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PROTECTIVE EFFECT OF POMEGRANATE (PEEL AND MOLASSES) INCORPORATED INTO ROASTED MEAT BALLS ON RENAL FAILURE IN RATS

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ABSTRACT

this research aimed to study the effect of pomegranate peel and molasses incorporated in roasted meat balls on the general acceptability of the product and its protective effects on renal failure (RF) induced by excessive dietary arginine in rats. PG peel powder ant PG molasses, and their mix were incorporated into meat balls at levels of 3, 5 and 8%. Proximate chemical composition and sensory properties of meat balls were evaluated. Meat balls with 3% PG peel, 5% PG molasses and 5% PG peel&molasses mix were chosen for biological evaluation. The results revealed that renal Failure induced by excessive L-arginine in rats caused a decrease in body weight gain, feed efficiency ratio, protein efficiency ratio, albumin, globulin, total protein, total antioxidants capacity, SOD, GSH, and CAT. It also increased serum biomarkers of renal function, C-reactive protein, tumor nicrosis factor-α (TNF-a) and TBARs. Coadministration of pomegranate derivatives alleviates oxidative stress and mitigated all renal failure signs. It can be concluded that meat balls contained pomegranate peel (3%), molasses (5%) and their mix (5%) received an acceptable sensory scores and exhibited renoprotective efficacy can be attributed to their antioxidant and anti-inflammatory aspects.

INTRODUCTION

The incidence of Chronic Renal Disease (CRD) appears to be on the increase, especially in some developing countries, imposing a very expensive and rising demand on health care systems already burdened by paucity of resources (Hossain *et al.* 2009). Chronic renal Failure (CRF) is a progressive and irreversible loss of large number of functional nephrons caused by wide variety of disorders of the blood vessels, glomeruli, tubules and renal interstitium. CRF is characterized by the structural and functional responses of remnant nephrons, which ultimately lead to glomerulosclerosis (Guyton and Hall, 2006). Chronic kidney disease (CKD) is a significant risk factor for premature cardiovascular disease and death. Increased oxidative stress in people with CKD has been implicated as a potential causative factor for some cardiovascular diseases. Antioxidant therapy may reduce cardiovascular mortality and morbidity in people with CKD (jun *et al.* 2012).

Plant derived products have been used for medicinal purposes for centuries and also being used in our daily food intake. Drugs of plant origin are known to play a vital role in the management of kidney diseases, and have protective effect against oxidative stress in rats (Ajith *et al.* 2007, and Ahmed *et al.* 2000). Pomegranate also serves as a remedy for diabetes in the Unani system of medicine practiced in the Middle East and India (Saxena and Vikram 2004). Pomegranate peel ethanol extract (500 mg/kg b.wt.) has ameliorative effect against chlorpyrifos-ethyl-induced oxidative stress in rats

(Ahmed and Zaki 2009). Pomegranate peel contains substantial amounts of polyphenols such as ellagic tannins, ellagic acid and gallic acid (Nasr *et al.* 1996).

There is continuing concern about the health effects of red meat. Population studies have linked higher red meat intake with increased risk of coronary heart disease (Hu *et al.* 2000 and van Dam *et al.* 2003) and type 2 diabetes (Schulze *et al.* 2003 Song *et al.* 2004), but it remains uncertain whether these associations are causally related to unprocessed lean red meat. Hodgson *et al.* 2006 suggest that partial replacement of dietary carbohydrate with protein from lean red meat does not elevate oxidative stress or inflammation.

Arginine, a semi essential amino acid is used in the synthesis of body proteins and it is a material for transport, storage and excretion of nitrous products. In particular, arginine is essential for ammonia detoxification via urea synthesis. Physiological requirement of tissues and organs for arginine should be supplied by endogenous synthesis and the diet (Reyes *et al.* 1994). Kidney is the main site of endogenous L-arginine synthesis. However, administration of excess arginine causes imbalance of amino acids and changes in protein metabolism. In addition arginine is the key substance of nitric oxide and guanidine compounds such as creatinine and methylguanidine which are considered to be uremic toxins responsible for renal failure (Natelson *et al.* 1979; Yokozawa *et al.* 1991 and Moncada 1997). The present study has been undertaken to determine the possible effect of pomegranate peel powder and molasses incorporated into meat balls on excessive Arginine induced renal damage in rats.

MATERIALS AND METHODS

Plant materials: Pomegranate *Punica granatum* peels, pomegranate molasses (provided by Alfa Inter Food Co. Lebanon), beef red meat, onion, salt, black pepper, burgul were obtained from the local market in Cairo city, Egypt.

***Chemicals*:** Arginine, casein, vitamins, minerals, cellulose and choline chloride were purchased from El-Nasr Pharm. and Chem. Ind. Comp. Cairo, Egypt. Corn oil and corn starch were obtained from local market. Kits used to determine serum biochemical parameters were purchased from Biodiagnostic Company, Dokki, Giza, Egypt.

