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Research Article
Biological Effects of Red Chili Pepper (*Capsicum annuum*) Consumption on High Fat Diet Female Albino Rats

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Abstract

Background and Objective: The evidence for safe consumption of chili pepper to gain health benefits is insufficient as high doses reported to cause gastric erosion. The objective of this study was to investigate the contribution of red chili pepper ethanol extract (RCEE) to weight loss in high fat dieted female albino Wistar rats while monitoring adverse effects of high dose consumption. **Materials and Methods:** Body weight gain, lipid profile, oxidative stress parameters, liver, kidney and thyroid biochemical functions were determined in addition to histopathological examination of liver and kidney to determine biological effects of 2 ingested concentrations of red chili pepper extract (5 and 10 mg). **Results:** Red chili pepper ethanol extract (RCEE) showed anti-obesity potentials and hypolipidemic effects through decreased levels of total cholesterol (T.Ch), low density lipoprotein (LDL), triglycerides (TG) and very low density lipoprotein (VLDL) reached 25, 55, 45 and 45%, respectively, in parallel with increased high density lipoprotein (HDL) levels by 22.6%. The antioxidative potentials of RCEE were pronounced through significant increases in glutathione peroxidase (GPx) and catalase (CAT) activities (346.43 mU mL^{-1} and 15.33 U L^{-1} in group 2, respectively). Histological examination of liver and kidney showed adverse results but that did not significantly reflect on their functional biochemical parameters. **Conclusion:** Results introduced scientific evidence that red chili pepper possesses functional pharmacological properties but the excessive consumption induces liver and kidney damage.

Key words: Red chili pepper, lipid profile, biochemical function parameters, oxidative stress, liver/kidney histological examination

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Chili pepper (*Capsicum annuum*) constitutes one of more consumed spices in the world which is used in a wide range in foods for flavor, aroma and to avoid food spoilage and in the pharmaceutical industries for its richness in capsaicin and vitamin C[1,2]. It was found to decrease appetite, energy intake and reduce the risk of overweight and obesity. The bioactive agents have also shown beneficial roles in reducing cardiovascular risk factors such as hypertension, smoking, diabetes, hypercholesterolemia and hyperlipoproteinemia, gastrointestinal conditions, various cancers, neurogenic bladder, dermatological conditions, diabetes, liver cirrhosis, prevent alcohol-induced acute liver injury in addition to, antibacterial activity, antioxidant potentials, anti-lithogenic, anti-inflammatory and analgesia[3-5].

Unfortunately, an inverse relationship between spicy food consumption and mortality was reported and red pepper is known to be harmful if consumed in excess as it possesses some chemical and pharmacological properties that are capable of inducing tissue damage[6,7]. As the human intake of capsaicinoids the main ingested as naturally occurring component of capsicum -in India, Thailand and Mexico, where capsicum spices are heavily consumed estimated with 25-200 mg/day, this high consumption reported to be associated with cancer of the upper digestive tract. The ideal dosage is needed to significantly contribute to weight loss with safe consumption still warrants further research[8].

To our knowledge, limited research has been reported concerning the effect of red chili pepper on the histopathology and biochemistry of liver and kidney. The aim of this work was to investigate the contribution of red chili pepper ethanol extract (RCEE) consumption to weight loss applying 2 concentrations to high fat diet female albino Wistar rats. The parameters of body weight gain, lipid profile, oxidative stress, liver and kidney biochemical and histopathological functions were assessed as an attempt to highlight positive and adverse effects of dose-dependent impact of red chili pepper consumption.

MATERIALS AND METHODS

Plant materials and extract preparation: Red chili pepper (*Capsicum annuum* L.) was obtained from local market, Al-Taif, Kingdom of Saudi Arabia originated from India, http://www.theplantlist.org/tpl/record/kew-2698415. The fruits were dried in circulating air oven at 35°C for seven days, then grinded using an electric grinder to powder. To obtain ethanol extract, extracted in 95% ethanol (1:10 w/v) by maceration for 72 h, filtered using Whatman No1 filter paper, evaporated to dryness in a rotary evaporator and hereafter preserved under -80°C for use[9].

