.21 [b]	14.55 [ab]	15.28 [ab]	14.69 [ab]	16.06 [a]
SD	2.07	2.11	3.55	0.99	2.11	0.79	2.39
% of Change	0.00	-34.17	-28.01	-20.71	-16.73	-19.95	-12.48
Glutathione reductase (GSH-Rd, U/g Hb)							
Mean	11.71 [a]	8.52 [c]	9.05 [ab]	9.25 [ab]	9.81 [ab]	9.68 [ab]	10.67 [a]
SD	0.94	1.18	1.62	0.69	0.77	1.14	0.85
% of Change	0.00	-27.24	-22.72	-21.01	-16.23	-17.34	-8.88
Catalase (CAT, U/g Hb)							
Mean	161.44 [a]	122.90 [d]	130.55 [bc]	137.57 [bc]	142.92 [b]	140.82 [b]	146.42 [b]
SD	21.14	10.87	5.67	14.87	11.79	21.76	6.98
% of Change	0.00	-23.87	-19.13	-14.79	-11.47	-12.77	-9.30
Superoxide dismutase (SOD, U/g Hb)							
Mean	3.98 [a]	2.76 [c]	3.11 [b]	3.23 [b]	3.31 [b]	3.27 [b]	3.46 [a]
SD	0.44	0.32	0.91	0.51	0.48	1.11	0.75
% of Change	0.00	-30.65	-21.86	-18.84	-16.83	-17.84	-13.07

*PPP, prickly pear peel methanolic extract; ROSME, red onion skin methanolic extract; PP, potato peel methanolic extract and Mix, mixture of PPP+ ROS+ PP by equal parts. Means in the same row with different superscript letters are significantly different at $p < 0.05$.

selenium and the reduced form of the enzyme then react with hydrogen peroxide. The ratio of nine *GSH lGSSG* in normal cells are kept high. So there must be a mechanism of reducing GSSG back to GSH. This is achieved by GSH-Rd enzyme which catalyze the reaction:GSSG + NADPH + $H^+ \rightarrow$ 2GSH + $NADP^+$. GSH-Rd can also catalyse reduction of certain mixed disulphides such as that between GSH and Co-enzyme A (Thomas *et al.*, 1990).

Mammalian RBC's operate the pentose phosphate pathway in order to provide NADPH for GSH reduction (Harmon, 1986). Many studies such Galinier *et al.,* (2004) and Cao, (2014) reported that antioxidant enzymes systems are active in liver cells. Decreasing the activity of the antioxidant enzyme, glutathione-*S*-transferase in cell cultures or its invalidation in mice both results in increased ROS production and mitochondrial dysfunction (Curtis *et al.,* 2010). The phyto extracts elected in the present study and their mixtures feeding are rich in bioactive compounds such phenolics, organosulphur compounds, carotenoids , kaempferol, quercetin etc which exhibited antioxidant activities in different biological systems (Elhassaneen *et al.,* 2016-a and and Mashal, 2016). Such antioxidant properties are important in manipulation of the diabetes development through ROS scavenging processes in RBC's.

Antioxidant vitamins concentration in plasma

Antioxidant vitamins concentration in plasma of diabetic rats consumed phyto extracts applied in beef meatballs were shown in Table (6). From such data it could be noticed that biabetes induced a significant decreased ($p \leq 0.05$) in vitamins A, C and E in plasma by -37.30, -31.16 and -26.73% compared to normal controls, respectively. Supplementation of the rat diets with meatballs (20%) increased these values which recorded -33.33, -29.64 and -29.64%, respectively. The increasing rate in plasma antioxidant vitamins level were elevated with the blending of the meatballs with 0.25% w/w by PPP, ROS, PP and their mixture which recorded -21.43, -23.30 and -12.98; -14.29, -20.34 and -9.17; -19.84; -22.74 and -11.17; and -12.70, -19.15 and -8.36, respectively. The highst level was recorded with the mixture treatment. It could be mean that a combination of different phyto extracts may be more efficient for elevating the plasma vitamins levels, the biomarkers of enhancing the antioxidative status plasma, because the interactive effects occurred by different catagories of bioactive compounds of extracts applied.