Methods

Meat balls preparation

100g of pomegranate fresh peel (79.22% moisture) were washed with water, cut into small pieces and placed in a hot air oven at 60 0C for 18 h to be dried. The dried materials (66.41% moisture) were crushed by food grinder in to powder form up to completely pass through 0.5 mm size sieve. Powder was transferred polyethylene bags until use (Kushwaha *et al.* 2013). Bulgur was soaked in double amount of water (20°C±1) for 15 minutes and drained. Fats were removed from the meat to get the lean tissues. Three formulas of meat balls were prepared. Meat balls (MB) consists of meat 86%, bulgur 10% (to reduce loss of water and nutrients during cooking), onion 1%,

black pepper 1%, salt 2%. PG peel MB, PG molasses MB and PG peel& molasses mix (1:2) MB were prepared by substitution of 3, 5 or 8% with meat. Fresh garlic and onion were peeled and minced (grinder, 2000 r.p.m. for 1 minute) before the addition to minced meat, salt, black pepper and bulgur were added and minced again (grinder, 2000 r.p.m. for 2 minute). Balls shaped pieces were prepared.

Sensory evaluation

Ten randomized volunteers were invited to score samples (30 minutes after cooking) from the control and test groups in terms of color, flavor, texture and overall acceptability according to Zhang and Zhang (2007**).**

Proximate chemical composition

Moisture, crude protein and crude fat estimations were carried out according to the methods of **A.O.A.C. (1995)**; Ash contents were carried out according to the method of **A.O.A.C. (2000);** Carbohydrates was calculated by the following equation:

% Carbohydrates = 100 – (% moisture + % protein + % fat + % ash)

Induction of Renal Failure: *Rats were fed on basal diet containing 2% (w/w) arginine to induce CRF according* to Yokozawa *et al.* 2003.

Animals and experimental design

Thirty six albino weighing 160-180 g were used in the present study. Rats were housed in temperature-controlled conditions under a 12:12-h light/dark photocycle with food and tap water supplied ad libitum. The basal diet comprised of 20% casein (protein > 80%), corn oil 4%, cellulose 5%,vitamin mixture 1%, salt mixture 3.5%, choline chloride 0.2% and the 66.3 corn starch**.** After one week acclimatization period, the rats were divided into six equal groups (n=6 rats per cage): All groups were fed on Arginin (20g/kg diet (w/w) daily on the first fourteen days) except the normal control. After fourteen days, rats groups were fed as follows:

Group 1 (Normal control): received normal basal diet for six weeks; Group 2 (Positive control): received basal diet for 6 weeks with 2% arginine (20g/kg diet (w/w)) on the first two weeks; Group 3 (Meat balls treatment): received basal diet with 30% MB substitution for six weeks and 2% arginine on the first two weeks; Group 4 (Pomegrenate peel treatment): received basal diet with 30% PG peel MB substitution and 2% arginine on the first two weeks; Group 5 (Pomegrenate molasses treatment): received basal diet with 30% pomegrenat molasses MB substitution and 2% arginine on the first two weeks; Group 6 (Pomegrenate molasses&peel mix treatment): received basal diet with 30% PG peel & molasses MB substitution and 2% 2% arginine on the first two weeks.

During the experimental period, consumed food and body weights were recorded twice weekly. Biological evaluation for different groups was carried out by determination of food intake, body weight gain% and relative kidney weight (kidney weight/body weight %). FER and PER were calculated according to Chapman *et al.* (1950). At the end of the experimental period the rats were fasted overnight and sacrificed. Blood samples were withdrawn from the orbital plexus of veins in the inner canthus of eye using capillary microtubes. Blood was left for 10 min. at room temperature to clot. Serum

samples were obtained by centrifugation at 4000 rpm for 15 min. and directly frozen at -18°C till biochemical analyses.

Biochemical analysis

Assessment of renal functions: Serum creatinine (mg/dl), serum uric acid (mg/dl), total protein (g/dl), serum blood urea nitrogen (BUN) (mg/dl) were assayed according to Henry (1974), Wybenga *et al.* (1971), Sonnenwirth and Jaret (1980) and Fawcett and Soctt (1960), rrespectively. Albumin and globulin (g/dl) were determined according to Doumas *et al.* (1971) (Globulin concentration is calculated by subtracting albumin from total protein).

Assessment of inflammation markers: C-reactive protein (CRP) level (mg/l) was determined in serum using kits provided from Biodiagnostic Company, Dokki, Giza, Egypt, according to the methods of Peltola *et al.* (1983). Serum tumor necrosis factor-α (TNF-α) (pg/ml) was measured using ELIZA technique according to Aggarwal *et al.* (1985).

