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Onion, Orange and Prickly Pear Peel Extracts Mixed with Beef Meatballs Ameliorate the Effect of Alloxan Induced Diabetic Rats

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Abstract: The present study was carried out to investigate the effectiveness of three plant parts methanol extracts (orange peel extract, OPE, red onion skin powder, ROSE, and prickly pear extract, PPE) mixed with beef meatballs in modulating hyperglycemia using alloxan induced diabetic rats model. Treatment of animals with alloxan caused a significant increase ($p \leq 0.05$) in serum glucose concentration (49.47%) compared to normal control group. Compared to the diabetic control, hypoglycemic capability of beef meatballs supplemented with 0.1% plant parts methanolic extracts was demonstrated by significant ($p \leq 0.05$) decreasing of glucose concentration in serum. The rate of decreasing was recorded 29.91, 39.99, 31.15 and 41.75% for meatballs supplemented with OPE, ROSE, PPPE and their mixture, respectively. The same behavior was recorded for liver tissue malonaldialdehyde (MDA) level, the biomarker of oxidative stress in liver. Activities of AST, ALT and ALP were increased significantly ($P<0.05$) in alloxan-induced diabetic rats in comparison with normal control group. Treatment with meatballs mixed with selected methanolic extracts to diabetic rats significantly ($P < 0.05$) decreased the elevated AST, ALT and ALP almost near to normal levels. In conclusion, OPE, ROSE and PPPE have ameliorated the effect of alloxan induced diabetes in rats. So, we advise to use these plant parts extracts in human nutrition as natural food additives for their anti-hyperglycemic effects.

Keywords: Plant Parts, Liver Functions, Aminotransferases, Glucose, Malonaldialdehyde, Glutathione

1. Introduction

Diabetes is defined as a state in which homeostasis of carbohydrate and lipid metabolism is improperly regulated by insulin. This results primarily in elevated fasting and postprandial blood glucose levels. If this imbalanced homeostasis does not return to normalcy and continues for a protracted period of time, it leads to hyperglycemia that in due course turns into a syndrome called diabetes mellitus (DM) [1]. DM is widely distributed all over the world. There were 171 million people in the world with diabetes in the year 2000 and this is projected to increase to 366 million by 2030 [2]. Therefore, the human population worldwide appears to be in the midst of an epidemic of diabetes. Reports from the World Health Organization (WHO) indicate that diabetes mellitus is one of the major killers of our time, with people in Southeast Asia and Western Pacific being most at risk.

There are two main categories of this disease. Type 1 (T1DM) diabetes mellitus also called insulin-dependent diabetes mellitus (IDDM) and Type 2 (T2DM), the non-insulin dependent diabetes mellitus (NIDDM).T2DM is one of the world's most common chronic diseases as changing lifestyles lead to reduced physical activity and increased obesity [2]. Early phenomenon of T2DM is insulin insensitivity, which not only has negative metabolic consequences but also contributes subsequent pancreas ß-cell exhaustion, resulting in the onset of clinical hyperglycemia [3]. Hyperglycemia is associated with reduced life expectancy,

significant morbidity due to specific diabetes related microvascular complications (retinopathy, nephropathy and neuropathy), increased risk of macrovascular complications (ischaemic heart disease, stroke and peripheral vascular disease), and diminished quality of life [4-5]

A number of ways to improve diabetic complications have been proposed, because early treatment and prevention play a pivotal role in reducing the population burden of diabetes. Lifestyle changes such as losing weight, exercising, and watching the diet are often recommended. Benefits of pharmaceutical factors to treat the disease aggressively early have been recommended, but medications may have unwanted side effects. Thus, there has been a growing interest in herbal remedies that can be but have been difficult to maintain over a long term introduced into the general population with the least side effects and the maximal preventive outcome [6]. In this context, many phytochemicals naturally occurring in plant foods would be desirable options. Amongst all of these bioactive compounds flavonoids, phenolic compounds, organosulfur compounds and anthocyanins are represent the central position. Such compounds have been reported to improve diabetic status by decreasing oxidative stress [7-9] or by reducing the disturbance of hepatic gene expressions [10-11].