The data of the present study indicated that the reducing in antioxidant enzymes defense potential of RBC's was contrary with significant decreasing ($p > 0.05$) in antioxidant vitamins in rats plasma as a consequence of diabetes injury. Beside the RBC's, plasma is rich

Table 6. The effect of phyto by-products methanolic extracts applied in beef meatballs on Plasma antioxidants vitamins concentration of diabetic rats*

Value	Control (ve-) Std diet	Control (ve+) Diabetic	Control (ve+) 20% meatballs	Meatballs + Phyto by-product methanolic extracts (0.1%, w/w)			
				PPP	ROS	PP	Mix
Vitamin A (Retinol, µmol/L)							
Mean	1.26 [a]	0.79 [b]	0.84 [b]	0.99 [ab]	1.08 [ab]	1.01 [ab]	1.10 [ab]
SD	0.25	0.38	0.15	0.32	0.19	0.36	0.22
% of Change	0.00	-37.30	-33.33	-21.43	-14.29	-19.84	-12.70
Vitamin C (Ascorbic acid, µmol/L)							
Mean	59.54 [a]	40.99 [c]	41.89 [c]	45.67 [ab]	47.43 [a]	46.0 [ab]	48.14 [a]
SD	3.52	1.99	1.87	3.43	4.55	3.98	4.87
% of Change	0.00	-31.16	-29.64	-23.30	-20.34	-22.74	-19.15
Vitamin E (Tocopherol, µmol/L)							
Mean	29.89 [a]	21.9 [c]	22.87 [c]	26.01 [ab]	27.15 [ab]	26.55 [ab]	27.39 [ab]
SD	3.11	2.32	7.21	5.45	3.54	2.15	3.62
% of Change	0.00	-26.73	-23.49	-12.98	-9.17	-11.17	-8.36

*PPP, prickly pear peel methanolic extract; ROSME, red onion skin methanolic extract; PP, potato peel methanolic extract and Mix, mixture of PPP+ ROS+ PP by equal parts. Means in the same row with different superscript letters are significantly different at $p < 0.05$.

in natural antioxidants compounds, as liposoluble vitamins (e.g., vitamins A and E) or β-carotenoids (Wohaieb and Godin, 1987). Our previous studies indicated that food processing by-products selected in the present study and their mixtures feeding are rich in bioactive compounds including caroteoids and vitamins which exhibited antioxidant activities in different biological systems (Elhassaneen *et al.,* 2016-a,b,c). Such antioxidant properties are important in manipulation of the diabetes development through ROS scavenging processes subsequently excess the bioavailability of the vitamins in plasm cells.

Correlation studies

In the correlation analysis, important differences were found between oxidative stress and antioxidant defense system in diabetic rats

feeding some selected phyto extracts (PPP, ROS and PP) applied in beef meatballs (Table 7). 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 = -0.951), antioxidant vitamins in plasma [vitamin A (r^2 = -0.793) and vitamin E (r^2 = -0.892)], antioxidant enzymes in RBC's [GSH-Px (r^2 = -0.925), CAT (r^2 = -0.857) and SOD (r^2 = -0.826)] and TBARS concentration in plasma. While, moderate negative significant ($p \leq 0.05$) relationship between water soluble antioxidant vitamins in plasma [vitamin C (r^2 = -0.601) and TBARS concentration in plasma. On the same time, there was a strong negative significant ($p \leq 0.05$) relationship between GSH concentration in plasma (r^2 = -0.881), antioxidant vitamins in plasma [vitamin A (r^2 = -0.819) and vitamin E (r^2 = -0. 849)], antioxidant enzymes in RBC's [GSH-Px (r^2 = -0. 886), CAT (r^2 = -0.872) and SOD (r^2 = -0.881)] and NO_2 concentration in plasma. While, moderate negative significant ($p \leq 0.05$) relationship between water soluble antioxidant vitamins in plasma [vitamin C (r^2 = -0.623) and NO_2 concentration in plasma. These correlations confirm that if there were no change in the antioxidant defense system of diabetes rats, it would be difficult to observe high concentrations of TBARS and NO_2. In this direction, Bohm *et al*., (1997) in some model systems, a combination of α-tocopherol and β-carotene interact synergistically to inhibit lipid peroxidation subsequently increased TBARS. Also, Shalaby, (2014) reported that high levels of MDA in the plasma of diabetic rats were associated with rather low levels of antioxidant vitamins and enzymes.

