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Research Article

Potential Hepato- and Reno-Protective Effect of Artichoke Callus Culture and Its Alcohol Extract in Galactosamine Hydrochloride Treated Rats

Al-Okbi S Y[1*], Mohamed D A [1], Gabr A M M[2], Mabrok H B [1], Hamed T E[1]

[1]*Nutrition and Food Sciences Department, National Research Centre, Cairo,12622, Egypt.*
[2]*Plant Biotechnology Department, National Research Centre, Cairo, 12622, Egypt.*

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ABSTRACT

Plant cell culture provides an alternative source of valuable secondary products which can be used as food supplements and nutraceuticals. The aim of the current work was to study the protective effect of artichoke callus culture and its alcohol extract towards hepatorenal syndrome (HRS) model in rats. Total phenolic, flavonoids, anthocyanin, antioxidant capacity, chlorogenic acid and cynarin contents were determined in the callus. The safety of the extract was studied through application of acute toxicity test. For HRS experiment, two weeks accommodation period for rats was followed by assigning four groups. Group one and two served as control normal and control HRS, respectively. Group three and four were treated by daily oral dose of artichoke callus (200 mg/kg) and its alcohol extract (100 mg/kg), respectively for three weeks. Rats of the four groups were fed on balanced diet all over the experiment. At the 20th day of experiment rats of group 2, 3 and 4 were treated intraperitoneally by D-(+)-Galactosamine hydrochloride for induction of HRS. In HRS control; plasma creatinine, urea and endotheline 1 were significantly elevated. In the same group plasma transaminases activity, total and direct bilirubin, and ammonia showed significant increase with reduction in albumin compared to control normal. Plasma malondialdehyde (MDA) and tumor necrosis factor alpha were increased significantly in HRS control. Urinary N-acetyl-β-D-Glucosaminidase was significantly elevated along with reduction of creatinine clearance in HRS control compared to control normal. Administration of either artichoke callus or its alcohol extract improved all biochemical parameters significantly; the callus was superior in improving plasma albumin and MDA. SDS-PAGE technique applied on liver tissue showed variation in protein bands among the different experimental groups. The callus showed higher contents of total phenolic and flavonoids compared to different parts of artichoke head, however anthocyanin of the inner purple artichoke petals was higher than that of the callus. Chlorogenic acid level was 252 µg/g dry callus while cynarin was 60 µg/g. The callus alcohol extract showed DPPH radical-scavenging activity % of 46.35 and was very safe up to the highest tested dose (12 g/kg mice).

Keywords: Callus culture, chlorogenic acid, cynarin, globe artichoke, hepatorenal syndrome**,** rat model, SDS-PAGE technique**,** *in-vitro* antioxidant activity**.**

INTRODUCTION

The Artichoke (*Cynara scolymus* L., Family Asteraceae) is an edible vegetable from the Mediterranean region. The globular flower heads are used in the immature state as a salad or vegetable; only the lower part of the fleshy bracts (leaves) and the center (heart) are eaten. Artichoke is a good source of natural antioxidant such as vitamin C, hydroxycinnamic acids and flavones. In fact, artichoke is a potential good source of antioxidant and anti-inflammatory bioactive constituents because it contains large amounts of caffeic acids[1,2]. Caffeic acid derivatives are the main phenolic compounds in artichoke heads, with a wide range of caffeoylquinic acid derivatives[3] with chlorogenic acid (5-O-caffeoylquinic acid) as the most important of these derivatives[4]. Other phenolics such as the flavonoids apigenin and luteolin (both glucosides and rutinosides) as well as different cyanidin caffeoylglucoside derivatives have been identified[5,6]. Preparation of artichoke callus through tissue culture utilizing biotechnology is an important way to increase the level of different bioactive constituents, thereby producing callus rich in functional food ingredients that could be of highly potential health benefits compared to natural artichoke itself.

Plant biotechnology has been known as a suitable and sustainable tool for studying both the biosynthesis and the production of plant secondary metabolites[7]. Plant biotechnology application for globe artichoke through micropropagation technology is well-developed for different artichoke species[8-12].