Animals: Twenty-one female albino Wistar rats (260-277 g body weight) were bred and maintained under standard conditions (22±1°C, 12 h light/12 h dark cycle), fed laboratory chow (fat 2.8%, protein 18.5%, fiber 11.2%) and water *ad libitum*, for 1 week before experiments, in the Experimental Animals Facility, Home Economics Department, Faculty of Agriculture, Alexandria University, Egypt. All of the experimental procedures were performed in accordance with the guidelines approved by Alexandria University Institutional Animal Care and Use Committee (AlEXU-IACUC), a member of International Council for Laboratory Animal Science (ICLAS) (permission number: AU08190319317). The biological experiment started in March 2019, while extract was previously prepared.

Induction and experimental design: After a week of adaptation, the rats were pretreated with high fat high cholesterol diet which contained, (23% fat, 17% casein, 27% sucrose, 20% starch, 6% cellulose, 4.3% minerals and 1.2% vitamins) based on a modification of the AIN-93 diet[10]. The 21 rats were randomly divided into 3 groups of 7 animals/cage (41×34×16 cm), receiving oral gavage daily for 25 days with, control group, high fat high cholesterol diet+0.5 mL saline, group 1, high fat high cholesterol diet+5 mg RCEE in 0.5 mL saline and group 2, high fat high cholesterol diet+10 mg RCEE in 0.5 mL saline.

Sampling and preparation: Body weight was monitored once/week. At the end of 25 days period, final weights were recorded and rats were sacrificed after overnight fasting under light diethyl ether anesthesia. Blood samples were collected from abdominal aorta in plain tubes, centrifuged at 4000 rpm for 20 min at 4°C for serum separation and stored at -20°C.

Plasma biochemical analysis: Plasma lipid profile, serum urea and creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin and thyroid-stimulating hormone (TSH) analysis were determined. Plasma biochemical analysis were carried out via enzymatic colorimetric methods using commercial diagnostic kits in accordance with the manufacturer's instructions. The levels of oxidative stress parameters in kidney and liver of female rats

Table 1: Red chili pepper extract impact on body weight gain of treated rats

Parameters	Control	Group 1	Group 2
Initial body weight	270.67±6.03[a]	268.33±2.89[b]	266.67±7.64[b]
Final body weight	324.00±9.64[a]	295.33±5.03[b]	297.00±10.82[b]
Body weight gain (%)	19.70±1.99[a]	10.06±0.92[b]	11.37±1.80[b]
Body weight gain (g)	53.33±5.69[a]	27.00±2.65[b]	30.33±5.03[b]

Control: High fat high cholesterol diet, group 1: High fat high cholesterol diet +5 mg red chili pepper ethanol extract, group 2: High fat high cholesterol diet +10 mg red chili pepper ethanol extract, data are the mean for 7 rats/group, [a,b,c]Means values in the same row marked with unlike letters are significantly different ($p<0.05$)

Table 2: Red chili pepper impact on plasma biochemical profile of treated rats

Parameters	Units	Control	Group 1	Group 2
Plasma lipid profile				
Total cholesterol	mg dL^{-1}	78.27±8.58[a]	60.67±3.21[b]	58.67±1.53[b]
Triglyceride	mg dL^{-1}	63.90±2.81[a]	36.07±5.38[b]	35.20±4.53[b]
High density lipoprotein	mg dL^{-1}	28.93±2.94[a]	35.47±2.45[b]	33.33±2.08[b]
Low density lipoprotein	mg dL^{-1}	36.55±2.35[a]	17.99±1.07[b]	18.29±0.69[b]
Very low density lipoprotein	mg dL^{-1}	12.78±0.56[a]	7.21±1.08[b]	7.04±0.91[b]
Liver function				
Aspartate aminotransferase	U mL^{-1}	95.00±5.19[a]	100.00±5.00[a]	97.00±2.00[a]
Alanine aminotransferase	U mL^{-1}	48.33±1.53[a]	45.67±2.08[a]	48.67±8.08[a]
T. Bilirubin	g dL^{-1}	0.23±0.06[c]	0.37±0.06[b]	0.61±0.02[a]
Kidney function				
Urea	g dL^{-1}	26.07±3.68[a]	26.00±4.36[a]	26.83±5.01[a]
Creatinine	g dL^{-1}	0.62±0.07[a]	0.76±0.03[a]	0.70±0.10[a]
Thyroid function				
Thyroid-stimulating hormone	mU L^{-1}	0.34±0.30[b]	0.39±0.04[b]	0.70±0.09[a]