Extensively studied sources of such natural compounds are fruits, vegetables, seeds, cereals, berries, wine, tea, onion bulbs, olive oil, spices and herbs. Attempts are also extended 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. In this context, we will focus in this study on some food processing wastes/by-products most commonly produced in Egypt which including onion skin, orange peel and prickly peer peel.

Skins/peels are the major waste of onion (*Allium cepa* L., family *Alliaceae*) dehydration processing. They are a source of flavour components and fiber as well as rich in phenolics particularly quercetin glycosides [12-13]. The major flavonoids of mature onion bulbs and probably their peeling wastes are quercetin 3,4-*O*-diglucoside and quercetin 40-*O*-monoglucoside, accounting for more than 85% of the total flavonoids [14]. Since quercetin from onions is rapidly absorbed and slowly eliminated, it could contribute significantly to antioxidant defense [15]. Few studies presented antidiabetic effects of onion skin extract *in vivo* [16-17]. Also, Jung *et al.,* [9] reported that quercetin derivatives in onion peel extracts have been regarded as the most important flavonoids to improve diabetic status in cells and animal models.

Prickly pear, commonly known as prickly pear, belongs to the family *Cactaceae*. The prickly cactus pear is widely distributed in Latin America, South Africa and the Mediterranean area including Egypt, [18]. Many studies indicated that the fruits pulp and peel of prickly pears contained phenolics and other antioxidants such as biothiols and concluded that they had a positive effect in the Redox balance of humans mainly due to reduced LDL hydroperoxides levels [19 20]. So, different plant prickly pear parts have been traditionally used in folk medicine to treat diabetes, hypertension, asthma, burns, edema, and indigestion [21-22].

Due to the large amounts of citrus (*Citrus Sinensis* L.) being processed into juice, a considerable by-product industry has evolved to utilize the residual peels, membranes, seeds, and other compounds. Among of those residues, citrus peel are a rich source of many bioactive compounds including fiber-pectins and flavonoids mainly hesperidin and eriocitrin. Flavonoids are found to possess high antioxidant activity and demonstrated many health protecting effects including diabetes [23].

Although there are a few studies presented antidiabetic effects of such wastes/by-products extracts *in vivo*, more evidence to support such roles is still needed. Therefore, the present study was performed to evaluate the potential effectiveness of onion, orange and prickly pear peel extracts mixed with beef meatballs in ameliorating hyperglycemia by using alloxan induced diabetic rat model.

2. Materials and Methods

2.1. Materials

2.1.1. Plant Parts

Red onion skin was obtained from the New Beni Suef company for Preservation, dehydration and Industrialization of Vegetables, Beni Suef Elgudida City, Nile East, Beni Suef, Egypt; Prickly pear (*Opuntia ficus-indica*) and orange "*Citrus sinensis* L." fruits samples were obtained from a local supermarket, Cairo, Egypt.

2.1.2. Meat Samples

Rose meat samples were obtained from the Egyptian local markets, transported to the lab, cut into small pieces using sharp knife and minced using electrical mixer (Toshiba ElAraby, Benha, Egypt) and used for meatballs processing.

2.1.3. Chemicals

Alloxan, used for induction of diabetes mellitus among rats, was obtained from Sigma Chemical Co., St. Loues, CA; Casein, as main source of protein from Morgan Company for Chemicals. Cairo, Egypt and Vitamin and salt mixtures, from El- Gomhoriya Company for Drugs, Chemical and Medical Instruments, Cairo, Egypt. All solvents, buffers and specific kits were high analytical grade and purchased from El-Gomhoriya Company for Drugs, Chemical and Medical Instruments, Cairo, Egypt.

