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# Evaluation of Functional Foods for Prevention of Hepatorenal Syndrome in Rat Model.

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**ABSTRACT**

Hepatorenal syndrome (HRS) is a functional renal impairment occurs in patients with liver disease or liver injury. The disease is associated with significant morbidity and mortality. The aim of the present study was preparation of two functional foods (A and B) in form of bakery products to prevent the progression to HRS. The functional foods were evaluated in HRS rat model induced by intraperitoneal injection of D-(+)-Galactosamine hydrochloride. The chemical composition and sensory evaluation of functional foods were assessed. Sensory attributes showed no significant changes between the two functional foods. Proximate composition of functional foods revealed that functional food A was higher in protein, ash and carbohydrate contents, while functional food B was higher in moisture, fat and fibers levels. HRS control rats showed significant elevation in plasma levels of creatinine, urea, uric acid and activities of transaminases with significant reduction in angiotensin-1 converting enzyme (ACE-1) compared to control normal. Feeding rats on diet containing 30% functional food A or B produced reduction in transaminases activity compared with HRS control. Also a reduction in the levels of creatinine, urea and uric acid was observed, whilea plasma level of ACE-1 was elevated significantly. Plasma levels of malondialdehyde (MDA), plasma nitrite (NO) and C-reactive protein (CRP) increased significantly in HRS control compared to normal. Both functional food diets produced significant improvement in plasma levels of CRP, NO and MDA compared with HRS control. Creatinine clearance was reduced significantly in HRS control group compared to normal control, while improved significantly in rats fed on functional food A or B nearly to the normal level. The results of nutritional parameters revealed non-significant changes in all experimental groups. **Conclusion;** The studied functional foods showed beneficial effects towards HRS; functional food B was slightly more promising than functional food A.

**Keywords:** Hepatorenal syndrome, rat model, Galactosamine hydrochloride, functional food.

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## Introduction

Hepatorenal syndrome (HRS) is characterized by renal dysfunction in severe liver diseases. During HRS there are renal vasoconstriction, reduced glomerular filtration rate (GFR), subsequent rise in creatinine, and impaired sodium and water excretion (1). No changes in renal histopathology were reported in HRS (2). Several mechanisms are involved in the development of HRS, including circulatory changes, kidney factors and systemic inflammation (3).Over the past 20 years, the incidence and prevalence of HRS has decreased, much of which is attributed to medical advancements in cirrhosis management and the availability of prophylactic antibiotics for spontaneous bacterial peritonitis (4). Oxidative stress and inflammation plays an important role in the progression of HRS. So reduction in both oxidative stress and inflammation are a major target in the treatment of HRS. In the present study two functional foods were prepared in form of bakery products. These functional foods contain functional ingredients from edible plants which previously showed beneficial effects towards HRS and for healthy liver in animal models (5-7). The aim of the present study was to prepare two functional foods in form of bakery products to prevent the induction of HRS. The effect of these functional foods towards HRS was evaluated in rat model using D- (+)-galactosamine hydrochloride. The proximate composition and organoleptic characteristics of the functional foods were assessed.

## MATERIALS

**Plant materials.** Green tea powder, *Nigella sativa* seeds, corn oil, brown rice, oat, mannitol, *Nigella sativa* oil, wheat germ oil, whey protein, yoghurt, honey, wheat flour, vanilla, and yeast were purchased from local markets, Cairo, Egypt.

**Chemicals:** D-(+)-Galactosamine hydrochloride was purchased from Sigma, USA. All other chemicals used in the experiment were of analytical grade.

**Animals** Male Sprague Dawley rats weighing 167–210 g (187.8 ± 12.25 as mean ± SD) were used in the present study. Animals were obtained from Animal house of National Research Centre, Cairo, Egypt. Animals were kept individually in stainless steel metabolic cages; water and food were given *ad-libtium*.

## METHODS

**Preparation of functional foods:** Two functional foods (A &B) were prepared inform of bakery products for prevention of hepatorenal syndrome. Ingredients of formula A were brown rice, oat, mannitol, *Nigella sativa* oil, wheat germ oil, whey protein, yoghurt and honey. Ingredients of formula B were wheat flour, green tea powder, *Nigella sativa* powder, corn oil and yeast.

**Organoleptic characteristics (Sensory evaluation) of functional foods**. Organoleptic characteristics of functional foods were evaluated according to Hoojjat and Zabik (8) where each formula was subjected to sensory analysis by 20 panelists. Each panelist was asked to assign scores 0-20 for color, odor, taste, texture, appearance and overall acceptability (0-100). A sensory score of 10 or above for an individual characteristic was deemed acceptable, and a sensory score below 10 was considered unacceptable.