Control: High fat high cholesterol diet, group 1: High fat high cholesterol diet+5 mg red chili pepper ethanol extract, group 2: High fat high cholesterol diet+10 mg red chili pepper ethanol extract, data are the mean for 7 rats/group, [a,b,c]Means values in the same row marked with unlike letters are significantly different ($p<0.05$)

represented in glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD) were determined as described by Aebi[11], Arthur and Boyne[12], De Quiroga *et al.*[13] and Esterbauer and Cheeseman[14]. Thiobarbituric acid reactive substances (TBARS) were determined in the liver and kidney using Enzyme-linked immunosorbent assay (ELISA)[15].

Histopathology examination: Immediately after necropsy, the rats' organs, liver and kidneys were dissected out carefully from 3 animals/group and fixed in 10% (v/v) formalin for 24 h. The fixed samples were washed and embedded in paraffin blocks and sectioned into 5-6 µm sections using Microtome (Reichert-Jung, Germany) for serial specimens then mounted on glass slides and stained with hematoxylin and eosin (H and E) stain[16]. The slides were observed under histological light microscope (Olympus, Tokyo, Japan) at 400X magnification.

Statistical analysis: The statistical analysis of the results was carried out by using linear-year style. The difference between the averages of groups was compared using the least significant difference when the relationship level $p<0.05$.

RESULTS AND DISCUSSION

Red chili pepper impact on body weight gain: Table 1, illustrates red chili pepper extract impact on body weight gain of 2 treated rats groups comparing to control. At the beginning of the experiment, the initial body weights were not different among the three groups. After 25 days at the end of experiment, the body weight gain of the 2 red chili pepper supplemented groups group 1 and 2 showed significant decrease comparing to control by 26.33, 23 g with percent of 9.64, 8.33%, respectively ($p<0.05$). Whilst, the differences were insignificant between the two concentrations of the red chili pepper extract treated groups. These results correlate with the previous studies that reported the anti-obesity potency of capsaicin containing foods consumption with different suggested mechanisms[17-19].

Red chili pepper impact on plasma biochemical profile: Table 2 showed red chili pepper impact on plasma biochemical profile of treated rats represented in plasma lipid profile, liver, kidney functions and thyroid function. The hypolipidemic effect of RCEE was announced in plasma lipid profile where oral ingestion of RCEE induced significant

Table 3: Red chili pepper impact on oxidative stress parameters in rats' liver and kidney

Parameters	Units	Control	Group 1	Group 2
Oxidative parameters in liver				
GPx	mU mL^{-1}	227.80±9.87[b]	329.67±11.55[a]	346.43±19.09[a]
CAT	U L^{-1}	11.43±0.25[a]	11.77±0.32[a]	15.33±0.49[b]
SOD	U mg^{-1} protein	2.77±0.60[a]	2.63±0.31[a]	2.37±0.72[a]
TBARS (MDA)	nmol mg^{-1} protein	24.57±1.05[a]	18.70±1.00[b]	16.00±1.12[b]
Oxidative parameters in kidney				
GPx	mU mL^{-1}	316.27±12.61[b]	427.13±19.75[a]	448.73±21.04[a]
CAT	U L^{-1}	11.33±0.41[a]	11.70±0.56[a]	15.57±0.31[b]
SOD	U mg^{-1} protein	2.53±0.93[a]	2.07±0.72[a]	2.87±0.49[a]
TBARS (MDA)	nmol mg^{-1} protein	15.43±0.15[a]	11.60±0.20[b]	10.67±0.47[b]