2.2. Methods

2.2.1. Preparation of the Plant Parts Extracts

After arriving of the selected plant parts samples, they were

prepared for drying process by manual sorting, washing, chopped separately into small pieces and drying in under vacuum oven (*Across International,* Livingston, NJ) at 55°C until arriving by the moisture in the final product to about 8%. The dried selected plant parts were homogenized in electric blender (Toshiba ElAraby, Benha, Egypt), sieved (60 mesh.inch^{-1}), packed in polyethylene bags and kept in -20°C. Powders of the selected plant parts were used for their different types extracts according to the method of Amin et al., [24] with some modifications. In aqueous extraction, 20 g from dried plant +180 ml deionized water 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 aqueous extract was removed under reduced pressure at 55°C using a rotary evaporator (Laborata 4000; Heidolph Instruments GmbH & Co. KG, Germany). In organic solvents extraction, the same previous extraction procedure was carried out by using different organic solvent separately including 80% (v/v) methanol, 80% (v/v) ethanol and 100% hexane as an extraction medium. The yield of the extracts were weighted and evaluated to use the high.

2.2.2. Meatball Manufacture

(i). Meatballs formulation

Egyptian-style meatballs were formulated as follow: 80% minced beef (~20% fat content), 14.5% potato powder, 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 + 0.1% (w/w) orange peel extract (OPE), beef meatballs + 0.1% (w/w) red onion skin extract (ROSE), beef meatballs + 0.1% (w/w)prickly pear peel extract (PPPE) and beef meatballs + 0.1% (w/w) mixture, OPE+ ROSE+ PPPE by equal parts. Plant parts were used at the concentrations suggested by previous studies [20, 25-26].

(ii). 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 (Moulinex Egypt, ElAraby Co., Egypt) 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 (15 g, 2-3 cm in diameter) and then subjected to a two stage cooking process. First, the meatballs were flash fried into sunflower oil at 190°C for 30 seconds to seal the surface of the ball and produce the characteristic browned look. They were then thoroughly cooked in a forced draught oven (Zanussi, Italy) at 250°C during 4 min to reach an internal temperature of 72°C in the center of the meatball. The temperature was monitored using an Omega digital thermometer (Omega Engineering, Inc., Stamford, CT) with a chromel–alumel (Omega K) thermocouple probe positioned in the geometric center of the product samples. When the endpoint temperature was achieved, the samples were immediately placed in a chiller (4°C) to reach a product temperature below 12°C. Three replications of this experiment were made.

2.2.3. Biological Experiments

(i). Animals

Animals used in this study, adult male albino rats (130-180 g per each) were obtained from Helwan Station, Ministry of Health and Population, Helwan, Cairo, Egypt.

(ii). Basal Diet

The basic diet prepared according to the following formula as mentioned by AIN [27] 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, [28] while the salt mixture used was formulated according to Hegsted, [29].

(iii). 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, [30]. Rats (n=42 rats), were housed individually in wire cages in a room maintained at 25 ± 2°C and kept under normal healthy conditions. 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 (36 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 20% meatballs without plant parts extracts.
- Group (4): Fed on standard diet containing 20% beef meatballs formula with 0.1% (w/w) OPE.
- Group (5): Fed on standard diet containing 20% meatballs with 0.1% (w/w) ROSE.
- Group (6): Fed on standard diet containing 20% meatballs with 0.1% (w/w) PPPE.
- Group (7): Fed on standard diet containing 20% meatballs with 0.1% (w/w) mixture, OPE+ ROSE+ PPPE by equal parts.

(iv). 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 [31]. Immediately after injection animals were received 5% glucose solution over night to overcome drug induced hyperglycemia [32-33]. After five days blood glucose was analyzed by a drop of blood obtained from tail vein and subjected to a strip of haemogluco test. All rats with fasting blood sugar > 126 mg.dl^{-1} were considered to be diabetics and included in the experiment.

(v). 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 anesthesia. 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 Stroev and Makarova [34]. Serum was carefully aspirate, transferred into clean covet tubes and stored frozen at -20°C until analysis.

2.2.4. Hematological Analysis

(i). Liver functions

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phsphatase (ALP) were determined according to Yound, [35], Tietz, [36], Moss [37], respectively.

(ii). Serum glucose

Enzymatic determination of serum glucose was carried out colorimetrically according to Dohnal *et al.,* [38].

(iii). Reduced glutathione (GSH)

GSH was measured by the spectrophotometric recycling method of Tietze [39] in the presence of 5,5'-ditiobis (2-nitrobenzoic acid) (DTNB), NADPH and glutathione reductase (GR) in a DU70 Beckman spectrophotometer (CA).