Assessment of lipid peroxidation: lipid peroxidation (TBARs) (nM/ml) level was determined in serum according to the method of Okhawa *et al.* (1979).

Assessment of antioxidant parameters: kidneys were quickly removed and washed in cold isotonic saline 0.9% NaCL with 50 to 100 of ice cold solution for estimation of superoxide dismutase (SOD) according to Nishikimi *et al.* (1972) and reduced glutathione (GSH) activity in tissue according to Aebi (1974). Catalaze (CAT) activity was determined according to Ellman (1959). Total antioxidant capacity was determined by colorimetric method according to the method of Koracevic *et al.* (2001). Kits were provided From Biodiagnostic Company, Dokky, Cairo, Egypt.

Statistical analysis

Data were presented as means ± SD and statistically analyzed using one way analysis of variance (ANOVA) test ($p<0.05$), followed by Least Significant Difference (LSD) and Duncans multiple range test using computerized SPSS program version 20.0 software for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Proximate chemical composition of meat balls: Moisture, protein, fat ash and carbohydrates content (g/100g) of meat balls contained PG peel, molasses and peel& molasses mix are described in Table 1. Moisture content was higher in meat balls (MB) contained PG derivatives than control meat balls except PG peel MB.The highest content of moisture (55.52±0.30g/100g) was for PG molasses MB. Data show that there were a decrease in both of protein and fat content of PG derivatives MB; however an increase in ash and carbohydrates content was observed. The highest content of protein (14.50±0.03 g/100g) and fat (13.32±0.28 g/100g) was for MB. Meanwhile PG peel MB recorded the highest content of ash (2.31±0.03 g/100g) and carbohydrates (20.47±0.27 g/100g). These results are in line with Ikhlas *et al.* (2011) who found proximate composition of cooked wheat meat balls was 65.94±0.10 % moisture, 13.53±0.20 % protein, 10.59±0.48 % fat, 2.30±0.13% ash and 7.63±0.57 % carbohydrate.

Table (1): Mean values ± SD of proximate composition of cooked meat balls with or without pomegranate derivatives

Groups	Moisture	Crud protein	Fat	Ash	Carbohydrates
Meat balls (MB)	55.4^{a}±0.10	14.50^{a}±0.03	13.32^{a}±0.28	2.05^{a}±0.02	14.72^{d}±0.32
PG peel MB	51.56^{c***}±0.30	13.32^{b***}±0.01	12.33^{b***}±0.01	2.31^{a***}±0.03	20.47^{a***}±0.27
PG molasses MB	55.52^{a}±0.05	13.14^{d***}±0.02	12.03^{c***}±0.03	2.05a±0.03	17.25^{c***}±0.07
PG peel with molasses MB	53.43b***±0.02	13.24^{c***}±0.03	12.21^{bc***}±0.02	2.16^{a***}±0.03	18.97^{b***}±0.05
LSD	0.000	0.000	0.000	0.000	0.000

PG: Pomegranate; MB: Meat balls; * P<0.05, ** P<0.01 or *** P<0.001 is significant with meat balls sample; Mean values in each raw having different superscript (a, b, c) denote significant difference.

Sensory evaluation of meat balls: Sensory evaluation of meat balls (MB) and meat balls contained different percentage (3, 5 and 8 %) of PG derivatives (peel, molasses and peel&molasses mix) was performed for taste, color, odor, appearance and overall acceptability (OA). Samples of meat balls which had the highest sensory characteristics with insignificant differences with control were used in the biological experiment. As shown in Table 2, the control sample (meat balls without PG derivatives) recorded the highest scores in all sensory parameters (9.43±0.06 taste, 9.70±0.26 color, 9.27±0.25 appearance and 9.28±0.13 overall acceptability, respectively) except odor (8.70±0.26). However significant decrease in color for PG derivatives MB was noticeable. Significant decrease ($p<0.05$) was observed in all parameters at level of 8 % PG derivatives MB, followed by level of 5%. Meanwhile, the highest OA score in PG treatments was 3% and 5 % peel&molasses mix MB (9.34±0.09 and 9.20±0.18 respectively), followed by 5% molasses MB and 3% peel MB (9.10±0.15 and 9.06±0.13, respectively). These findings were partially similar to those obtained by Narsaiah *et al.* (2011) who indicated that tenderidazation of goat meat with PG seed powder (PSP) improved the textural products properties, however got lower score for color in sensory evaluation and there was adverse effect on taste of treated meat. Devatkal *et al.* 2010 also observed similar darkening of goat meat patties containing PG products which agree with our results.