Control: High fat high cholesterol diet, group 1: High fat high cholesterol diet+5 mg red chili pepper ethanol extract, group 2: High fat high cholesterol diet+10 mg red chili pepper ethanol extract, data are the mean for 7 rats/group, [a,b,c]Means values in the same row marked with unlike letters are significantly different ($p<0.05$)

decrease in serum total cholesterol and LDL concentrations by 22-25 and 55-35%, respectively in group 1 and 2 compared to that of the control group ($p<0.05$). On the other hand, significant reduction announced by lower values of TG and VLDL in the two RCEE treated groups were recorded (36.07, 35.20 and 7.21, 7.04 mg dL^{-1}, respectively). Obtained results agreed with Sanati *et al.*[20] and Zhang *et al.*[21]. The HDL exhibited reverse results where significant increase recorded in group 1 by 6.54 mg dL^{-1} (22.6%). Generally, the effects of RCEE on plasma lipid profile were shown to be dose-independent as no significances were recorded between the 2 concentrations treated groups (group 1 and 2). These data agreed with the body weight gain results (Table 1) supporting that the red chili pepper is a body weight-loss food via fat metabolism mechanisms as previously reported by Varghese *et al.*[8] and Morales-Martinez *et al.*[22], that chili pepper intake facilitates weight loss representing different suggested mechanism of actions such as fat metabolism, energy expenditure and thermogenesis.

Not withstanding the adverse effects on liver and kidney tissue structure revealed by the histological examination (Fig. 1 and 2), the biochemical results represented in Table 2 indicated the insignificant effect of ingested RCEE on assessed kidney and liver function parameters except for the levels of bilirubin. The aim of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and total bilirubin determination was for hepatotoxicity evaluation. The oral intake doses of RCEE supplemented in the current study did not significantly affect the plasma levels of liver function parameters, AST and ALT in examined rats as demonstrated by the results in (Table 2), which showed to be comparable to control group. On the other hand, significant dose-dependent hyperbilirubinemia was obvious in the RCEE 2 treated groups comparing with the control group (0.37 and 0.61 g dL^{-1}) ($p<0.05$). Bilirubin is one of the end products of heme-catabolism, the significant increases in total bilirubin levels exhibited in current results indicated destruction of the hepatic cells results in more hepatic releases to exacerbate hepatic dysfunction which causes an elevation in serum bilirubin as explained by Zhang *et al.*[23], which was announced in histological examination in (Fig. 1). It is noteworthy that, the normal level of bilirubin is less than 0.3 g dL^{-1}, mild or moderately elevated serum bilirubin was reported to be beneficial for its antioxidant and anti-inflammatory properties[24]. These results encourage recommending the use of RCEE but with preferred consumption levels less than 5 mg/day to gain its benefits and avoid undesirable adverse effects on liver with high consumption levels.

Exhibited kidney function parameters levels of the 2 treated groups (Table 2) represented in urea and creatinine concentration levels (g dL^{-1}) were comparable to control group. These data agreed with what previously reported by Kim *et al.*[19].

The serum thyroid stimulating hormone (TSH) (Table 2), showed significant increase ($p<0.05$) in RCEE treated group 2 (0.7 mU L^{-1}) compared with control group. Meanwhile, the obtained results of control group and group 1 (0.34 and 0.39 mU L^{-1}), respectively were within the normal range of the hormone (0.4-4.5 mU L^{-1}) as reported by Schneider *et al.*[25], which indicated tolerance of the ingested dose of 5 mg/day of RCEE with no adverse effect on thyroid function.

Red chili pepper impact on oxidative stress parameters: Table 3 illustrates RCEE impact on oxidative stress parameters in liver and kidney. Reactive oxygen species (ROS) have short half-lives that are difficult to be assessed directly. Instead, several products of the damage produced by oxidative stress, such as TBARS are assessed. Estimation of lipid peroxidation in the tissues involved the assessment of thiobarbituric acid reactive substances (TBARS) via malondialdehyde (MDA). MDA is one of the end products of the hydrolytic decomposition of some primary and secondary lipid peroxidation products[26].