(iv). Malondialdehyde (MDA)

The extent of lipid peroxidation was measured by the reaction of MDA with thiobarbituric acid (TBA) to form a colorimetric (532 nm) product, proportional to the MDA present (Sigma-Aldrich, St. Louis, MO). The activity of lipid peroxidation was expressed as the amount of MDA normalized by the amount of cellular protein.

(v). Measurement of protein concentration

Protein concentration was measured by a BCA kit (Pierce Biotechnology, Rockford, lL) following the manufacturer's protocol.

2.2.5. 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).

3. Results and Discussion

3.1. The Effect of Plant Parts Methanolic Extracts Applied in Beef Meatballs on Serum Glucose Concentration ofAlloxan Induced Diabetic Rats

Serum glucose concentration of alloxan-induced diabetic rats consumed the tested plant by-product extracts applied in beef meatballs were shown in Table (1). From such data it could be noticed that treatment of animals with alloxan caused a significant increased ($p \leq 0.05$) in serum glucose concentration (49.47%) compared to normal controls. Compared to the diabetic control, hypoglycemic capability of beef meatballs supplemented with 0.1% plant parts methanolic extracts was demonstrated by significant ($p \leq 0.05$) decrease in glucose concentration in serum. The rate of decrease was recorded 29.91, 39.99, 31.15 and 41.75% for meatballs supplemented with OPE, ROSE, PPPE and their mixture, respectively. So, the decrease in serum glucose was depending on the type of the plant parts extracts applied in beef meatballs. The highest hypoglycemic yield was recorded for applying the mixture of the selected plant parts extracts followed by ROSE, PPPE and OPE, respectively.

Significant research has been done on the effect of onion consumption on diabetic conditions. The organosulfur compounds *S*-methyl cysteine sulfoxide (SMCS) and *S*-allyl cysteine sulfoxide (SACS) were linked to significant amelioration of weight loss, hyperglycemia, low liver protein and glycogen, and other characteristics of diabetes mellitus in rats [40]. They found that the use of SMCS and SACS (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. Similarly, Suresh and Srinivasan [41] found that a 3% onion powder diet also reduced hyperglycemia, circulating lipid peroxides, and blood cholesterol (LDL-VLDL exclusively). *In vivo* analysis of the effects of quercetin on human diabetic lymphocytes showed a significant increase in the protection against DNA damage from hydrogen peroxide at the tissue level. Antioxidant activity was shown, but non-diabetic controls were not used and symptom relief was not mentioned. Jung *et al.,* [9] reported that onion peel extract (OPE) 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. Moreover, in most cases, OPE showed greater potency than pure quercetin equivalent. These findings provide a basis for the use of onion peel to improve insulin insensitivity in type 2 diabetes. OPE 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. Moreover, in most cases, OPE showed greater potency than pure quercetin equivalent. These findings provide a basis for the use of onion peel to improve insulin insensitivity in T2DM.

The same effect was recorded for OPE and PPPE which displayed potent hypoglycemic action in alloxan-induced diabetic rats. For example, Abd El-Razek and Hassan, [18] found that treatment of diabetic rats with single or repeated dose of prickly pear fruit juice significantly ($P < 0.05$) decreased the levels of serum glucose by about 50%. Such activity may be related to diverse bioactive compounds present in those extracts including phenolics, alkaloids and carotenoids [18, 20, 42]. 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 [9, 43-44]. Also, all of the above data partially explain why the highest hypoglycemic yield was recorded for applying the mixture of the selected plant parts extracts? It could be due to the

interactive effects occurred by different categories of bioactive compounds of the plant parts extracts used.