Development of acute renal failure in a patient with advanced liver disease is called hepatorenal syndrome[13]. Pathophysiological changes in HRS are still needed to be elucidated. Among the reported biochemical changes in HRS are the elevated oxidative stress and inflammatory biomarkers together with severe reduction of plasma

**Author for Correspondence: S_Y_alokbi@hotmail.com*

albumin and elevated endothelin-1[14,15]. Bioactive food ingredients that possess hepatoprotective effects as those present in artichoke could have a therapeutic impact on HRS. Inflammation and oxidative stress are among the different factors that contribute in the progression of HRS. It is hypothesized that artichoke callus could prevent induction of hepatorenal syndrome due to the presence of functional food ingredients of strong bioactivity as antioxidant, anti-inflammatory and hepatoprotective effect.

The aim of the present research was to use biotechnology to increase the level of bioactive ingredients in artichoke through tissue culture. The *in-vitro* antioxidant effect of the callus alcohol extract was assessed. The main goal is to study the protective effect of artichoke callus and its alcohol extract towards hepatorenal syndrome (HRS) model in rats. Total phenolic, flavonoids and anthocyanin contents were determined in the callus and compared to different parts of original artichoke head obtained from the market. The safety of the extract was evaluated by acute toxicity test.

MATERIALS AND METHODS

Seed sterilization and germination

Seeds of Global Artichoke, (purchased from Haraz trade, Cairo, Egypt), were used as starting plant material for *in vitro* callus culture. Seeds were washed with distilled water and then immersed in 70 % ethanol for 30 sec followed by 20 % commercial Clorox (containing 5.25 % sodium hypochlorite) for 10 min and finally washed three times with distilled sterilized water. The steps of disinfestations were took place under aseptic conditions in a laminar air-flow cabinet. Seeds were placed in 250 ml Erlenmeyer flasks contain 50 ml liquid free MS-basal medium on a rotary shaker at 120 rpm (as a sprout culture technique)[16]. MS is Murashige and Skoog medium. All cultures were maintained at 25 ± 2°C and under light irradiation 16/8 h light/dark.

Callus induction

Cotyledons were obtained after 18 to 21 days of the sprout culturing which were taken and re-cultured on fresh solidified MS medium contained 2 mg/l kinetin + 100 mg/l adenine sulfate to get stock plant material.

Leaves were taken and re-cultured on fresh solidified MS medium contained different concentrations (0.5, 1.0 and1.5 mg/l) of 2,4-Dichlorophenoxyacetic acid (2,4-D) + 0.5 mg/l of Benzyladenine (BA) for callus induction.

Callus proliferation

For mass proliferation of callus cultures, callus obtained from the previous step was transferred on MS medium containing 0.5 mg/l of 2,4-D + 0.1 mg/l of 1-naphthaleneacetic acid (NAA) + 0.5 mg/l of 2-isopentenyladenine (2ip). After 3 times subculture on the mass proliferation medium, callus were harvested, immersed directly in liquid nitrogen and lyophilized and stored in -80°C for further analysis.

Determination of total phenolics, flavonoids, total anthocyanins, chlorogenic acid and cynarin contents in artichoke

Total phenolics were determined colorimetric in artichoke tissue culture. Also, total phenolics were determined in different parts of artichoke head obtained from local market according to the modified Folin-Ciocalteu micro method[17]. Absorbance was measured at 760 nm using UVPC spectrophotometer. Gallic acid was used as standard for the calibration curve. Total phenolic content was expressed as gallic acid equivalent (GAE) in mg per g. dry weight.

Total flavonoids were determined in artichoke callus and in different parts of artichoke head purchased from local market according to the method of Ordon et al.[18]. Quercetin was used as standard for the calibration curve. Total flavonoids content was expressed as quercetin equivalent (QE) (mg QE g^{-1} dry weight).

The contents of anthocyanins were determined in artichoke callus and in different parts of artichoke head purchased from local market**.** Total anthocyanin was measured according to Sims and Gamon[19] with minor modification. Total anthocyanin was expressed as mg cyanidin-3-O-glucoside/Kg.

Total phenolics, flavonoids and total anthocyanins were determined in triplicate and then averaged.

Chlorogenic acid and cynarin were quantified with High-Performance Liquid Chromatography with Diode-Array Detection system (Perkin Elmer serie 200, Germany). The column was Prevail™ organic acid (250 × 4.6 mm, 5µ) and the temperature of column was 40°C.The flow rate was 1.0 ml/min and the injection volume was 10µl. The mobile phase was solvent A (formic acid: water 0.1%, v/v) and solvent B (formic acid: acetonitrile, 0.1%, v/v). Chromatograms were integrated at 330 nm. The concentrations of chlorogenic acid and cynarin were quantified by measuring peak area and comparing them to that of standards chlorogenic acid and cynarin obtained from Sigma-Aldrich, Germany.

