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Original article

Protective effect of *Acorus calamus* on kidney and liver functions in healthy mice

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ABSTRACT

Acorus calamus (AC), is an herbal medicine commonly used as traditional practice in pharmacological applications. Present study initiated was evident to proof the hepatoprotective and nephroprotective activity with supporting histopathological status of kidneys and liver. Investigation done with the 5% (w/v) of AC dissolved in tap water (50 g/l) given for 15 days compared with control tap water to 5-7 week old C57Bl/6 mice both sexes. Renal function, liver function, biochemical and complete blood count was evaluated. AC significantly reduced food intake, body weight, also plasma concentration of electrolytes such as Na^+, K^+, Ca^{2+}, were reduced as the excretion of electrolytes were increased in urine, significantly increased Fluid Intake, with Urinary urea, Urinary creatinine, Glomerular Filtration Rate, creatinine clearance, High-density lipoproteins, Mean Corpuscular Volume. The biochemical findings showed the hepatoprotective and histopathological changes showed the nephroprotective nature of AC by normal structure with no necrosis.

1. Introduction

Obesity is the serious disease spreading worldwide creating negative impact on human system mentally and physically. Overweight and obesity leads to health problems such as diabetes, heart disease, osteoarthritis and sometimes cancer (Kyrou et al., 2018). The onset of obesity is due to factors such as economic development, industrialization, mechanized transport, urbanization, as well as sedentary lifestyles (Hruby and Hu, 2015). The other cause of obesity is the food with nutritional transition; processed foods and high calorie diet. Many countries have witnessed obesity prevalence increasing in the number of citizens (Bhurosy and Jeewon, 2014). The complex disorder deals with numerous human factors such as genetic, behavioral, socioeconomic, and increases the risk of morbidity and mortality, with an growing incidence worldwide. (Ahmad et al., 2010). Obesity is viewed as a lipid metabolism disorder and the enzymes involved in this mechanism (Klop et al., 2013). lipase inhibitors production has initiated for antiobesity drugs (Rodgers et al., 2012). Due to changes in the human lifestyle there is increase in high energy diet due to which there is increased prevalence of obesity and have also affected the children's population. Several pharmacological agents are available as antiobesity medications, however, most of the licensed and marketed anti-obesity medications have now been discontinued due to significant adverse effects (Rodgers et al., 2012). Due to adverse side effects natural products have been used in many Asian countries to treat obesity. Natural products potentiality in treating obesity remains unexplored but it is the excellent alternative to the safe and successful production of antiobesity drugs (Liu et al., 2017). Since ancient times, the naturopathic treatment of obesity has been widely studied and has gained importance in the modern scenario. Medicinal plants well known for phytoconstituents have been used for the treatment of obesity and its related secondary complications. Clinical trials were tested using active medicinal plants with their respective bioactive compounds for obesity treatment (Patra et al., 2015). Moreover, due to high costs and potentially dangerous side effects natural products have gained importance in developing anti-obesity drugs (Sun et al., 2016).

Sweet flag, calamus, a flowering perennial herb, also known as *Acorus calamus*, belongs to Acoraceae family, in the genus Acorus, consists of tufts basal leaves arising from rhizome, 30–100 cm tall. It is commonly used as herbal medicine traditionally practised in pharmacological applications. Ayurvedic medicine has reported all parts of the Calamus; stem, leaf, root, bark plays magnificent

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role (Imam et al., 2013). It has many activities in pharmacology, such as antidiabetic, antiproliferative, immunosuppressive, antidiarrhoeal, and hypolipidemic. Often used as a sedative, laxative, diuretic, carminative agent (Rajput et al., 2014). Used in Siddha, Unani and recently used in homeopathic remedy as a well-known mother tincture in treatment of intestinal worms (Nath and Yadav, 2016), digestive disorders including flatulence, also relieves stomach pain, bloating, fever, nausea, vomiting, fatigue, exhaustion, treats mouth ulcers by preventing bleeding of the gums and bad breath, it has an antiseptic properties in healing cuts and wounds, Sweet flag is the most common medicinal plant used by Chipewyan people.

The present research deals with phytoextracts *Acorus calamus* with their mechanism of action as potential antiobesity with its preclinical experiment showing no adverse effects in biochemical, supporting as hepatoprotectivity and nephroprotecivity.