**Proximate composition of different prepared functional foods.** Moisture, protein, fat, crude fiber and ash of cookies were determined according to AOAC (9). Carbohydrates were calculated by differences.

**Total sugars, reducing and non-reducing sugars.** Total sugars, reducing and non-reducing sugars were assessed as described previously (9).

**Diets:** Experimental diets were prepared as in table (1). Salt mixture and vitamin mixtures were prepared according to Briggs and Williams (10) and Morcos (11), respectively. Oil soluble vitamins were given orally in a dose of 0.1 mL/rat per week. Functional foods were dried and grinded into powder before being added to the diets.

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**Table (1): Composition of different diets (g per 100 g).**

| Ingredients | Balanced diet | DietA[a] | Diet B[b] |
|---|---|---|---|
| ***Casein** | 12 | 12 | 12 |
| **Corn oil** | 10 | 10 | 10 |
| **Starch** | 70.5 | 40.5 | 40.5 |
| **Salt mix.** | 3.5 | 3.5 | 3.5 |
| **Vitamin mix.** | 1 | 1 | 1 |
| **Cellulose** | 3 | 3 | 3 |
| **Functional food A powder** | - | 30 | - |
| **Functional Food B powder** | - | - | 30 |

*12 g. casein has been estimated to contain 10 g protein using AOAC (2000).

[a] Diet A: Balanced diet supplemented by functional food A powder.

[b]Diet B: Balanced diet supplemented by functional food B powder.

**Experimental procedures**

Twenty four rats were divided into four groups, each comprised six rats. All rats were fed on balanced diet for three weeks. The first was normal group where rats received a balanced diet. Rats of group two and three were fed on balanced diet containing 30% of functional food A and B, respectively for three weeks. The fourth group was hepatorenal syndrome control where rats were fed on balanced diet. During the experiment, body weight and food intake were recorded weekly. At the end of the study, total food intake, body weight gain and food efficiency ratio (Body weight gain/total food intake) were calculated. At the 20$^{th}$ day all rats except normal control were received 1.1 g/kg body weight of D- (+)-galactosamine hydrochloride via intraperitoneal injection as a 200 mg/ml saline for induction of HRS according to Saracyn *et al*. (12). Twenty-four-hours urine samples were collected during 24 h from the galactosamine hydrochloride injection for determination of creatinine (13) for calculation of creatinine clearance. Blood samples were collected on heparin from all rats after an overnight fast and plasma was separated by centrifugation. Plasma malondialdehyde (MDA) was determined according to Satoh (14) as indicator of lipid peroxidation. Plasma nitrite (NO) was assessed by the method of Montogomery and Dymock (15) as representative of both oxidative stress and inflammation. Plasma C-reactive protein (CRP) (an inflammatory biomarker) was determined by ELISA (16). The activity of aspartate transaminase (AST) and alanine transaminase (ALT) was estimated as reported by Reitman and Frankel (17) as indicator of liver function. Plasma creatinine, uric acid and urea were determined according to Houot (13), Watts (18) and Fawcett and Scott (19), respectively as indicator of kidney function. Plasma angiotensin-1 converting enzyme (ACE-1) was determined adopting ELISA by the method of Oliveri *et al*. (20). The animal procedure was performed according to approved protocols and in accordance with the Ethics Committee of the National Research Centre, Cairo, Egypt and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

**Statistical analysis.** The data of animal experiment are expressed as the mean±SE and they are analyzed statistically using the one-way analysis of variance ANOVA followed by Duncan's test. In all cases $p<0.05$ was used as the criterion of statistical significance. The results of organoleptic characteristics and proximate composition were evaluated statistically using analysis of variance as reported by McClave and Benson (21).

## RESULTS

**Sensory evaluation**

Functional foods are shown in Fig 1. Sensory evaluation of the prepared functional foods (table 2) showed no significant changes in the different sensory attributes between the two formulas. Both functional foods were accepted by the panelists.

**Proximate composition of functional foods**

Proximate compositions of the functional foods are present in table 3. Percentage of protein in functional food A (10.6) was significantly higher than that in B (8.86). Moisture content was 19.79% and

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26.33% in functional food A and B, respectively with significant difference. Ash was 1.54% and 1.27 in formulas A and B, respectively. Fat content in functional food A (4.96) was significantly lower than in functional food B (8.25%). Fibers of formula B (1.77%) were significantly higher than formula A (0.85%). Total carbohydrate showed significant higher level in formula A (62.26%) compared to formula B (53.52%).Total and non-reducing sugars in functional food A were significantly higher than that in functional food B while reducing sugar showed significant lower level in functional food A compared to B.