Determination of free radical scavenging activity using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) method

Free radical scavenging activity of artichoke callus and different parts of artichoke head purchased from local market was determined utilizing the DPPH test according to the method of Lee et al.[20] with some modifications.

DPPH radical scavenging activity (%) = [Abs_{blank} – (Abs_{sample} /Abs_{blank})] x 100

Where

Abs_{blank} = Absorbance of DPPH radical of the blank

Abs_{sample} = Absorbance of DPPH radical of the sample

Animals

Male Sprague Dawley rats weighing 130–170 g were used in the present study in HRS experiment. Rats were kept individually in stainless steel metabolic cages; water and food were given *ad-libtium*. Male and female senile mice were used in the acute toxicity test. Animals were obtained from Animal house of National Research Centre, Cairo, Egypt**.** The animal experiments were carried out according to 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).

Diets

Experimental balanced diet was prepared and fed to rats all over the animal experiment. The diet contains 12% casein, 10% corn oil, 47% starch, 23.5% sucrose, 3.5% salt mixture, 1% vitamin mixture, and 3% cellulose.

Preparation of crude ethanol extract of artichoke callus

The powder of lyophilized artichoke callus culture was placed in a continuous extraction apparatus and subjected to extraction by ethanol. The solvent was completely removed by evaporation under reduced pressure at a temperature not exceeding 40°C. The extract was kept in deepfreeze till further studies.

Preparation of callus and ethanol extract emulsion

Callus powder and ethanol extract were prepared in emulsion form to facilitate their administration to animals. Callus was finely grinded and emulsified in distilled water using gum acacia as emulsifying agent. Likely, the ethanol extract emulsion was prepared. A vehicle containing only the same quantity of gum acacia in water was prepared to be given to control groups in HRS experiment.

The protective effect of artichoke callus and its alcohol extract in hepatorenal syndrome model in rats

All rats were fed on balanced diet for two weeks as adaptation period, and then divided into 4 groups, each comprised six rats. Rats of group three and four were treated by daily oral dose of 200 mg whole powder of artichoke callus /kg rat body weight or 100 mg artichoke callus ethanol extract /kg rat body weight (Both in emulsion form), respectively for three weeks. The first and second groups only received daily oral dose of gum acacia vehicle for the same period. Rats were fed on balanced diet all over the study. During the experiment, body weight and food intake were recorded weekly. At the 20th day (The 34th day if counting the adaptation period) all rats except those of normal healthy control group (group 1) received 1.1 g D-(+)-Galactosamine hydrochloride (Sigma, USA) per kg rat body weight via intraperitoneal injection in a concentration of 200 mg/ml saline according to Saracyn et al.[21]. Group 2 served as control HRS group. At the end of the study, total food intake, body weight gain and food efficiency ratio (Body weight gain/total food intake) were calculated. Twenty-four-hours urine samples were collected after galactosamine hydrochloride injection for determination of N-acetyl-β-D-Glucosaminidase (NAG) according to Price and Whiting[22], also creatinine was determined in the collected 24h urine following the method of Houot[23]. Blood samples were collected from all rats after an overnight fast for determination of malondialdehyde (MDA) according to Satoh[24] as indicator of lipid peroxidation. Plasma tumor necrosis factor-α (TNF-α) was estimated adopting ELISA according to Stepaniak et al.[25] as an inflammatory biomarker. Plasma activity of aspartate transaminase (AST) and alanine transaminase (ALT)[26], plasma albumin[27], total and direct bilirubin[28] and ammonia[29] were determined for assessing liver function. Plasma creatinine[23] and urea[30] were estimated as indicator of kidney function. Plasma endothelin-1 (ET-1)[31] was assessed as a proposed biomarker of hepatorenal syndrome. Creatinine clearance was calculated. Liver and kidney were separated and weighed to calculate organ weight/body weight %. A part of liver (only 3 samples from each group) was used to identify the different liver proteins through their molecular weights. This is implemented by separating proteins by electrophoresis using a discontinuous polyacrylamide gel as a support medium and sodium dodecyl sulfate (SDS) to denature the proteins, the method is called sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