Table 7. Correlation between oxidative stress and antioxidant defense systems in diabets rats feeding some selected phyto extracts applied in beef meatballs*

Parameters	**R^{2*}**	**Parameters**	**R^2**
TBARS/GSH	- 0.951	NO_2/GSH	- 0.881
TBARS/ Vit A	- 0.793	NO_2/ Vit A	- 0.819
TBARS/Vit C	- 0.601	NO_2/Vit C	- 0.623
TBARS/Vit E	- 0.892	NO_2/Vit E	- 0.849
TBARS/GSH-Px	- 0.925	NO_2/GSH-Px	- 0.886
TBARS/CAT	- 0.857	NO_2/CAT	- 0.872
TBARS/SOD	- 0.826	NO_2/SOD	- 0.881

* $P \leq 0.05$

In conclusion, the present study has demonstrated the potency of the selected phyto extracts including PPP, ROS, PP and their mixture to partially ameliorate hyperglycemia and its complications in diabetic rats. The complications include improved the antioxidant enzymatic and nonenzymatic defense systems and suppressed the oxidative stress status in both serum and RBC's. All of these effects could be attributed to the high antioxidant activities as the result of high levels of many bioactive compounds found in the all tested phyto extracts. These findings provide a basis for the use of phyto extracts for the prevention and/or treatment of type-2 Diabetes mellitus.

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إضافة المستخلصات النباتية الى كرات اللحم البقرى لمعالجة التغيرات فى ميكانيكيات دفاع الشقوق الحرة فى مرض السكرى المستحث بالألوكسان فى الفئران

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يعد مرض السكري من النوع الثانى (T2DM) واحدا من الأمراض المزمنة الأكثر شيوعا في العالم لأن أنماط الحياة المتغيرة تؤدي إلى انخفاض النشاط البدني وزيادة معدلات الإصابة بالسمنة التى تعد جميعها من أسباب الاصابة بهذا المرض. وقد تم اقتراح العديد من الاستراتيجيات لتحسين مضاعفات السكري، لأن العلاج المبكر والوقاية تلعب دورا محوريا في الحد من عبء السكان من مرض السكري. لذلك أجريت الدراسة الحالية الى إستبيان فعالية ثلاثة مستخلصات ميثانولية نباتية قادمة من قشور التين الشوكى، قشورالبصل الأحمر ، وقشور البطاطس والتى تم خلطها فى كرات اللحم البقري لمعالجة التغيرات فى ميكانيكيات دفاع الشقوق الحرة فى مرض السكرى المستحث بالألوكسان فى الفئران. ولقد أظهرت المستخلصات النباتية المختارة درجات عالية من النشاط المضاد للأكسدة تراوحت بين 70,62 – 89,78% وكذلك مستويات عالية من المركبات النشطة بيولوجيا ومنها الفينولات والكاروتينات . ولقد أوضحت النتائج أن معاملة الفئران بالألوكسان قد تسبب فى زيادة معنوية ($p≤0.05$) فى جلوكوز الدم وذلك بنسبة 118,09% مقارنة بالمجموعة الضابطة الطبيعية. كما أدى تدعيم الوجبات الخاصة بالفئران بكرات اللحم بنسبة 20% الى حدوث إنخفاض فى تلك النسبة من سكر الدم لتسجل 93,63%. وعند خلط كرات اللحم بالمستحضرات الميثانولية لقشور التين الشوكى والبصل الأحمر والبطاطس 0,25% (وزن/وزن) ومخلوط منها بنسب متساوية قد أحدث إنخاضا معنويا ($p≤0.05$) فى نسبة سكر الدم لتسجل معدل إنخفاض 61,59، 41,28، 52,10، 37,15% للمستخلصات السابقة على الوالى. كما تم تسجيل نفس السلوك للمواد الفعالة لحمض الثيوباربيتوريك وأكاسيد النيتروجين الفعالة (أكسيد النتريت والنترات) فى بلازما الدم والتى تعد من المؤشرات الحيوية للإجهاد التأكسدي. وفي الوقت نفسه، تم تسجيل تحسن في أنظمة الدفاع الإنزيمية والغير إنزيمية المضادة للأكسدة في كل من مصل الدم وكرات الدم الحمراء. وفي النهاية، فإن المستخلصات النباتية المختبرة قد حسنت فى التغيرات المتعلقة بميكانيكيات دفاع الشقوق الحرة فى مرض السكرى وذلك خلال الأنشطة العالية المضادة للأكسدة نتيجة للمحتوى العالى للعديد من المركبات النشطة بيولوجيا الموجودة في هذه المستخلصات .أيضا، تشكل نتائج الدراسة الحالية أساسا علميا لاستخدام المستخلصات النباتية فى الوقاية أو علاج مرض السكرى من النوع الثانى.

الكلمات المفتاحية: قشر التين الشوكى، قشر البصل الأحمر، قشر البطاطس، النشاط المضاد للأكسدة، الإجهاد التأكسدى، الإنزيمات المضادة للأسدة، الفيتامينات المضادة للأكسدة، كرات الدم الحمراء.