2. Material and methods

2.1. Acorus calamus

Dried leaves of *Acorus calamus*, were purchased from the local market of Turabah City, Saudi Arabia, and identified by botanical specialist. *Acorus calamus*, were grained, very fine then added in normal water and left overnight and then filtered and given to the treated mice group.

2.2. Animals

Experiment were carried on 5–7 week old C57Bl/6 mice of both sexes (n = 20) from, (King Fahd Center for Medical Research, King Abdulaziz University, Jeddah, Saudi Arabia). All animals were housed under controlled environmental conditions (22–24° C, 50–70 percent humidity and a 12-h light/dark cycle). All animals had free access to standard granulated food (C1310, Altromin, Heidenau, Germany). Animals were divided into control group had tap water and experimental group which received the same type of diet, but 5% (w/v) of *Acorus calamus* dissolved in tap water (50 g/l). During the treatment time, fresh extracts were revised every 3 days. All animal experiments were, conducted according to the guidelines of international law for the care and welfare of animals and were approved by local authorities.

During the treatment period (15 days), body weight, were monitored for control and treated group at baseline and last day of experiment to see the effect of 5% of *Acorus calamus* drink on body weight. For the determination of food, fluid intake, and urinary output all mice were housed in metabolic cages for five days. During the metabolic experiment all mice had free access to normal diet and tap water or 5% of *Acorus calamus* drink as indicated. On the last day of the experiment, blood samples were taken by puncturing the retro-orbital plexus while they were under diethylether anaesthesia. (Roth, Karlsruhe, Germany) and blood was withdrawn into blood collecting tube as required for different biochemical analysis.

2.3. Blood biochemistry

Plasma concentrations of Na^+, K^+, and $Ca2^+$, were measured by flame photometer (AFM 5051, Eppendorf, Germany) (Dean, 1960). Plasma and urinary creatinine concentrations were measured using methods of kinetic, urinary urea concentration were measured by (UREA, colorimetric), blood cholesterol (CHOD PAP methods), AST GOT, ALT, GPT, HDL, cholesterol (direct method), triglycerides (GOPt method), uric acid (Uricase method), random glucose levels were measured using kits from (BIOLABO, Les Hautes Rivers, 02160, Maizy, France), www.Biolabo.fr. The complete blood count (CBC), examination was determined using an electronic hematology particle counter, equipped with a photometric unit for determination of hemoglobin (MDM 905 of Medical Diagnostics 140 Marx; Butzbach, Germany). All measurements were preformed according to manufacturing instructions.

2.4. Histopathology

On the last day of experiment, all mice were sacrificed, kidney organs were removed and processed for further histological analysis. Tissues were deparaffinized, sections of 5-μm-thick tissue sections were fixed in formaldehyde and stained with hematoxylin/eosin (HE), for microscopic evaluations (Kiernan, J.A. 1999). The stained sections were viewed and evaluated for pathological changes using a light microscope (Nikon, Eclipse i80). The images were taken in different magnifications with Nikon mounted digital camera (OXM 1200C, Nikon, Japan).

2.5. Statistics

Data are given as a means ± SEM, n is the number of independent experiments. All data were tested for significance unpaired Student's *t*-test, as appropriate using GraphPad Prism 8.4.3.686 (San Diego, California, USA). A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Body weight analysis

The changes in the body weight of the experimental animals were recorded on day 1 after the treatment on day 15 (Table 1), the data were graphically represented in (Fig. 1). The results showed a significant reduction in the body weight of mice with 5% (w/v) of *Acarus calamus* (AC) when compared with control.

3.2. Food and fluids analysis

The parameters such as food intake, fluid intake, fecal wet, dry weight, urinary output, plasma concentrations and urinary excretion of electrolytes (Table 2), in the experimental rats (7-wk-old of C57Bl/6) treated with 5% (w/v) of *Acarus calamus* (AC) dissolved in tap water (50 g/l) given for 15 days were measured and compared with control tap water. The data in (Fig. 2), reveals the decreased food intake, fecal wet, and fecal dry weight compared to control. The increase in fluid intake were gradual with urinary output.

The electrolytes concentration in plasma were reduced and followed with increase in urinary electrolytes concentration in treated mice as compared with control group (Table 3) and (Fig. 3).

Table 1
Body weight analysis.