SDS-PAGE

SDS-PAGE was carried out according to Laemmli[32]. Liver samples were ground in liquid nitrogen using a mortar and pestle. Tissue materials were homogenized manually with lysis buffer composed of 30 mMTris, 7 M urea, 2 M thiourea, 4% CHAPS detergent, and supplemented freshly with protease inhibitor (1:100; v/v). The homogenized samples were centrifuged (15,000 × *g* for 30 min at 4 °C), then the supernatant was collected and protein concentration was measured. Protein extract for each samples (50 µl) were added to the same volume of loading buffer. Samples were boiled for 10 minutes in water bath, then 10 µl bromophenol blue was added to each tube before sample loading (50µl). SDS-polyacrylamide gel electrophoresis was performed[32] using 10% running gel and 5% stacking gel. The protein bands were visualized by Commassie staining and were analyzed using gel analyzer 2010 software.

Acute lethal toxicity test of the alcohol extract of artichoke callus

Acute oral lethal toxicity test of crude ethanol extract of artichoke tissue culture was carried out[33]. The 24 h mortality counts (if any) among equal sized groups of mice (8 animals/group) receiving progressively increasing oral dose levels of the alcohol extract were recorded.

Statistical analysis

The results of animal experiment were expressed as the mean±SE and they were 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. Different phytochemical parameters and free radical scavenging activity were represented as mean ±SD.

RESULTS

Artichoke Callus culture

Fig 1A showed callus induction from cotyledons of artichoke on MS medium contained 1.5 mg of 2,4-D /l + 0.5 mg of BA /l. Callus proliferation on MS medium containing 0.5 mg of 2,4-D /l + 0.1 mg of NAA /l + 0.5 mg of 2ip /l is seen in Fig 1B.

Phytochemical contents and antioxidant activity of artichoke

The yield of ethanol extract of the callus was 51.96%; so, the dose of the alcohol extract tested in the present study (100mg/Kg rat body weight) was half that of the whole callus (200mg/kg rat body weight). Table 1 represents total phenolics, flavonoids, anthocyanins content and DPPH scavenging activity in globe artichoke callus and in different parts of artichoke head purchased from the local market. Artichoke callus showed the highest values of total phenolic content (559.24±1.99) and flavonoids

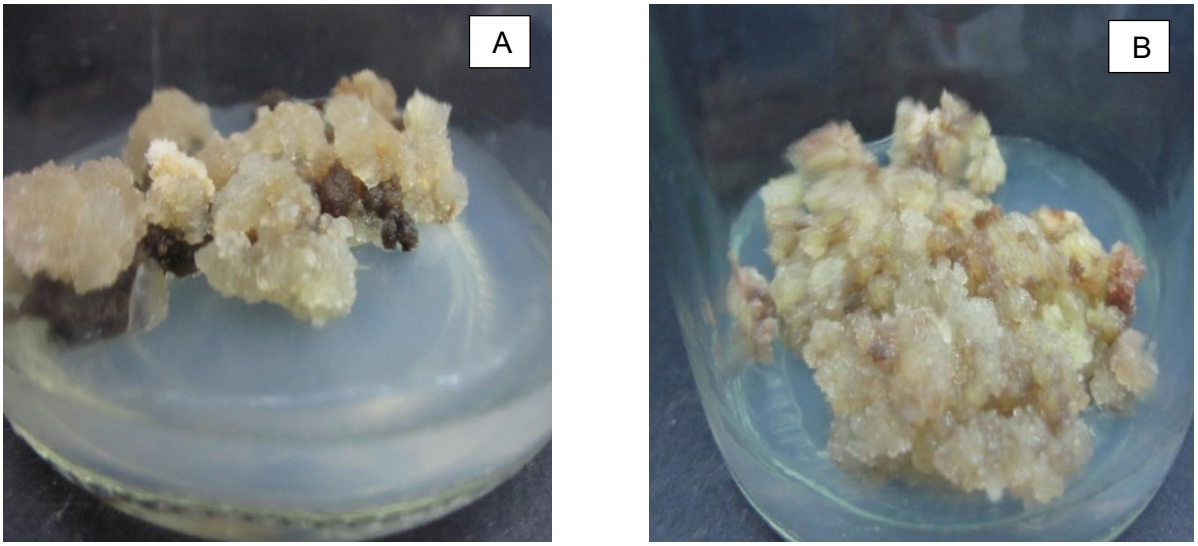

Figure1: Artichoke callus induction and proliferation.
A: Callus induction from cotyledons of Globe Artichoke on MS medium contained 1.5 mg/l of 2, 4-D + 0.5 mg/l of BA.
B: Callus proliferation on MS medium containing 0.5 mg/l of 2,4-D + 0.1 mg/l of NAA + 0.5 mg/l of 2ip.
MS medium: Murashige and Skoog medium
2, 4-D: 2,4-Dichlorophenoxyacetic acid
BA: Benzyladenine
NAA: 1-naphthaleneacetic acid
2ip: 2-isopentenyladenine

Table 1: Total phenolics, flavonoids, anthocyanins content and free radical scavenging activity of globe artichoke callus and different parts of artichoke head purchased from market.