**Fig1: Functional foods A and B**

**Table (2): Sensory properties of functional foods (Mean ±SE).**

| Parameters | Functional food A | Functional food B |
|---|---|---|
| **Taste(20)** | $15^{a}$± 2.16 | $17.2^{a}$±0.512 |
| **Oder(20)** | $18^{a}$.± 3.09 | $17.1^{a}$±0.658 |
| **Color(20)** | $15^{a}$± 2.15 | $14.8^{a}$±0.611 |
| **Texture(20)** | $13^{a}$± 1.59 | $16.8^{a}$±0.490 |
| **Appearance(20)** | $15^{a}$± 3.09 | $15.1^{a}$±0.482 |
| **Overall acceptability(100)** | $76^{a}$±3.65 | $86.2^{a}$±2.319 |

In each raw same letters mean non-significant difference at 0.05probability.

**Table (3):Chemical composition of fresh functional foods formulas (Mean±SE).**

| Parameters | Functional food A | Functional food B | LSD at 0.05 |
|---|---|---|---|
| **Moisture** | $19.79^{d}$±0.11 | $26.33^{b}$±0.19 | 0.088 |
| **Protein** | $10.6^{a}$±0.5 | $8.86^{b}$±0.07 | 0.083 |
| **Fat** | $4.96^{c}$±0.09 | $8.25^{b}$±0.05 | 0.062 |
| **Ash** | $1.54^{a}$±0.04 | $1.27^{b}$±0.05 | 0.038 |
| **Fiber** | $0.85^{d}$±0.01 | $1.77^{b}$±0.03 | 0.057 |
| **Total carbohydrates** | $62.26^{b}$±0.45 | $53.52^{d}$±0.65 | 0.083 |
| **Total sugars** | $11.91^{a}$±0.07 | $8.98^{c}$±0.08 | 0.042 |
| **Reducing sugars** | $2.37^{b}$±0.02 | $4.08^{a}$±0.01 | 0.073 |
| **Non Reducing sugars** | $9.54^{a}$±0.09 | $4.9^{b}$±0.03 | 0.32 |

In each raw different letters mean significant difference at 0.05probability.

Table 4 showed the different biochemical parameters of the studied experimental groups. Plasma activities of AST and ALT were significantly higher in HRS control than control healthy group, indicating liver dysfunction. Feeding rats on balanced diet containing 30% of functional food A or B reduced the activity of ALT and AST compared with HRS control but still higher than normal control. Plasma levels of creatinine, urea and uric acid were significantly high in HRS control rats compared with normal rats, while ACE-1 plasma level was reduced significantly in HRS control compared with normal control indicating kidney dysfunction. Supplementation of balanced diet with functional food A or B reduced the elevation observed in the plasma

levels of creatinine, urea and uric acid to reach levels similar to normal control, while plasma levels of ACE-1 was elevated significantly in both treated groups but still lower than normal control. The results indicate that rats fed on balanced diet containing 30% of functional food A or B showed significant improvement in liver and kidney functions with different degrees compared with HRS control group. Plasma levels of MDA and CRP (as indicator to lipid peroxidation and inflammation, respectively) increased significantly in HRS control group compared with normal control. Also plasma nitrite level was increased significantly in HRS control compared with normal control. Both treatments showed significant reduction in plasma levels of CRP, NO and MDA compared with HRS control group while still higher than the normal level. Creatinine clearance was reduced significantly in HRS control group compared to normal control group. Feeding rats on balanced diet containing 30% of functional food A or B showed significant increase in creatinine clearance levels nearly to normal control.