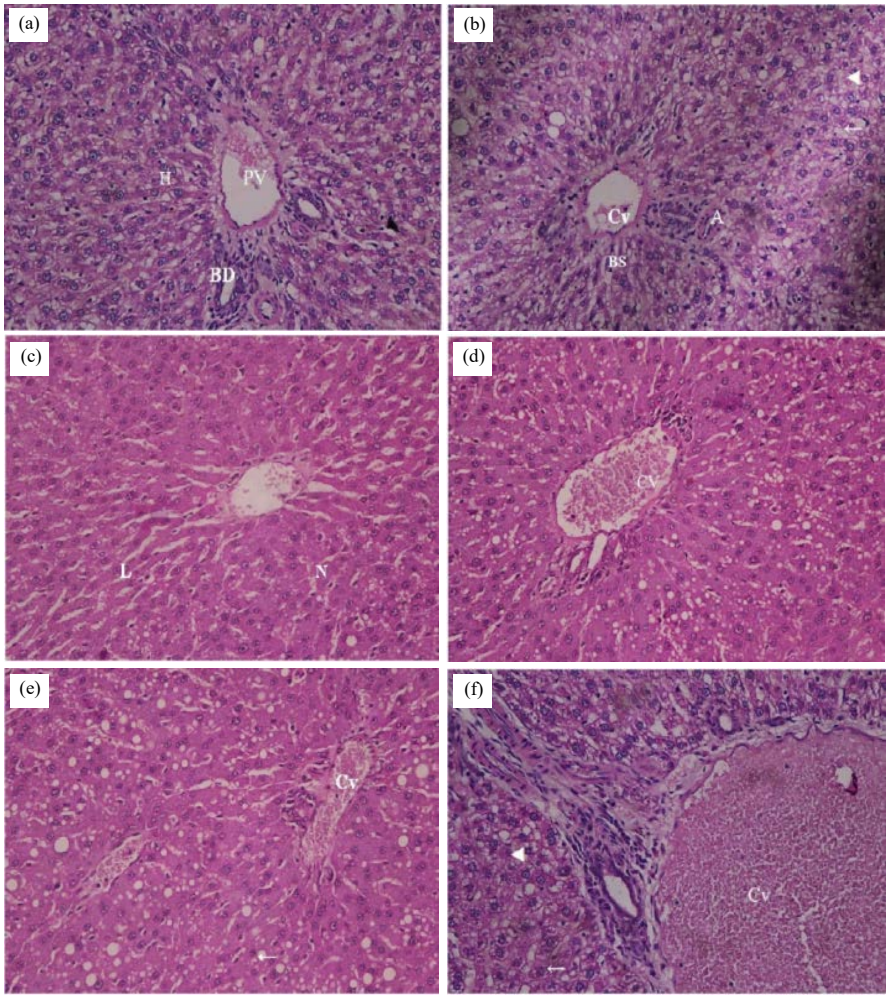

Fig. 1(a-f): Representative hematoxylin and eosin stained sections of female albino Wistar rats' liver (H and E stain X 400) (a) Portal area with portal vein (PV) bile ductile (BD) and regular distinct hepatocytes with sinusoidal spaces arranged radially around the central vein (H), (b) Normal hepatic architecture central vein (Cv), normal hepatic artery (A), normal hepatocytes strands by blood sinusoids (BS) and hepatocytes with normal basophilic nucleus (◂) and eosinophilic cytoplasm (←), (c) Group 1 treated rats with 5 mg of red chili pepper ethanol extract showing slight necrosis of hepatocyte (N) marked by a homogenization of the structure of cytoplasm that becomes eosinophilic with disappearance of limit cells (L), (d) Group 1 treated rats with 5 mg of red chili pepper ethanol extract showing relatively congested central vein (Cv) with less pathological changes and improved liver architecture, (e) Photomicrograph of liver of Group II treated rats with 10 mg of red chili pepper ethanol extract showing congested central vein (Cv), distorted hepatic strands, hepatocytes with vacuolated cytoplasm (←) and (f) Group 2 treated rats with 10 mg of red chili pepper ethanol extract showing dilated and congested central vein (Cv), hepatocytes with vacuolated cytoplasm (←) and nucleus (◂)