	Total phenolics (mg GA/g)	Total flavonoids (mg QE/g)	Total anthocyanin (mg cyanidin-3-O-glucoside/Kg)	DPPH radical-scavenging activity% of ethanol extracts
Artichoke heart	363.73±1.27	383.39±2.16	10.38±0.39	41.59±0.007
Inner purple artichoke petals	432.05±0.86	440.01±1.65	102.02±0.81	43.03±0.007
Center choke (Hairy part)	402.63±0.85	387.92±1.09	18.33±0.23	40.43±0.001
Globe artichoke Callus	559.24±1.99	527.77±2.53	50.41±0.54	46.35±0.00

Values were expressed as mean± SD (n= 3)

Table 2: Biochemical parameters of different experimental groups.

Parameters	Normal healthy control	Hepatorenal control	Whole artichoke callus	Artichoke callus ethanol extract
Plasma creatinine (mg/dl)	0.682^{a}±0.031	1.03^{b}±0.021	0.726^{a}±0.022	0.717^{a}±0.021
Plasma urea (mg/dl)	28.3^{a}±1.488	45.8^{b}±1.176	31.2^{a}±0.499	32.8^{a}±1.301
Plasma albumin (g/dl)	4.1^{a}±0.159	2.72^{b}±0.141	3.7^{a}±0.088	3.3^{c}±0.109
Plasma AST (U/l)	55^{a}±0.816	85.5^{b}±0.991	75^{c}±1.825	73.5^{c}±1.784
Plasma ALT (U/l)	41.7^{a}±0.843	91.8^{b}±1.195	70.2^{c}±1.447	67.7^{c}±1.873
Plasma T. Bilirubin (mg/dl)	0.377^{a}±0.015	0.543^{b}±0.015	0.45^{c}±0.017	0.431^{c}±0.011
Plasma D. Bilirubin (mg/dl)	0.159^{a}±0.004	0.266^{b}±0.006	0.235^{c}±0.004	0.207^{c}±0.005
Plasma ammonia (µg/dl)	163.8^{a}±3.113	217.5^{b}±4.232	198.3^{c}±2.788	193.3^{c}±4.409
Plasma ET-1 (ng/ml)	24.7^{a}±0.989	41.2^{b}±1.815	29.3^{c}±0.715	29.5^{c}±1.784
Plasma MDA (nmol/ml)	6.6^{a}±0.256	22.7^{b}±1.620	12.8^{a}±0. 792	14.8^{c}±0.601
Plasma TNF-α (pg/ml)	17.5^{a}±0.764	32.8^{b}±0.654	21.8^{c}±0.792	20.7^{c}±1.453
Urinary NAG (IU/l)	36.7^{a}±0.361	48.6^{b}±1.307	41.5^{c}±0.991	40.5^{c}±0.846
Creatinine clearance	0.976^{a}±0.041	0.529^{b}±0.047	0.865^{a}±0.075	0.893^{a}±0.081

Values were expressed as mean± SE. In each raw same letters mean non-significant difference; different letters mean significance difference at 0.05 probabilities.

(527.77±2.53) compared to that in the different parts of artichoke head from local market. Anthocyanins showed the highest level in the inner petals followed by the callus, the heart and choke showed the least level. Percentage free radical scavenging activity was the highest in case of the callus, followed by inner purple artichoke petals, artichoke heart, then center choke (Hairy part). Chlorogenic acid was found to be 252 µg/g dry callus while cynarin was 60 µg/g.