**Table (4): Biochemical parameters of different experimental groups (Mean+SE).**

| Parameters | Normal control | HRS control | HRS + Functional Food A | HRS + Functional Food B |
|---|---|---|---|---|
| **Plasma parameters:** | | | | |
| **Creatinine (mg/dl)** | $0.746^{a}$±0.032 | $1.10^{b}$±0.043 | $0.829^{a}$±0.026 | $0.81^{a}$±0.024 |
| **Urea (mg/dl)** | $28.3^{a}$±1.489 | $44.6^{b}$±1.071 | $29.4^{a}$±0.507 | $29.0^{a}$±0.808 |
| **Uric acid (mg/dl)** | $1.0^{a}$±0.064 | $1.25^{b}$±0.018 | $1.04^{a}$±0.030 | $0.983^{a}$±0.049 |
| **ALT (U/l)** | $27.2^{a}$±1.469 | $83.5^{b}$±2.045 | $71.2^{c}$±1.815 | $68.5^{c}$±2.232 |
| **AST (U/l)** | $37.8^{a}$±1.301 | $95.2^{b}$±2.599 | $75.5^{c}$±2.045 | $71.5^{c}$±1.944 |
| **ACE-1 (pg/ml)** | $525^{a}$±7.636 | $388.3^{b}$±10.773 | $443.3^{d}$±11.153 | $491.7^{c}$±7.922 |
| **NO (µmol/l)** | $10.2^{a}$±0.499 | $18^{b}$±1.064 | $14.5^{d}$±0.076 | $12.8^{c}$±0.601 |
| **MDA (nmol/ml)** | $9.2^{a}$±0.363 | $17.5^{b}$±0.588 | $13.2^{c}$±0.438 | $12.3^{c}$±0.519 |
| **CRP(ng/ml)** | $0.708^{a}$±0.039 | $1.23^{b}$±0.084 | $0.9^{d}$±0.029 | $0.81^{c}$±0.024 |
| **Urine parameter:** | | | | |
| **Creatinine clearance (ml/min)** | $1.01^{a}$±0.071 | $0.515^{b}$±0.030 | $0.894^{a}$ ±0.042 | $0.898^{a}$±0.034 |

In each row same letters mean non-significant difference; different letters mean significant difference at 0.05 probability.

Nutritional parameters of all studied groups are present in table 5. The results revealed non-significant changes among all nutritional parameters between the different experimental groups.

**Table (5): Nutritional parameters of different experimental groups (Mean± SE).**

| Parameters | Normal control | HRS control | HRS + Functional Food A | HRS + Functional Food B |
|---|---|---|---|---|
| **Initial BW(g)** | $185.67^{a}$±5.99 | $185.67^{a}$±2.56 | $185.67^{a}$±5.282 | $185.67^{a}$±4.37 |
| **Final BW (g)** | $261^{a}$±8.511 | $257.7^{a}$±5.851 | $261.3^{a}$±6.720 | $266.8^{a}$±3.771 |
| **Body weight gain (g)** | $75.3^{a}$±3.843 | $72^{a}$±5.452 | $75.7^{a}$±4.751 | $81.2^{a}$±3.299 |
| **Total Food intake (g)** | $348.8^{a}$±8.606 | $332.2^{a}$±11.13 | $310.7^{a}$±4.629 | $353.2^{a}$±9.377 |
| **Food efficiency ratio** | $0.218^{a}$±0.009 | $0.216^{a}$±0.010 | $0.245^{a}$±0.012 | $0.229^{a}$±0.007 |

In each row same letters mean non-significant difference; different letters mean significant difference at 0.05 probability.

## DISCUSSION

Hepatorenal syndrome is a serious complication of advanced chronic liver disease consisting of functional but not morphological renal failure, progressive decline in GFR, sodium with holding, oliguria and azotemia (22). High percentages of end-stage de-compensated liver disease patients (40-80%) develop HRS (23). During HRS there is reduction in renal blood flow due to severe liver dysfunction induced by cirrhosis and ascites (24, 25). Progression of cardiac dysfunction which is known as cirrhotic cardiomyopathy might further worsen renal impairment. The features of cirrhotic cardiomyopathy include diastolic and diastolic dysfunction

and electrophysiological abnormalities (4). The dysfunction in systolic is attributed to malfunction of β adrenergic receptors and enhancement of myocyte apoptosis along with the increase in endogenous cannabinoids and cardio suppressants such as inflammatory cytokines and nitric oxide. Diastolic dysfunction could be related to salt retention and rennin angiotensin system activation. Myocardial dysfunction is recently reported as precipitating factor in HRS (26, 27).

In the present study galactosamine hydrochloride was used for induction of HRS in rats. Galactosamine (GAL) is a 6-carbon amino sugar derivative of galactose. Under physiological conditions, it is a component of specific glycoprotein hormones, such as follicle-stimulating hormone or luteinizing hormone (28). GAL is a potent hepatotoxic substance, which can cause hepatocyte death both by necrosis and apoptosis. It inhibits the synthesis of liver RNA via the production of uridine diphosphate hexosamines, which block the transcription of genetic material (29). Short-term administration of GAL to experimental animals causes liver damage and acute kidney failure without changes in renal histopathology (6, 7, 29, 30) which simulate HRS in human.