Table (2): Mean values ± SD for sensory evaluation of cooked meat balls with or without pomegranate derivatives

Groups		Taste	Color	Odor	appearance	OA
Meat balls (MB)	0%	9.43^{a}±0.06	9.70^{a}±0.26	8.70^{bcd}±0.26	9.27^{a}±0.25	9.28^{a}±0.13
PG peel MB	3%	9.23^{a}±0.25	8.60^{c***}±0.10	9.07^{abc}±0.06	8.27^{bc**}±0.25	8.79^{b**}±0.08
	5%	7.27^{b***}±0.25	6.87^{e***}±0.32	9.10^{abc}±0.17	7.87^{c***}±0.15	7.78^{c***}±0.06
	8%	5.23^{c***}±0.85	5.13^{f***}±0.15	7.43^{e***}±0.60	6.37^{d***}±0.31	6.04^{d***}±0.25
PG molasses MB	3%	9.30^{a}±0.26	8.53^{c***}±0.12	9.33^{ab}±0.15	9.07^{a}±0.40	9.06^{ab}±0.13
	5%	9.40^{a}±0.10	8.90^{bc***}±0.10	9.30^{ab}±0.26	8.80^{ab}±0.60	9.10^{ab}±0.15
	8%	5.17^{c***}±0.96	7.03^{de***}±0.21	8.47^{cd}±0.50	8.03^{c***}±0.21	7.18^{d***}±0.41
PG peel & molasses MB	3%	9.47^{a}±0.06	9.00^{b**}±0.10	9.67^{a**}±0.15	9.23^{a}±0.25	9.34^{a}±0.09
	5%	9.40±0.10	8.87^{bc***}±0.15	9.47^{a}±0.25	9.03^{a}±0.25	9.20^{a}±0.18
	8%	5.83c***±0.76	7.37^{d***}±0.40	8.33^{d}±0.61	7.93^{c***}±0.38	7.37^{d***}±0.29

PG: Pomegranate; MB: Meat balls; OA: Overall acceptability; * P<0.05, ** P<0.01 or *** P<0.001 is significant with meat balls sample; Mean values in each raw having different superscript (a, b, c) denote significant difference.

Effect of PG meat balls on nutritional status: Effect of feeding pomegranate derivatives meat balls on food intake, body weight gain, relative kidney weight, FER and PER of rats with renal failure is represented in Table 3. Data show significant decrease in food intake ($p<0.01$) and body weight gain ($p<0.05$) and insignificant decrease in kidney relative weight, food efficiency ratio and protein efficiency ratio in rats fed excessive L-arginine comparing to normal control group. On the other hand, rats fed PG derivatives showed a decrease in all previous parameters. Rats fed PG peel MB recorded the highest value of food intake (16.69±0.31g/d), kidney relative weight (2.28±0.21), however PG molasses MB showed the highest mean value of body weight gain (59.95±4.64g), food efficiency ratio (0.12±0.02) and protein efficiency ratio (0.61±0.05) comparing to other treatments. These results are in agreement with El-Habiby 2013 as observed that Co-treatment of pomegranate increased body weight of rats suffering from renal failure in comparison to positive control group rats.

Table (3): The mean values ± SD of body weight gain, food intake, FER and PER of the experimental rat groups.

Groups	Food Intake (g/d))	FER	PER	Body Weight Gain (g)	Kidney Weight/Body Weight %
Normal control	16.14^{c***} ±0.10	0.136^{a}±0.05	0.68^{a}±0.24	65.95^{a*}±13.68	1.73^{ab}±0.16
Positive control	14.47^{a}±0.30	0.08^{a}±0.02	0.38^{a}±0.12	33.07^{a}±11.22	1.41^{b}±0.17
Meat balls	15.41^{b**} ±0.38	0.08^{a}±0.03	0.4^{a}±0.14	36.89^{a}±12.63	1.72^{ab}±0.18
PG peel MB	16.69^{d***} ±0.31	0.11^{a}±0.03	0.55^{a}±0.19	55.06^{a}±14.33	2.28^{a*}±0.21
PG molasses MB	16.32^{cd***} ±0.28	0.12^{a}±0.02	0.61^{a}±0.05	59.95^{a}±4.64	2.10^{ab*}±0.41
PG peel & molasses MB	15.83^{bc***}±0.01	0.11^{a}±0.04	0.57^{a}±0.2	53.99^{a}±13.44	2.11^{ab*}±0.72
LSD	0.000	0.321	0.328	0.211	0.111

PG: Pomegranate; MB: Meat balls; FER: Food efficiency ratio; PER: Protein efficiency ratio; * P<0.05, ** P<0.01 or *** P<0.001 is significant with control positive control group; Mean values in each raw having different superscript (a, b, c) denote significant difference.