Both GPx and CAT activities showed significant increase in both rats' liver and kidney with oral consumption of RCEE in groups 1 and 2 in comparison with control group ($p<0.05$). GPx enzyme activities exhibited elevation by 44.7 and 52.1% in liver, 35 and 41.8% in kidney for both group 1 and 2 respectively than that of the control group. The CAT enzyme activities were greater by 3 and 34.12% in liver, 3.26 and 33.1% in kidney for both group 1 and 2, respectively than that of the control group.

The activity of SOD, an antioxidant enzyme which reduces superoxide radicals to H_2O_2, which in turn is excreted as H_2O, based on the activity of CAT and GPx, thereby protecting the body from oxygen toxicity. Results of insignificant changes comparing to control group of SOD activity levels in both liver

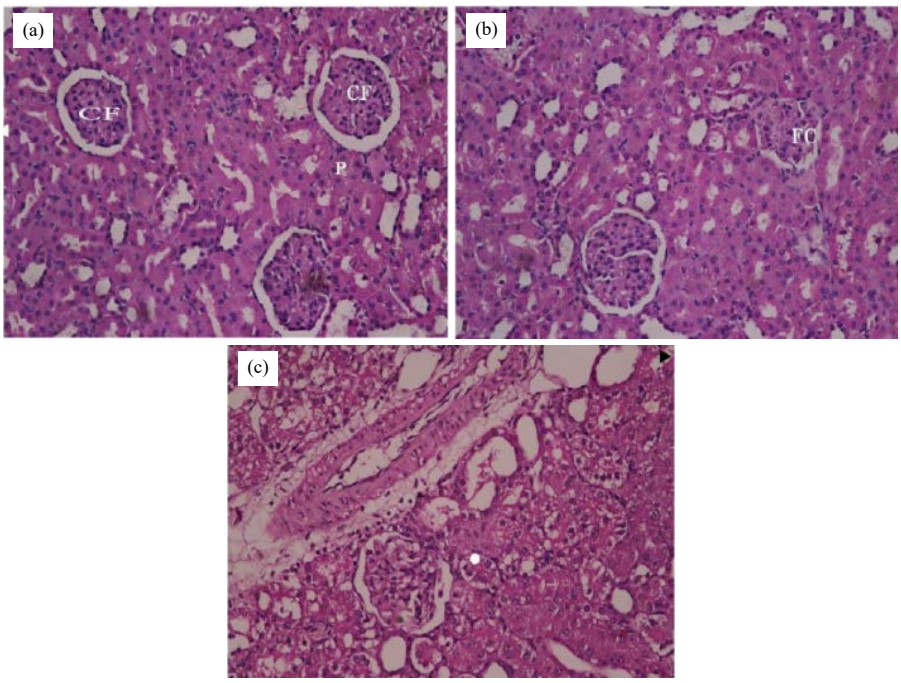

Fig. 2(a-c): Representative hematoxylin and eosin stained sections of female albino Wistar rats' kidney (H and E stain X 400), (a) Glomerulus with the capillary flocculates (CF), glomerular room (◂), the vascular pole (P) and the excretory ducts, (b) Group 1 treated rats with 0.5 g of red chili pepper ethanol extract showing structural changing marked by the thickening of the interstitium with compresses of the flocculus capillary and disappearance of glomerular room (FC) and (c) Photomicrograph of kidney of group 2 treated rats with 1 g of red chili pepper ethanol extract showing glomerular vacuolation (▸), obstruction of the glomerular renal space, vascular congestion (arrowhead) and cellular infiltration (○)

and kidney indicated the safe use of ingested RCEE that did not affect the cells oxidative stress.