Antioxidant status of HRS control in the present study was reduced significantly through elevation of NO and MDA pointed to the increased oxidative stress. MDA is considered as a highly reactive product which produced from reactions´ series due to lipid peroxidation induced by reactive oxygen species in the tissues (31). Elevated oxidative stress is one of the main factors that could lead to inflammation reflected in the increased CRP and damage of both liver and kidney.

GAL in the present study induced liver damage reflected in a very significant increase in the level of cellular enzymes alanine and aspartate aminotransferases. These results agreed with that of Cuesta *et al*. (33). GAL also produced elevation of inflammatory markers represented by of CRP and NO.

In the present study, renal dysfunction developed rapidly in a typical manner, with a parallel increase in nitrogen retention parameters and a decrease in creatinine clearance. Renal function tests demonstrate a marked decline in renal blood flow and the glomerular filtration rate, with a simultaneous increase in urea and creatinine in plasma.

The results revealed that plasma uric acid was significantly high in HRS rat model. Experimental studies revealed that the elevated uric level could induce renal disease without the deposition of uric acid crystals (34, 35). This might be due to induction of oxidative stress and endothelial dysfunction by the increased uric acid level leading to both glomerular and systemic hypertension that associated by elevation in renal vascular resistance and reduction in renal blood flow (36, 37).

In the present study the results revealed that in HRS model; ACE-1 was reduced significantly. Angiotensin I converting enzyme type-1 is the second rate limiting enzyme that controls the liberation of angiotensin II (Ang II) which is the most active component in renin-angiotensin system (38). Ang II has numerous biological activities, including vasoconstriction, antinatriuresis, and antidiuresis, actions which are closely affiliated to renal clearance functions and blood pressure regulation (39). It is most probable that under physiological conditions renal and systemically produced Ang II work synergistically, where renal Ang II acts as the principle paracrine regulator of the kidneys' clearance function determinants, including renal hemodynamics, glomerular filtration rate, and tubular handling of electrolytes and water (40). Evidences suggest that Ang II enhances cellular reactive oxygen species (ROS) production such as hydrogen peroxide and superoxide anion leading to kidney damage (41). Elevated oxidative stress due to liberation of ROS is one of the most important factors that contribute to this reperfusion injury. Oxidative stress might also increase due to reduction in ROS scavenging activity. Disorganization of cell structure and function occurs due to reaction of membrane lipids with ROS resulting in peroxidation. After re-perfusion and re-oxygenation, massive generation of superoxide anion results due to imbalance between restoration of oxygen supply and mitochondrial respiratory function (42, 43). In this condition, the defensive system represented by the antioxidant enzymes and other body antioxidants cannot guard against the escape of ROS in the mitochondria and other intracellular sites (43).

In the present study supplementation of balanced diet containing functional foods reduced plasma ACE-1, creatinine, urea and uric acid levels significantly compared with HRS control pointing to prevention of progression of liver disease to HRS. It was reported that serum uric acid, creatinine and urea are considered as

biomarkers of glomerular filtration rate (44). Functional foods also showed significant improvement in antioxidant status through reduction of both NO and MDA and reduction of inflammation as could be seen from the decreased level of CRP. As expected due to reduction of oxidative stress and inflammation liver function parameters (ALT and AST) were improved significantly in HRS rats fed on balanced diet containing functional food A or B compared with HRS control rats. These effects may be attributed to the presence of bioactive ingredients in whey protein, green tea, wheat germ oil, oat, brown rice, honey, yoghurt and *Nigella sativa* in the functional foods. All the ingredients used in the present study are rich sources of antioxidants that could protect the components of living cells including protein, lipids and DNA from oxidation. If imbalance in the ratio between antioxidants and free radicals occurs, this could lead to pathological changes that induce cellular damage (45).

In a previous study green tea extract reduced serum uric acid significantly in healthy subjects (46). It was reported that tea polyphenols showed significant reduction of creatinine levels and in the same time attenuate the increment in the inflammatory markers TNF-α, IL-6 and IL-1βas well as elevate the antioxidant enzymes superoxide dismutase and glutathione peroxidase in rat model of kidney injury. Tea polyphenol pretreatment could suppress TLR4/NF-κB p65, the apoptotic signaling pathway, thereby prevent ischemic/reperfusion injury by protecting renal tubular epithelial cells from apoptosis (47). Green tea polyphenols markedly attenuate nephrotoxicity caused by cyclosporine A with subsequent improvement in renal dysfunction. This polyphenols effect could be due to stimulation of mitochondrial biogenesis (48). Al-Okbi *et al*. (7) reported hepato and reno-protective effect of green tea extract in HRS rat model. These cited literatures could explain the improvement in HRS condition in the present study on feeding the functional food that contains green tea.