Effect of PG meat balls on renal functions: Effect of feeding PG derivatives on serum Creatinine, uric acid, blood Urea nitrogen, Albumin, Globulin and total protein of rats suffering from kidney failure is illustrated in Table 4. Results show significant elevation ($p<0.01$) in uric acid and blood urea nitrogen in both of rats fed L-arginine and those fed on L-arginine with meat balls comparing to normal control group. Infected rats received meat balls contained PG molasses or peel&molasses mix exhibited significant decline in blood urea nitrogen level comparing with positive control group (30.37±2.10 and 27.80±2.55 vs 35.14±2.01mg/dl), respectively). On the other hand, results showed a reduction in albumin, globulin and total protein in positive control group comparing with normal control group. Supplemental dietary Arginine may have adverse effect in advanced glycation end product - induced kidney inflammatory response and oxidative damage in rats (Ya-Mei Hu *et al.* 2012). Prophylaxis with PG peel extract for week resulted in reduction in serum creatinine and urea values in oxidative damage induced by Ferric nitrilotriaacetate (Fe-NTA) of rats (Ahmed and Ali 2010).

Administration of PG juice and PG peel methanol extract in rats with chronic renal failure significantly decreased serum levels of creatinine, blood urea nitrogen, uric acid (El-Habibi 2013).

Table (4): The Mean values ± SD of serum Creatinine, Uric acid, Urea nitrogen, Albumin, Globulin and T protein of the experimental groups

Groups	Creatinine (mg/dl)	Uric acid (mg/dl)	BUN (mg/dl)	Albumin (g/dl)	Globulin (g/dl)	T protein (g/dl)
Normal control	0.74^{a}±0.05	2.70^{b**} ±0.46	19.25^{e***} ±1.09	3.57^{a}±0.25	3.27^{a}±0.35	6.83^{a}±0.12
Positive control	0.84^{a}±0.02	3.65^{a}±0.23	35.14^{ab}±2.01	3.33^{a}±0.80	3.23^{a}±0.70	6.57^{a}±0.15
Meat balls	0.87^{a}±0.20	3.76^{a}±0.36	36.73^{a}±1.55	3.27^{a}±0.15	3.14^{a}±0.22	6.41^{a}±0.36
PG peel MB	0.86^{a}±0.23	3.67^{a}±0.39	32.07^{bc}±2.00	3.33^{a}±0.32	3.17^{a}±0.70	6.50^{a}±0.46
PG molasses MB	0.83^{a}±0.09	3.55^{a}±0.34	30.37^{cd*}±2.10	3.54^{a}±0.04	3.04^{a}±0.55	6.58^{a}±0.51
PG peel&molasses MB	0.83^{a}±0.09	3.52^{a}±0.38	27.80^{d**}±2.55	3.45^{a}±0.51	3.09^{a}±0.43	6.54a±0.40
LSD	0.972	0.039	0.000	0.932	0.994	0.801

PG: Pomegranate; MB: Meat balls; BUN: Blood urea nitrogen; * P<0.05, ** P<0.01 or *** P<0.001 is significant with control positive control group; Mean values in each raw having different superscript (a, b, c) denote significant difference.

Effect of PG meat balls on inflammation markers and oxidation status: Results in Table 5 revealed that serum C-reactive protein (CRP), tumor necrosis factor (TNF-a) were increased in L-arginine administered group as well as meat ball treated group comparing to normal control group except PG peel&molasses MB. Meanwhile, concomitant treatment with PG caused significant improvement (p<0.05) towards the normal levels. CRP was at the lowest level in PG peel MT (11.47b±1.35 mg/l), however PG peel & molasses MT caused the lowest TNF-a level (9.12±1.56 pg/ml). The levels of oxidative stress markers (Thiobarbituric acid reactive substances (TBARs) and free radicals (FRs)) were elevated in positive control groups however ameliorated in PG derivatives treated groups. The lowest total antioxidant level was for meat balls (1.77±0.06 mm/l) followed by positive control group (1.78a±0.04 mm/l), however PG molasses MB recorded the highest level (1.82±0.04 mm/l).

Pomegranate Juice caused a significantly decrease malondialdehyde (MDA) in the kidney cortex of rats under oxidative stress (Ilbey *et al.* 2009). A significant decrease in MDA level, by-product of lipid peroxidation, of kidney samples of rats received pomegranate methanol extract of peel were observed Abdel Moneim *et al.* (2011). pomegranate juice and pomegranate peel methanol extract lowered thiobarbituric acid reactive substance and decreased significantly serum tumor necrosis factor-α (TNF-α) and C-reactive protein concentrations accompanied by increase in nitric oxide level (El-Habibi 2013). pomegranate juice was found to have inhibitory effects on renal tubular cell injury and oxidative stress (Ilbey 2009). Antioxidant activity of pomegranate is referred to its polyphenolic capacity such as ellagic acid and ellagitannis (Seeram *et al.* 2005), which may suggest its role as an electron donor in scavenging free radicals (Kaur *et al.* 2006). Previous investigation

revealed the ability of pomegranate fruit extract (Sudheesh and Vijayalakshmi 2005 and Noda *et al.* 2002) and peel extract (Singh *et al.* 2002) to suppress lipid peroxidation.