The TBARS concentration levels in both liver and kidney showed dose-dependent significant decrease (23.9, 34.9% in liver and 25, 31% in kidney for group 1 and 2, respectively) compared with control group. This depression assured the free radical defense role of RCEE and its positive alterations in the activities of antioxidant enzymes. These results are in agreement with Omoregie and Osagie[27]. Antioxidative role of hot red chili pepper was previously documented by Sanati *et al.*[20], Amarowicz[28], Gougoulias *et al.*[29] and Otunola *et al.*[30].

Histopathology analysis: Representative hematoxylin and eosin stained sections of female albino Wistar rats' liver of control group and 2 concentrations of RCEE treatments (group 1 and 2) are illustrated in Fig. 1a-f. The control group revealed normal hepatic architecture, the hepatic lobule appeared to be made up of hepatocytes with sinusoidal spaces radiating from the central vein. They were polyhedral in shape with granular eosinophilic slightly vacuolated cytoplasm and rounded vesicular, hepatocytes with normal basophilic nucleus (Fig. 1a). The portal vein showed the normal appearance with one bile duct radical, branch of hepatic artery and branch of portal vein, all were enclosed in scanty amount of connective tissue (Fig.1b). The histological sections of rats of group 1 ingested 5 mg of RCEE (Fig. 1c, d), revealed less preserved hepatic architecture. Slight necrosis of hepatocytes arranged in cords radiating from relatively congested central vein (Cv) with less pathological changes and separated by blood sinusoids marked by homogenized cytoplasm structure that becomes slightly eosinophilic with disappearance of limit cells. In group 2 fed 10 mg of RCEE (Fig. 1e, f), there was diffusing affection of the hepatic lobule. The central vein appeared dilated and congested and the hepatic architecture was disorganized showing distorted hepatic strands, hepatocytes with vacuolated cytoplasm and nucleus. Similar results were reported by Dkhil and Al-Quraishy[7], when they studied the effect of extensive consumption of hot red pepper on the histopathology and biochemistry of rabbit's liver (2 g kg^{-1}/day for 10 days).