3.2. The Effect of Plant Parts Methanolic Extracts Applied in Beef Meatballs on Liver Functions of Alloxan Induced Diabetic Rats

Liver function enzymes activities (aspartate aminotransferase, AST; alanine aminotransferase, ALT and alkaline phosphatase, AP) in serum of alloxan-induced diabetic rats consumed the tested plant by-product extracts applied in beef meatballs were shown in Table (2). From such data it could be noticed that treatment of animals with alloxan caused a significant increased ($p \leq 0.05$) in serum in AST, ALT and AP activities (21.26, 19.08 and 19.33%) compared to normal controls. Compared to the diabetic control, liver functions enhancement of beef meatballs supplemented with 0.1% plant parts methanolic extracts was demonstrated by significant ($p \leq 0.05$) decreasing of specific enzymes activities in serum. The rate of decreasing in AST, ALT and ALP was recorded 11.91, 20.06, 14.20 and 20.19; 11.05, 14.44, 11.99 and 16.13; and 9.34, 14.41, 11.57 and 16.23% for meatballs supplemented with OPE, ROSE, PPPE and their mixture, respectively. So, the decrease in serum liver enzymes activities was dependent on the type of the plant parts extracts applied in beef meatballs. The highest liver functions enhancement yield was recorded for applying the mixture of the selected plant parts extracts followed by ROSE, PPPE and OPE, respectively.

Aminotransferases are normally intracellular enzymes. Thus, the presence of elevated levels of aminotransferase in the plasma indicates damage to cells rich in these enzymes. For example, physical trauma or a disease process can cause cell lysis, resulting in release of intracellular enzymes into the blood. Two amino transferases were found in plasma are of particular diagnostic value AST and ALT. AST enzyme is one of the enzymes tested in the cardiac enzyme series. This enzyme is found in very high concentration within the heart muscles, skeletal muscle cells, and to a lesser degree in the kidney and panaceas. ALT is found predominately in the liver lesser quantities are found in the kidneys, heart and skeletal muscles. Elevated liver function in T2DM was observed by Harris [46] who found that individuals with T2DM have a higher incidence of liver function test abnormalities than individuals who do not have diabetes. Mild chronic elevations of transaminases often reflect underlying insulin resistance. Also, antidiabetic agents have generally been shown to decrease alanine aminotransferase levels as tighter blood glucose levels are achieved.

Such as reviewed in many studies plant parts including OPE, ROSE and PPPE are a rich source of different classes of phytochemicals such as flavonols, phenolic acids, anthocyanins, alkaloids, carotenoids, phytosterols and organosulfur compounds [20, 42, 47-48]. It is reported that the effect of many plant parts on decreasing the serum liver function enzymes activity could be attributed to their high level content of that phytochemicals. For example, El-Nashar, [49] found that different doses of cinnamon extract showed slight-decreased in serum AST, ALT and AP after 12 week of feeding when compared with control group. The same observation was reported in rats injected with nitrosamine and treated with apricot kernel extracts [18]. Also, active ingredients in sweet violet (*Viola odorata* L.) blossom powder prevented partially the rise of mean serum ALT, AST and AP activities induced by CCl_4 injection [50]. The possible mode of action of liver serum enzymes-lowering activity of the tested plant parts could be explained by one or more of the following process. Dawson, [51] reported that flavonoid found in all the tested plant parts is known to block the hepatocellular uptake of bile acids. Beattic *et al.,* [48] reviewed that flavonoids pretreatment improved the antioxidant capacity of the liver, diminished the bilirubin concentration compared with the groups without treatment. Also, flavonol glycosides reduced the elevated levels of the following serum enzymes, AST, ALT and AP. El-Nashar, [49] reported that pre-treatment with flavonoids were not only able to suppress the elevation of AST and ALT but also reduce the damage of hepatocytes *in vitro*. Also, they found that flavonoids have exhibited strong antioxidant activity against reactive oxygen species (ROS) *in vitro*. The hepatoprotective activity of flavonoids was possibly due to its antioxidant properties, acting as scavengers of reactive oxygen species (ROS). Also, treatment with prickly pear fruit juice to diabetic rats significantly ($P < 0.05$) decreased the elevated AST, ALT and ALP almost to normal levels [18] Furthermore, Sayed Ahmed [52] found that pre-treatment with plant parts rich in phytochemicals such as onion skin, potato peel and cauliflower leaves powders, were able to reduce the damage of liver i.e. suppress the elevation of AST, ALT and ALP through the improvement of antioxidant defense system in red blood cells. Such studies with the others confirmed the data obtained by the present study,liver functions enhancement was recorded for applying the mixture of the selected plant parts extracts. Such observation could be interpreted by the interactive effects occurred by different categories of bioactive compounds of the plant parts extracts used.