Hepatorenal syndrome experiment

Table 2 showed biochemical changes of different experimental groups. Plasma levels of total and direct bilirubin and plasma activities of AST and ALT were significantly higher in HRS control than control healthy group, indicating liver dysfunction. Also, plasma albumin was significantly low in HRS control rats compared with normal rats. Plasma levels of creatinine, urea and endotheline-1 (ET-1) and urinary NAG were significantly high along with significant reduction of creatinine clearance in HRS control rats compared with normal rats, indicating kidney dysfunction. Oral administration of whole artichoke callus and its ethanol extract, showed significant improvement in liver and kidney functions with different degrees compared with HRS control group. The improvement only reached the normal level concerning creatinine clearance, plasma creatinine and urea. Plasma albumin only reversed to normal level on treatment with the whole callus. Plasma levels of MDA and TNF-α as indicator to lipid peroxidation and inflammation, respectively increased significantly in HRS control group compared with normal control. Both callus and ethanol extract treatments showed significant reduction in MDA and TNF-α levels compared with HRS control group, only MDA reached the normal level on treatment with the callus.

Nutritional parameters, liver weight/body weight% and kidney weight/body weight % of all the experimental groups are shown in table 3. The results revealed non-significant changes between all nutritional parameters and organ weight/body weight %of the different experimental groups.

SDS-PAGE results

SDS-PAGE technique revealed 27 protein bands with different molecular weights ranged from 269 to 25KDa as shown in Table 4 and Fig. 2. Among those protein bands, four bands with 231, 82, 66 and 36 KDa were only seen in the normal control. Moreover, four different bands (42, 35, 29 and 25KDa) were detected in all groups. While 32 KDa protein band was existed in normal group and treatment groups but not hepatorenal syndrome control group; 33 KDa protein band was detected in the hepatorenal syndrome control group and treated groups but not normal group. 37 KDa is present in HRS control while absent in normal control and in HRS rats treated with either callus or its alcohol extract.

Acute toxicity test of ethanol extract

The acute oral lethal toxicity test revealed that crude ethanol extract of artichoke callus culture was very safe up to 12g/kg mice body weight.

DISCUSSION

In an effort to study the biosynthesis and the accumulation of secondary metabolites, *in-vitro* plant cell cultures have been established as a very useful tool allowing uniformity, accessibility and reduced complexity[34-37]. Few reports discussed the possibility of accumulating chlorogenic acid or cynarin in *in-vitro* plant cell culture of artichoke[38]. Being rich in the aforementioned bioactive constituents; artichoke callus was expected to possess health benefits towards HRS model in rats.

In the present research induction of hepatorenal syndrome in rats was verified on treatment with D-(+)-Galactosamine hydrochloride where both hepatic and renal dysfunction was achieved as reported previously[21]. The improvement in different biochemical parameters of HRS model in rats on treatment with artichoke callus or its alcohol extract could be attributed to their bioactive constituents that proved from the current research to possess antioxidant, anti-inflammatory, renoprotective and hepatoprotective effects.

Apigenin-7-rutinoside and narirutin, were found to be unique to artichoke heads[39].The isolated compounds from artichoke were determined as the four caffeoylquinic acid derivatives; chlorogenic acid, cynarin, 3,5-di-O-caffeoylquinic acid, and 4,5-di-O-caffeoylquinic acid, and the four flavonoids; luteolin-7-rutinoside, cynaroside, apigenin-7-rutinoside, and apigenin-7-O-beta-D-glucopyranoside, respectively[40]. Schütz et al.[41] reported that among the 22 major phenolic compounds, 11

Table 3: Nutritional parameters, liver weight/body weight % and kidney weight/body weight % of different experimental groups.

Parameters	Normal healthy control	Hepatorenal control	whole artichoke callus	Artichoke callus ethanol extract
Initial BW(g)	142.8^{a} ±3.599	143^{a}±5.353	143.2^{a}±5.306	142.8^{a}±5.405
Final BW (g)	224.5^{a} ±5.805	221.2^{a}±8.398	223.5^{a}±7.319	222^{a}±8.011
Body weight gain (g)	81.7^{a}±3.693	78.2^{a}±5.127	80.3^{a}±4.601	79.2^{a}±4.692
Total Food intake (g)	519.8^{a}±5.539	518.3^{a}±2.703	516.5^{a}±2.156	518.5^{a}±2.232
Food efficiency ratio	0.157^{a}±0.007	0.151^{a}±0.009	0.156^{a} ±0.009	0.153^{a} ±0.009
Liver weight/body weight %	2.6^{a}±0.135	2.5^{a}±0.221	2.5^{a}±0.221	2.4^{a}±0.217
Kidney weight/body weight %	0.650^{a}±0.036	0.608^{a}±0.029	0.574^{a}±0.017	0.621^{a}±0.043

Values were expressed as mean± SE. In each raw same letters mean non-significant difference; different letters mean significance difference at 0.05 probabilities.