	Control	*Acarus calamus*	P-value
Body weight at baseline, g	15.08 ± 0.35	15.36 ± 0.50	0.6441
Body weight at end, g	16.13 ± 0.47	14.34 ± 0.56	0.025
Body weight gain, g	1.05 ± 0.27	−1.02 ± 0.29****	<0.0001

Arithmetic means ± SEM (n = 10 each). ****indicates extremely significant (P < 0.0001) difference between control and 5% *Acarus calamus* (AC) drink after 15 days.

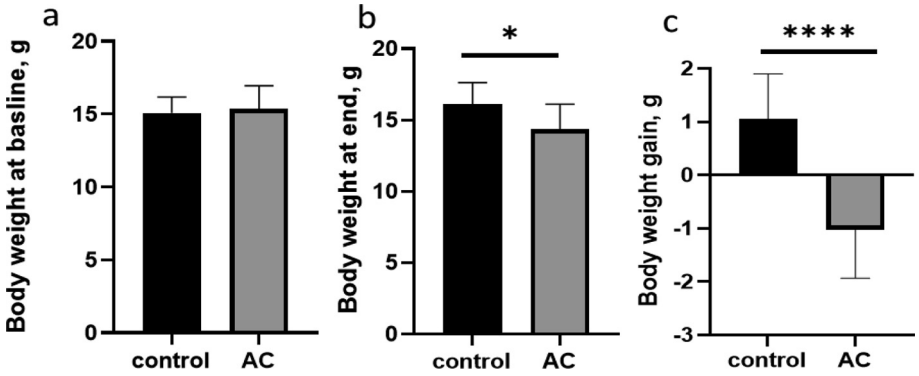

Fig. 1. Arithmetic means ± SEM of body weight at baseline (a), body weight at end (b) and body weight gain (c), ****indicates extremely significant (P < 0.0001), difference between control and 5% *Acarus calamus* (AC) drink after 15 days.

Table 2
Food and fluids analysis.

	Control	*Acarus calamus*	P-value
Food Intake, (g/24 h)	1.13 ± 0.06	0.97 ± 0.09	0.1679
Fecal wet weight, (g/24 h)	0.32 ± 0.06	0.29 ± 0.02	0.5483
Fecal dry weight, (g/24 h)	0.28 ± 0.06	0.25 ± 0.01	0.6113
Fluid Intake ,(mL/24 h)	2.04 ± 0.18	2.96 ± 0.16*	0.0015
Urine output, (mL/24 h)	0.74 ± 0.08	1.14 ± 0.07**	0.0013

Arithmetic means ± SEM (n = 10 each).*indicates significant (P < 0.05), **indicates highly significant (P < 0.001), difference between control and 5% *Acarus calamus* (AC) drink after 15 days.

3.3. Renal function test

Results shown in (Table 4) and Figs. 4–6, blood urea nitrogen, plasma creatinine were less significantly decreased, whereas, urinary urea excretion, plasma urea concentration, ureanary creatinine excretion, plasma creatine concentertion, glomerular filtration rate, Normalized 24-h. Creatinine Clearance, were increased in treated mice compared to control group.

3.4. Liver marker test

Table 5, shows the levels of liver marker; alkaline phosphatase (ALP) was mild increased, whereas alanine transaminase (ALT), aspartate transaminase (AST), were decreased when compared with control, not much change in Bilirubin was noted. The non-significant decreased levels of Random Blood Glucose level, (mg/dl) were obtained on treatment. The lipid profile on treated mice with the plant extract showed that Cholesterol and Triglycerides were little reduced, whereas High-density lipoproteins were increased.

3.5. Complete blood count (CBC) analysis

Table 6, shows Compete Blood Count (CBC); White Blood Cells, Lymphocytes Granulocytes, Monocytes showed normalcy in accordance with control. Mean corpuscular Corpuscular Volume was increased as the result Hemoglobin were also little increased in treated mice with 5% *Acarus calamus* (AC) drink for 15 days.