Table 1. *The effect of plant parts methanolic extracts applied in beef meatballs on Serum glucose concentration (mg /dl) of alloxan induced diabetic rats*.*

Value	Control (-)	Control (+)	Control (+) (20% meatballs)	Meatballs + Plant parts methanolic extracts (0.1%, w/w)			
				OPE	ROSE	PPPE	Mix
Mean	102.45^{e}	202.76^{a}	176.78^{b}	142.12^{c}	121.67^{d}	139.6^{c}	118.11^{d}
SD	10.22	18.93	5.12	12.87	8.66	10.67	9.43
% of Change (from the control $^{+}$)	--------	49.47	-12.81	-29.91	-39.99	-31.15	-41.75

* OPE, orange peel extract; ROSE, red onion skin extract; PPPE, prickly pear peel extract and Mixture, OPE+ ROSE+ PPPE by equal parts. Values with different superscript letters in the same raw are significantly different at $p \leq 0.05$.

Table 2. *The effect of plant parts methanolic extracts applied in beef meatballs on serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT) and alkaline phosphatase (AP) activities (IU/L) of alloxan induced diabetic rats*.*

Value	Control (-)	Control (+)	Control (+) (20% meatballs)	Meatballs + Plant parts methanolic extracts (0.1%, w/w)			
				OPE	ROSE	PPPE	Mix
AST							
Mean	31.67^{ab}	40.22^{a}	37.14^{a}	35.43^{ab}	32.15^{ab}	34.51^{ab}	32.10^{ab}
SD	2.18	3.1	2.88	1.18	2.2	4.32	3.2
% of Change (from the control +)	-------	21.26	-7.66	-11.91	-20.06	-14.20	-20.19
ALT							
Mean	50.23^{ab}	62.07^{a}	57.78^{a}	55.21^{ab}	53.11^{ab}	54.63^{ab}	52.06^{ab}
SD	5.32	4.11	6.32	2.11	9.1	5.32	2.99
% of Change (from the control +)	-------	19.08	-6.91	-11.05	-14.44	-11.99	-16.13
ALP							
Mean	98.55^{ab}	122.17^{a}	113.98^{ab}	110.76^{ab}	104.56^{ab}	108.04^{ab}	102.34^{ab}
SD	8.78	5.98	5.01	9.11	7.32	20.76	10.54
% of Change (from the control +)	-------	19.33	-6.70	-9.34	-14.41	-11.57	-16.23

* OPE, orange peel extract; ROSE, red onion skin extract; PPPE, prickly pear peel extract and Mixture, OPE+ ROSE+ PPPE by equal parts. Values with different superscript letters in the same raw are significantly different at p≤0.05.

3.3. The Effect of Plant Parts Methanolic Extracts Applied in Beef Meatballs on Malondialdehyde (MDA) Concentration in Liver Tissue of Alloxan Induced Diabetic Rats

Oxidative stress status was assessed by measuring the MDA formation in liver. Malondialdehyde (MDA) concentration in liver tissue of alloxaninduced diabetic rats consumed the tested plant by-product extracts applied in beef meatballs were shown in Table (3). From such data it could be noticed that treatment of animals with alloxan caused a significant increase ($p\leq0.05$) in liver MDA content (33.67%) compared to normal controls. Compared to the diabetic control, suppress oxidative stress of beef meatballs supplemented with 0.1% plant parts methanolic extracts was demonstrated by significant ($p\leq0.05$) decreasing of MDA content in liver. The rate of decrease was recorded 14.25, 21.12, 16.28 and 24.17% for meatballs supplemented with OPE, ROSE, PPPE and their mixture, respectively. So, the decreasing in liver MDA content was depending on the type of the plant parts extracts applied in beef meatballs. The highest oxidative stress suppression was recorded for applying the mixture of the selected plant parts extracts followed by ROSE, PPPE and OPE, respectively.