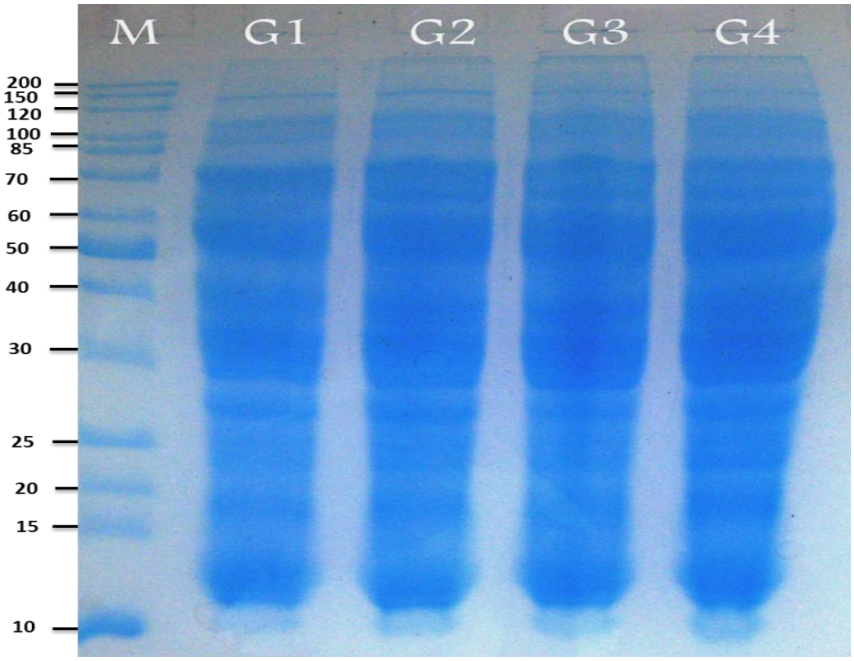

Figure 2: SDS-PAGE profile of proteins extracted from the liver of rats from different experimental groups, Marker (M); normal control (G1); hepatorenal syndrome control (G2); whole artichoke callus treated group (G3); artichoke callus ethanol extract treated group (G4).

caffeoylquinic acids and 8 flavonoids were detected in artichocke. Apigenin 7-*O*-glucuronide was found to be the major flavonoid. 1, 5-Di-O-caffeoylquinic acid represented the major hydroxycinnamic acid, with 3890 mg/kg in artichoke heads and 3269 mg/kg in the pomace, whereas in the juice 1,3-di-*O*-caffeoylquinic acid (cynarin) was predominant. Total phenolic contents of ~12 g/kg on a dry matter basis revealed that artichoke pomace is a promising source of phenolic compounds that might be recovered and used as natural antioxidants or functional food ingredients [41]. The present study showed that the prepared artichoke callus contains high levels of total phenolic and flavonoids in addition to anthocyanin, chlorogenic acid and cynarin which could have together synergistic therapeutic activity towards HRS due to their reported antioxidant and hepatoprotective effect. Artichoke extracts from industrial byproducts showed a high free radical scavenging activity (versus both DPPH• and ABTS•+ radicals) as well as capacity to inhibit lipid peroxidation according to ferric thiocyanate method[42]. Although the present study showed the alcohol extract of the callus to possess intermediate *in-vitro* free radical scavenging activity, however it significantly reduced in-vivo lipid peroxidation reflected in the reduced MDA. Jiménez-Escrig et al.[43] reported that artichoke extracts showed good efficiency in the inhibition of *in-vitro* LDL oxidation and possess in-vivo antioxidant activity. Among different antioxidant enzymes measured (superoxide dismutase, gluthatione peroxidase, gluthatione reductase, and catalase) in erythrocytes, only gluthatione peroxidase activity was elevated in the artichoke group compared to the control group. Also, the *in-vivo* study in the present work proved the significant anti-inflammatory activity of both artichoke callus and its alcohol extract through inhibiting the inflammatory biomarker TNF-α. Different studies about artichoke have demonstrated their health-protective potential, especially their hepatoprotective[44,45] and anti-inflammatory activities[46] which agreed with the present research that showed improvement in liver function in HRS rat model through reduction of transaminases activities and bilirubin with elevation of the reduced albumin. Hyperammonemia present in HRS rat model in the present study has been reported previously in chronic liver disease[47,48] which resulted from the reduced capacity of the sick liver to convert ammonia into urea. So, reduction of ammonia level on administration of either callus or its alcohol extract indicated further improvement of liver function. The current work also showed renoprotective effect of artichoke callus and its alcohol extract detected by reduction of plasma creatinine and urea and enhancing creatinine clearance along with reduction of urinary NAG in HRS rat model. Improvement of the level of endotheline1 previously reported as HRS biomarker in rat model[49,50] pointed to the protective effect of artichoke callus and its alcohol extract towards HRS.