Table (5): The Mean values ± SD of serum C-reactive protein (CRP), tumor necrosis factor (TNF-a), thiobarbituric acid reactive substances (TBARs) and total antioxidant capacity (TAC) of the experimental groups

Groups	CRP mg/l	TNF-a pg/ml	TBARs nM/ml	TAC (mm/l)
Normal control	11.68b±2.13	12.54b*±0.94	4.41a±0.84	1.83a±0.04
Positive control	14.17ab±1.86	15.35a±0.76	4.94a±0.99	1.78a±0.04
Meat balls	15.04a±1.91	16.60a±1.67	4.54a±1.09	1.77a±0.06
PG peel MB	11.47b*±1.35	11.05abc**±0.85	3.84a±1.18	1.81a±0.08
PG molasses MB	11.66b*±1.00	10.12abc**±2.33	3.45a±1.07	1.82a±0.04
PG peel&molasses MB	13.92ab±0.41	9.12c***±1.56	3.85a±1.18	1.80a±0.03
LSD	0.055	0.000	0.566	0.623

PG: Pomegranate; MB: Meat balls; * P<0.05, ** P<0.01 or *** P<0.001 is significant with control positive control group; Mean values in each raw having different superscript (a, b, c) denote significant difference.

The activities of antioxidant enzymes; catalase (CAT) in serum, superoxide dismutase (SOD) and reduced glutathione (GSH) levels in renal tissue of various rat groups are shown in Table 6. The results indicate that both of serum CAT, renal tissue homogenate SOD and GSH were significantly decreased in L-arginine and meat balls fed rats compared to normal control group, while increased in PG derivatives administered rats. Highly significant increase ($p<0.01$) in CAT level for PG molasses MB (60.68±2.24 µ/l) and PG peel & molasses MB (60.90±4.71 µ/l) fed groups comparing to positive control group. Glutathione (GSH) is the major intracellular antioxidant with multiple biological functions, including the maintenance of the thiol moieties of proteins and the reduced from of many other biologically active molecules (Ushio-Fukai *et al.* 1999). GSH depletion increases the sensitivity of organ to oxidative and chemical injury. Studies with a number of models show that the metabolism of xenobiotics often produced GSH depletion (Mitchell *et al.* 1973 and Ahmed and Zaki 2009). Administration of PG extract significantly increased GSH levels ($P<0.01$) compared to positive control group in rats under oxidative stress Ahmed and Zaki, 2009. Pretreatment with pomegranate flower for a period of one week significantly protected against oxidative damage induced by Ferric nitrilotriaacetate (Fe-NTA) by modulation of GSH content as well as antioxidant enzymes CAT and GST (Kaur *et al.* 2006). Middle and high doses of Pomegranate juice increased GSH levels ($p < 0.05$) in the kidney cortex in rats under oxidative stress caused by oxalate crystals (Ilbey *et al.* 2009). The present findings are in agreement with other investigations indicating that supplementation with pomegranate peel extract to treated animals showed significant inccrease in GSH content, GST and CAT activities in kidney (Ahmed and Zaki 2009). The effect of pomegranate on GSH level is due to its

polyphenols, where it is known to be able to modulate the transcription and expression of proteins related to the endogenous antioxidant defense by interacting with antioxidant response elements in gene promoter regions of genes encoding proteins related to oxidative injury management (Moskaug *et al.* 2005; Myhrstad *et al.* 2002). Molasses or juice were added to the drinking water of mice during 11 weeks leading to a significant increase in superoxide dismutase activity (Mounayar *et al.* 2012). Studies in rats and mice have confirmed that the antioxidant properties of a pomegranate by-product extract made from whole fruit without the juice showed a decrease in cellular lipid peroxide content and an increase in reduced glutathione levels (Rosenblat *et al.* 2006).

Table (6): Mean values ± SD of kidney catalase (CAT) and superoxide dismutase (SOD) enzymes of the experimental rats groups.

Groups	Catalase (µ/l)	SOD (u/g)	GSH (u/g)
Normal control	53.11^{ab}±7.46	27.67^{a}±3.73	6.37^{a*}±0.32
Positive control	48.80^{b}±3.10	24.77^{b}±2.46	5.27^{b}±0.67
Meat balls	48.75^{b}±3.60	23.69^{b}±2.80	5.24^{b}±0.36
PG peel MB	50.75^{b}±1.17	27.67^{a}±4.62	5.76^{ab}±0.30
PG molasses MB	60.68^{a**}±2.24	25.38^{ab}±4.75	5.86^{ab}±0.72
PG peel&molasses MB	60.90^{a**}±4.71	30.41^{a}±13.71	5.80^{ab}±0.45
LSD	0.008	0.138	0.127

PG: Pomegranate; MB: Meat balls; Significant with control (+ve) group * P<0.05 ** P<0.01 *** P<0.001; Mean values in each raw having different superscript (a, b, c) denote significant difference.