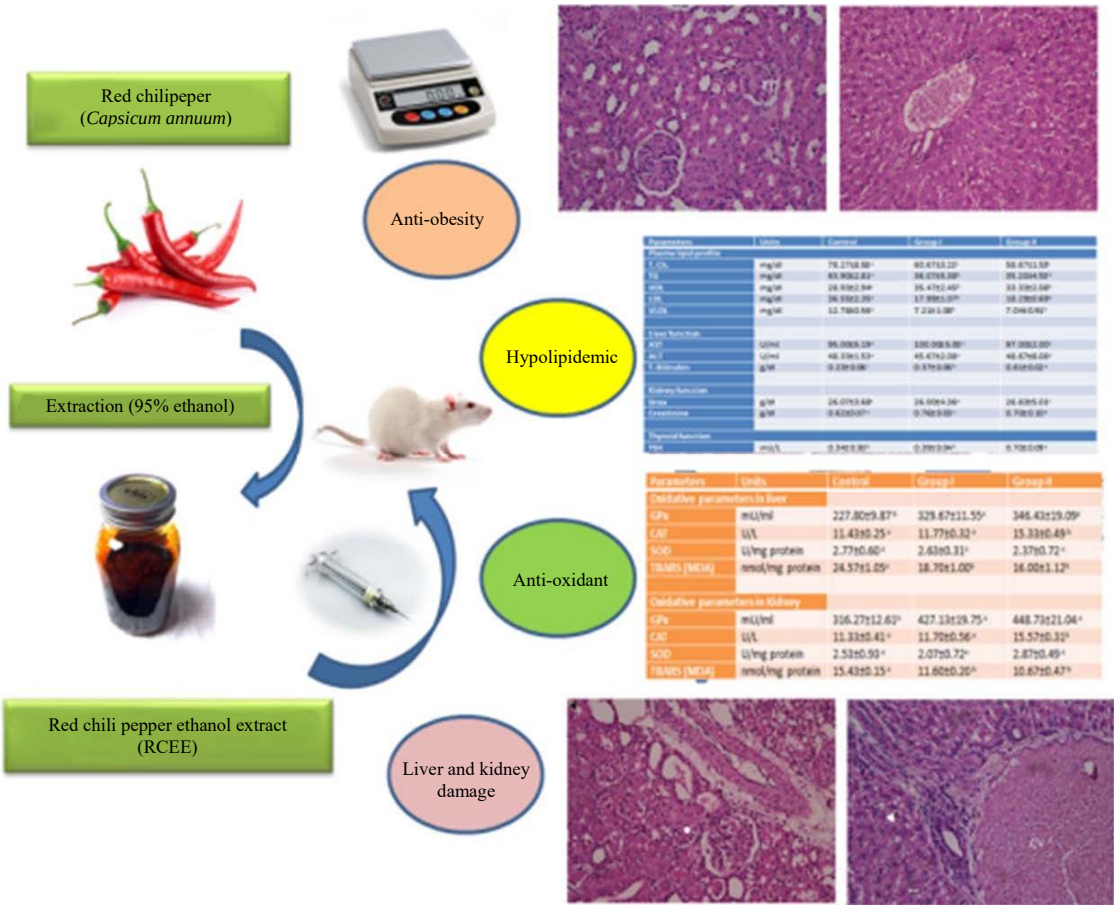

Fig. 3: Graphical abstract for theoretical description of results

Representative hematoxylin and eosin stained sections of female albino Wistar rats' kidney of control group and 2 concentrations of RCEE treatments (group 1 and 2) are illustrated in Fig. 2a-c. Kidneys of control group rats showed normal architecture of renal tissue, being composed of a number of glomeruli with the capillary flocculates, glomerular room, vascular pole (P) and the excretory ducts (Fig. 2a). Microscope examination of H and E stained kidney sections of group 1 treated with RCEE with concentration of 5 mg (approx. 0.015 mg kg^{-1}/day, for 25 days), showed structural changes marked by the thickening of the interstitium with compresses of the flocculus capillary and disappearance of glomerular room (Fig. 2b). Additional necrosis, glomerular vacuolation, obstruction of the glomerular renal space, vascular congestion and cellular infiltration was announced in group 2 rats subjected to RCEE with concentration of 10 mg (approx. 0.035 mg kg^{-1}/day, for 25 days) (Fig. 2c). Related results were reported by Rios-Silva *et al.*[31].

Although, the chili pepper extract exhibited adverse results through the histological examination of liver and kidney tissues but that did not significantly reflect on their functional biochemical parameters except for obvious increase in bilirubin. However, the Committee on Food could not establish a safe exposure level for capsaicinoids foods, while BfR (Bundesinstitut für Risikobewertung)[32] stated that the dosage of capsaicinoids ingested by affected persons is unknown.

A graphical abstract for concluded theoretical descriptions of above results is illustrated in Fig. 3.

CONCLUSION

Red chili pepper ethanol extract (RCEE) oral ingestion showed anti-obesity potentials could be relied to increased metabolism through 2 possible mechanisms, the hypolipidemic effect and activation of serum thyroid stimulating hormone (TSH). Furthermore, decreased TBARS levels in both liver and kidney assured the free radical defense role of RCEE in alterations of oxidative status through increased GPx and CAT enzyme activities. Neither kidney

functions, nor liver functions were significantly affected except for dose-dependent hyperbilirubinemia was noticed. Although bilirubin increase (up to 0.3 g dL^{-1}) is beneficial for its antioxidant and anti-inflammatory properties but as one of the end products of heme-catabolism, significant increases in total bilirubin levels exhibited in current results were paired with necrosis in the hepatic and kidney cells announced in histological examination.

SIGNIFICANCE STATEMENT

This study highlights the functional pharmacological properties of red chili pepper represented in anti-obesity, antioxidant and hypolipidemic effect. On the other hand, this study emphasis the adverse effect of excessive consumption such as hyperbilirubinemia, kidney and liver damage. Recommending a preferred consumption levels not more than 5 mg/day of red chili pepper ethanol extract to gain its benefits and avoid undesirable adverse effects. Obtained results, encourage more research in this area to determine safe dose, present new theories of functional properties mechanisms of red chili pepper that may be arrived at.

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