*Nigella sativa* oil and thymoquinone administration produced reduction in albuminurea in animal model of nephropathy through preservation of podocyte function and suppression of enhanced extracellular matrix gene expression by interfering with TGF- β1 (49). Also, *Nigella sativa* hydro-alcohol extract reduced oxidative stress in adriamycin treated animal (50). Inflammatory processes and oxidative stress have a crucial role in inducing glomerulonephritis, nephropathy and acute renal dysfunction (51). *Nigella sativa* is used for curing many diseases including renal disorders through lessening oxidative stress (52, 53). *Nigella sativa* aqueous ethanol extract normalzed both serum and urine parameters reflecting improved kidney function in cisplatin induced nephrotoxicity in rats (54). *Nigella sativa* extract possesses hepatoprotective action reflected in improving the changes in AST, ALT and serum protein (55, 56). Al-Okbi *et al*. (6) reported hepato and reno-protective effect of *Nigella sativa* oil. So, *Nigella sativa* powder and oil are good option as ingredients of the functional foods studied in the present research.

Wheat germ oil (WGO) is rich in the powerful lipid soluble antioxidants tocopherols and tocotrinols that present as 1300-2700 mg/kg. Other bioactive compounds of WGO were reported such as phytosterols (24-50 mg/Kg), plicosanol (10mg/Kg) and carotenoids (4.0–38 mg/kg) (57). The content of phytosterol in WGO is higher than in other commercial oils (58, 59). WGO is a good source of linoleic and alpha linolenic acid. WGO may reduce the intensity of lipid peroxidation processes by stimulating the tocopherol redox-system (60) because it is a rich source of the aforementioned natural antioxidant like tocopherols and sterols (61, 62). WGO has DNA protective effect in addition of reducing the autoxidation of unsaturated fatty acids (63).

Oat is an important source of water-soluble fibers especially $\beta$-glucan reported previously toimprove kidney function (64-66). In addition, oat is a source of antioxidants, such as tocopherolsand various phenolic compounds thereby promotes scavenging of reactive oxygen species (67-69).So oat ingredient of functional food could impart protective effect against HRS.

Mannitol is an osmotic diuretic sugar. Mannitol is used for certain cases of kidney failure with low urine output and to treat fluid buildup. It prevents cisplatin induced nephrotoxicity (70). Mannitol possesses free radical scavenging properties (71). Therefore mannitol inclusion in functional food A could participate in prevention of HRS seen in the present research.

Brown rice is covered by rice bran to which all the bioactivities are ascribed. Rice bran is rich source of phytochemicals and phytonutrients that possess antioxidant and anti-inflammatory activity. Such bioactive

constituents include phenolic compounds, phytosterols, tocopherols, tocotrienols, policosanol, oryzanol, ferulic acid and dietary fibers (72-74).

Whey protein was reported to improve malnutrition inflammation scores in hemodialysis patients (75). The inflammatory biomarker C-reactive protein was reduced in hemodialysis patients on supplementation of whey protein (76). Whey protein also has great beneficial health effect in liver injury and steatosis (77, 78). These literatures clarify the therapeutic benefits of whey protein towards kidney and liver as an ingredient of functional food.

Yoghurt present in functional food A is a rich source of the probiotic *lactobacillus* that could improve microbiota imbalance, reported in renal dysfunction, thus improving endothelial dysfunction, vascular inflammation, vascular oxidative stress, and renal hypertrophy (79).Even if the thermal effect during food processing might affect the viability of probiotic, dead probiotics were reported to still possess beneficial effect (80). Honey in the same functional food is considered as prebiotic (81) who could have a synergistic effect with *lactobacillus* in improving the microflora.

All ingredients of the two functional foods could act synergistically to elicit HRS protective effect in the present study.

## CONCLUSION

Both functional foods prepared and evaluated in the present study showed promising protection towards HRS. They could normalize renal function tests represented by creatinine, urea and uric acid however all parameters reflecting inflammation, antioxidant status and liver function were significantly improved without reaching the normal levels. Functional food B was slightly more efficient than A.

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