Effect of PG meat balls on kidney histopathological: The histopathological analysis of kidney in the meat balls, PG peel, molasses and peel&molasses mix meat balls treatments are presented in Fig. 1. Normal control group showed no histologial changes (Pic.1&2). Excessive arginine group showed striking histopathological findings represented in focal necrosis of renal tubules associated, inflammatory cells infiltration (Pic. 3), atrophy of glomerular tuft (Pic. 4) and vacuolation of epithelial lining renal tubules (Pic. 5). Meat balls treated group showed congestion of renal blood vessel (Pic. 6) with no histopathological changes in other section (Pic 7). A renal section of PG peel meat balls showed vacuolation of epithelial lining renal tubules (Pic 8), however there were no histopathological changes in other section (Pic. 9). While both of PG molasses treated group (Pic. 10&11) and peel&molasses mix meat balls treated group (Pic. 12&13) showed no histopathological changes. Indeed PG peel, molasses or peel and molasses allowed, to a certain extent, overcoming renal architecture aberrations with the preservation of parenchymal structure.

FIG

Hu *et al.* (2000) pointed out that supplemental dietary Arginine may have adverse effect in advanced glycation end-induced kidney inflammatory response and oxidative damage in rats. PG Juice was found to have inhibitory effects on renal tubular cell injury and oxidative stress caused by oxalate crystals by reducing ROS, iNOS, p38-MAPK, and NF-kB expression (Ilbey 2009). Toxicity of the polyphenol antioxidant punicalagin, abundant in pomegranate juice, was evaluated in rats. No toxic effects or significant differences were observed in the treatment group compared to controls, which was confirmed via histopathological analysis of rat organs (Cerda *et al.* 2003). The protective effects of the PM against ROS generated by the electrolysis were histologically demonstrated (Chalfoun-Mounayar *et al.* 2012).

In conclusion, the addition of pomjegranate peel, molasses or peel&molasses mix to meat balls recorded acceptability in all sensory parameters. The peel&molasses mix formulation recorded the highest overall acceptability scores. Renal function appears to be improved with meatballs incorporated into pomegranate peel, molasses or peel&molasses mix. Findings of the present study reveal that pomegranate may prove to be a suitable supplement to produce acceptable meat product with the ability to attenuate and protect against renal diseases complications by improving antioxidant defense system. Further studies are needed to confirm this effect in human.

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التأثير الوقائي لقشور و مولاس الرمان المدمجة مع كرات اللحم على الفشل الكلوي في الفئران