So far the pathophysiology of HRS is not clearly understood. So, in continuation of searching the variable changes that happens in HRS; the present research highlights the changes in liver proteins in this syndrome and to what extent it could be prevented on treatment by artichoke callus and its alcohol extract. To the best of our knowledge this might be the first study in this respect. Liver protein fingerprint in HRS rat model according to SDS-PAGE showed 15 protein bands of molecular weight ranged from 269 to 25KDa in HRS control group compared to 17 bands in normal control. The protein bands of the fingerprint pointed to the presence of two proteins with a molecular weight of 33 and 37 KDa in HRS control that were absent in the control normal. The previous fingerprint was accompanied with the disappearance of

Table 4: SDS-PAGE profile of total proteins extracted from the liver of rats of different experimental groups.

Band No.	MW (KDa)	Normal healthy control			Hepatorenal control			Whole artichoke callus			Artichoke callus ethanol extract		
		1	2	3	1	2	3	1	2	3	1	2	3
1	269	+	+	+		+							
2	233	+			+	+							
3	231		+	+									
4	212							+		+	+		+
5	207										+	+	
6	123			+		+							
7	121	+	+		+				+	+			
8	107		+	+					+				
9	103							+		+			
10	82	+	+	+									
11	78							+		+	+	+	
12	73						+						+
13	66	+	+	+									
14	64							+	+	+			
15	55	+	+	+	+			+	+	+			+
16	53					+					+	+	+
17	42	+	+	+	+	+		+	+	+	+		
18	39											+	+
19	36		+	+									
20	37					+	+						
21	35	+			+	+		+	+	+		+	+
22	34	+		+	+		+						
23	33				+	+		+	+		+		+
24	32	+	+	+				+	+	+		+	+
25	29	+	+	+	+	+	+	+	+	+	+	+	+
26	26		+		+								
27	25		+	+	+	+	+	+	+	+			+
	Total No.	11	13	13	11	10	5	11	10	11	7	7	10

1, 2, 3: Total protein extracted from liver samples (n=3); +: means protein band is indicated.

proteins with molecular weights of 231, 82, 66 and 36 KDa in HRS control compared to control normal group; artichoke callus and its alcohol extract could not prevent this change. Treatment with artichoke callus and its alcohol extract could not impart any effect on the presence of 33 KDa protein showed in HRS control. Both callus and its alcohol extract could eliminate 37 KDa protein from liver to be similar to the control healthy group in this respect. Also both treatments prevent the elimination or destruction of 32 KDa protein from liver on induction of HRS. Liver plays a major role in producing proteins including major plasma proteins, factors in hemostasis and fibrinolysis, carrier proteins, hormones, prohormones and apolipoproteins. Damaged liver induced imbalance in production of protein[51]. An electrophoretic investigation of quantitative protein content in rats' liver under chronic alcoholic intoxication showed significant increase of protein content with molecular mass 55, 48-50, 43-45, 39-41 KDa and the decrease-of protein fractions with molecular mass 46-48, 34-35, 27-30, 16-18 KDa[52].

In the present study the prepared artichoke callus utilizing biotechnological technique showed to contain higher contents of total phenolics and flavonoids compared to different parts of artichoke head purchased from local market. Also, the level of anthocyanin callus was high compared to artichoke heart and center choke. These results emphasized that the used biotechnology technique succeeded to elevate the bioactive constituents of artichoke.

Artichoke callus ethanol extract was very safe up to 12g/kg mice body weight as could be seen from acute toxicity test. This dose corresponds to 93g/70kg man body weight for human when following conversion scheme from mice to human according to Paget and Barnes[53]. This reflects the highest safety of the bioactive extract.

CONCLUSION

HRS protective effect of artichoke callus in the present research could be attributed to their content of total phenolic, flavonoids, anthocyanin, chlorogenic acid and cynarin. The protective effect of callus was superior to its alcohol extract.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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