Serum of diabetic patients increased oxidative stress (high levels of lipid peroxides and aldehydes, and low levels of SH groups). The increased serum oxidative stress in the diabetic patients could be the result of glycation and glycooxidation of LDL by glucose, or/and the decreased capability of the patients' HDL to protect LDL against oxidation [53]. Also, MDA which is a secondary product of lipid peroxidation is known to cause crosslinkage of membrane components containing amino groups and make the membrane fragile [54].

In similar study carried out by Jung *et al.,* [9] reported that oxidative stress and metabolic dysregulation of free fatty acids (FFAs) indiabetic condition were alleviated by onion peel extract administration. Plasma FFAs levels were decreased in a dose-dependent manner and one percent of onion peel extract administration showed significant lower level compared with the diabetic control ($P = 0.01$). Several studies suggested that impaired blood lipids are characteristic of subjects with insulin resistance, especially circulating FFAs [55-56]. These observations were supported by Nguyen *et al.,* [57] who found that FFAs directly activate macrophage to secrete pro-inflammatory cytokines that render muscle cells insulin resistant. Also, Jung *et al.,* [9] found that hepatic oxidant stress was reduced by 1% onion peel extract, as assessed by increasing SOD activity and blocking MDA formation. Moreover, hepatic expressions of TNF-a and IL-6 were suppressed by either 1% OPE or quercetin. These results are in agreement with Coskun *et al.,* [8] who reported that quercetin, dominant flavonoid in onion peel extract, had anti-oxidative and anti-inflammatory activities. Therefore, although detailed mechanisms of action await further investigation, it is proposed that onion peel extract leads to improve insulin sensitivity, at least in part, through enhancing lipid metabolism, reducing oxidative stress, or modulating pro-inflammatory cytokines in diabetic rats.

In the same context, prickly pear species have antioxidant activity that may be associated with their phenolic content. Short-term receiving 250 g of fresh fruit pulp in patients with familial isolated hypercholesterolemia reduced oxidative damage to lipids and improved oxidative stress status of treated patients [58]. Also, El-safty and Al-Masri, [59] found that treatment of diabetic rats with prickly pear juice could significantly ($P < 0.05$) lower the elevated MDA levels in serum compared with diabetic rats. Similarly, Akacha *et al.,* [60] found that prickly pear cladodes extract was effective in the protection of the small intestine against Methotrexate (MTX) -induced damage including MDA level. Furthermore, Motawe *et al.,* [61] reported that MDA was enhanced in rat CCl_4 induced hepatotoxicity for the groups manipulated with orange peel incorporated with CCl_4. The antioxidative action is one of many mechanisms by which prickly pear might exert their beneficial health effects [61-62]. The presence of several antioxidants such as ascorbic acid, carotenoids, reduced

glutathione, cysteine, taurine while flavonoids such as quercetin, kaempferol and isorhamnetin has been detected in different varieties of prickly pear and orange plant parts including fruit peels [59, 63].

Such studies with the others confirmed the data obtained by the present study, maximum lipid perioxidation/oxidative stress suppression in liver tissues was recorded for applying the mixture of the selected plant parts extracts. Such observation could be interpreted by the interactive effects occurred by different categories of bioactive compounds of the plant parts extracts used.

3.4. The Effect of Plant Parts Methanolic Extracts Applied in Beef Meatballs on Reduced Glutathione (GSH) Concentration in Liver Tissue of Alloxan Induced Diabetic rats

Reduced glutathione (GSH) concentration in liver tissue of alloxan-induced diabetic rats consumed the tested plant by-product extracts applied in beef meatballs were shown in Table (4). From such data it could be noticed that treatment of animals with alloxan caused a significant decrease ($p \leq 0.05$) in liver GSH content (27.36%) compared to normal controls. Compared to the diabetic control, biological antioxidant activity enhancement of beef meatballs supplemented with 0.1% plant parts methanolic extracts was demonstrated by significant ($p \leq 0.05$) increasing of GSH content in liver. The rate of increase was recorded 13.47, 19.20, 14.47 and 23.07% for meatballs supplemented with OPE, ROSE, PPPE and their mixture, respectively. So, the increase in liver GSH content was dependent on the type of the plant parts extracts applied in beef meatballs. The highest biological antioxidant activity enhancement was recorded for applying the mixture of the selected plant parts extracts followed by ROSE, PPPE and OPE, respectively.