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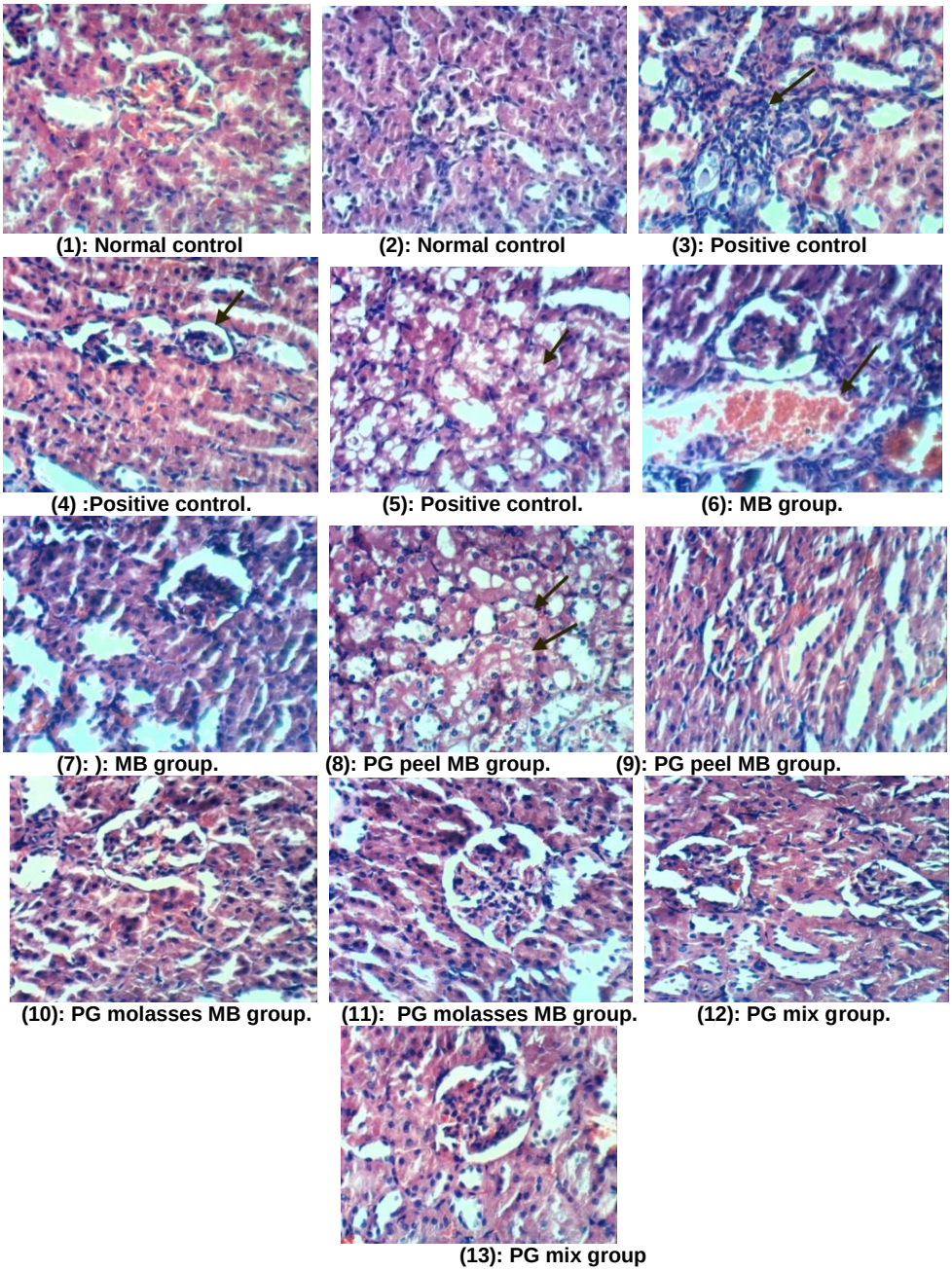
هدف البحث لدراسة تأثير استخدام قشور الرمان و المولاس (دبس الرمان) في اعداد كرات اللحم على الخواص الحسية و التركيب الكيميائي للمنتج و كذلك تأثيره على تطور الفشل الكلوي الناتج من تناول كميات مفرطة من الآرجنين في الفئران. أدمج مطحون قشر الرمان أو دبس الرمان أو مزيجهما في كرات اللحم عند مستوى ٣ و ٥ و ٨ %. تم تقدير التركيب الكيميائي و التقييم الحسي و اختيرت النسب ٣% قشر و ٥% مولاس و ٥% مزيج القشر و المولاس للتقييم البيولوجي. تم قياس الوزن و حساب المتناول اليومي من الغذاء و معدل الكفاءة الغذائية و معدل كفاءة البروتين. و تقيدر مستوى الكرياتينين و حامض اليوريك و يوريا الدم و الألبيومين و الجلوبيولين و البروتين الكلي و حامض الثيوباربيوتريك و C-reactive protein (CRP) و serum tumor necrosis factor-α (TNF-a) و السعة التأكسدية (TAC) في السيرم. كما تم قياس مستوى إنزيم الكتاليز في السيرم و مادة الجلوتاثيون (GSH) و انزيم السوبرأكسيددسميوتيز(SOD) في أنسجة الكلية. و قد أوحت النتائج بحدوث فشل في وظائف الكلية نتيجة تناول الآرجنين تسبب في انخفاض في الوزن و معدل الكفاءة الغذائية و معدل كفاءة البروتين و مستوى الأبيومين و الجلوبيولين و البروتين و السعة التأكسدية و مستوى السوبر أكسيددسميوتيز و مادة الجلوتاثيون و إنزيم الكتاليز. أدى أيضا لارتفاع مستوي حامض الثيوباربيوتريك و مؤشرات وظائف الكلى و مؤشرات حدوث الالتهابات (C-reactive protein و tumor nicrosis (TNF-a)). و قد خففت المعاملة المتزامنة بمشتقات الرمان من الضغوط التأكسدية و علامات الفشل الكلوي. قد خلصت النتائج إلى أن تناول كرات اللحم المحتوية على مطحون قشور الرمان بنسبة ٣% أو مولاس الرمان بنسبة ٥% أو مزيجهما بنسبة ٥% لها تأثير وقائي يمكن أن يعزى إلى تأثيراتها المضادة للأكسدة مع الحصول على طعم مقبول و رائحة أفضل للمنتج.

التوصيات: و توصي الدراسة باستخدام قشور الرمان و مولاس الرمان و مزيجهما في اعداد منتجات اللحوم على المستوى الصناعي. كما توصي ذوي الاستعداد للقصور الكلوي بادماج مشتقات الرمان في منتجات اللحوم.



(1): Normal control (2): Normal control (3): Positive control

(4) :Positive control. (5): Positive control. (6): MB group.

(7):): MB group. (8): PG peel MB group. (9): PG peel MB group.

(10): PG molasses MB group. (11): PG molasses MB group. (12): PG mix group.

(13): PG mix group

Fig. (1): Histopathological examination
MB: Meat balls; PG: Pomegranate