GSH, a tripeptide-thiol (γ-glutamylcysteinyl-glycine) present in millimolar concentrations in all the cells,is an important antioxidant [64]. Reduced glutathione normally plays the role of an intracellular radical scavenger and is the substrate of many xenobiotic elimination reactions [65]. A marked decreased level of reduced glutathione is reported in the plasma of diabetic patients. Results of our study are in agreement with other studies [66-69]. GSH systems may have the ability to manage oxidative stress with adaptational changes in enzymes regulating GSH metabolism. Long time ago, Paglia and Valentine [70] reported the link between hyperglycemia and GSH depletion. It could be interpreted by Lee and Chung, [71] 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. As GSH is an important antioxidant molecule, its depletion leads to the increase of oxidative stress.

The present study reported that feeding the diabetics rats with beef meatballs supplemented with plant parts methanolic extracts significantly removed some of the metabolic disorders induced by T2DM in liver cells through increasing the GSH synthesis. Many studies reported the potent antioxidant capacity of those plant parts extracts (OPE, ROSE and PPPE) in both *in vitro* and *in vivo* studies [20, 25]. Such effect leads to increase glutathione content and stimulate its related antioxidant enzymes activity i.e. GSH-peroxidase and GSH-reductase [25, 72]. The highest effect was recorded for applying the mixture of the selected plant parts extracts which could be attributed to the interactive effects occurred by different categories of bioactive compounds included in those extracts.

Table 3. *The effect of plant parts applied in beef meatballs on malondialdehyde (MDA) concentration (nmol/mg tissue protein) in liver tissue of alloxan induced diabetic rats*.*

Value	Control (-)	Control (+)	Control (+)(20% meatballs)	Meatballs + Plant parts methanolic extracts (0.1%, w/w)			
				OPE	ROSE	PPPE	Mix
Mean	2.94^{c}	3.93^{a}	3.53^{ab}	3.37^{ab}	3.1^{c}	3.29^{ab}	2.98^{c}
SD	0.21	0.12	0.1	0.55	0.44	0.22	0.27
% of Change(from the control +)	------	33.67	-10.18	-14.25	-21.12	-16.28	-24.17

* OPE, orange peel extract; ROSE, red onion skin extract; PPPE, prickly pear peel extract and Mixture, OPE+ ROSE+ PPPE by equal parts. Values with different superscript letters in the same raw are significantly different at $p \leq 0.05$.

Table 4. *The effect of plant parts applied in beef meatballs on reduced glutathione (GSH) concentration (nmol/mg tissue protein) in liver tissue of alloxan induced diabetic rats*.*

Value	Control (-)	Control (+)	Control (+)(20% meatballs)	Meatballs + Plant parts methanolic extracts (0.1%, w/w)			
				OPE	ROSE	PPPE	Mix
Mean	8.89^{a}	6.98^{c}	7.11^{abc}	7.92^{ab}	8.32^{ab}	7.99^{ab}	8.59^{a}
SD	2.09	1.89	2.65	1.11	1.77	1.53	0.97
% of Change(from the control +)	------	-27.36	1.86	13.47	19.20	14.47	23.07

* OPE, orange peel extract; ROSE, red onion skin extract; PPPE, prickly pear peel extract and Mixture, OPE+ ROSE+ PPPE by equal parts. Values with different superscript letters in the same raw are significantly different at $p \leq 0.05$.

4. Conclusion

In conclusion, the present study has demonstrated the potency of OPE, ROSE, PPPE and their mixture to ameliorate hyperglycemia in diabetic rats. Furthermore, OPE, ROSE, PPPE and their mixture lowered MDAand increased GSH content in liver i.e. suppressed oxidative and inflammatory stress in liver. These findings provide a basis for the use of OPE, ROSE, PPPE and their mixture also have important implications for the prevention and early treatment of T2DM.

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