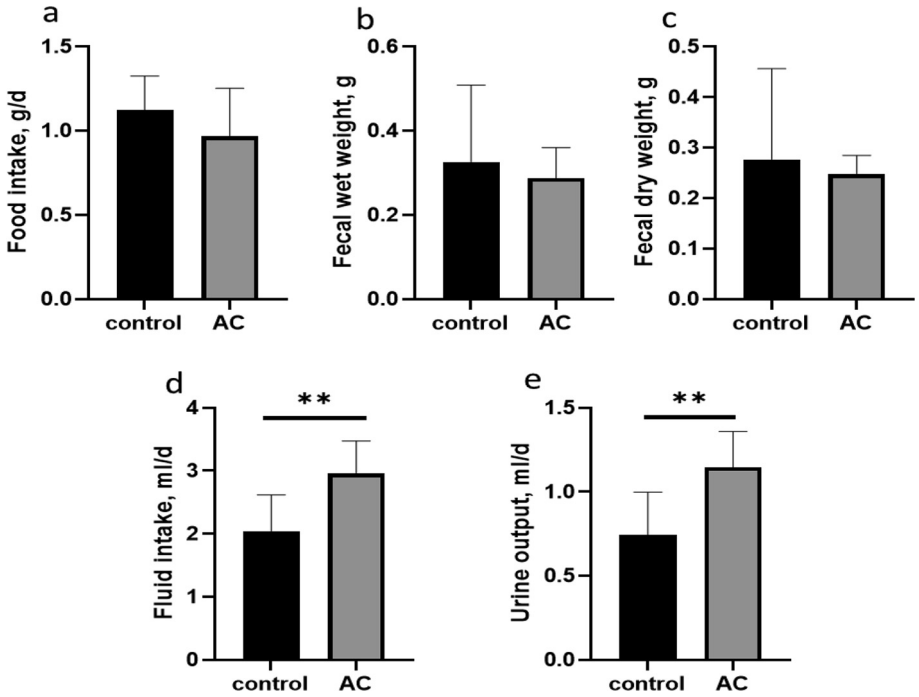

Fig. 2. Arithmetic means ± SEM of food intake (a), fecal wet weight (b) and fecal dry weight (c), fluid intake (d), and urine output (e), **indicates highly significant (P < 0.001), difference between control and 5% *Acarus calamus* drink after 15 days.

Table 3
Plasma and urine electrolytes.

	Control	*Acarus calamus*	P-value
Plasma concentration of electrolytes			
[Na^+]plasma, (mM)	143.19 ± 1.47	141.01 ± 1.41	0.2976
[K^+]plasma, (mM)	4.29 ± 0.13	4.19 ± 0.25	0.7172
[Ca^{2+}]plasma, (mM)	2.15 ± 0.05	2.09 ± 0.07	0.4882
Urinary excretion of electrolytes			
Urine Na^+, (mmol/l)	213.90 ± 5.79	237.84 ± 13.89	0.6975
Urine K^+, (mmol/l)	706.47 ± 10.41	715.51 ± 20.23	0.6958
Urine Ca^{2+}, (mmol/l)	8.24 ± 0.20	8.28 ± 0.42	0.9288

Sodium (Na^+) Potassium (K^+) and Calcium (Ca^{2+}).
Arithmetic means ± SEM (n = 10 each), difference between control and 5% *Acarus calamus* drink after15 days.

Table 4
Renal function test.

	Control	*Acarus calamus*	P-value
Blood Urea Nitrogen, (mg/dl)	19.26 ± 0.85	16.39 ± 1.06*	0.0489
Urinary urea, (mg/dl)	47.50 ± 2.85	55.27 ± 1.13*	0.0105
Plasma creatinine, (mg/dl)	0.27 ± 0.02	0.25 ± 0.04	0.6564
Urinary creatinine, (mg/dl)	40.39 ± 2.66	42.54 ± 1.53	0.4921
Glomerular Filtration Rate GFR, (μl/min)	10.77 ± 1.60	18.53 ± 2.94*	0.0322
Normalized 24-h creatinine clearance (μL/min/g body weight)	34.06 ± 2.77	52.64 ± 5.90*	0.0106

Arithmetic means ± SEM (n = 10 each),*indicates significant (P < 0.05), difference between control and 5% *Acarus calamus* drink after 15 day.

3.6. Histological analysis of Acarus calamus (AC) on kidney and liver

After 15 days of 5% *Acarus calamus* (AC) treatment, the histology section of section of kidney (Fig. 7), showed normal glomerulus and renal tubules compared to control group, also histology of liver section after 5% *Acarus calamus* (AC) treatment, showed normal hepatic tissues, hepatocytes, Kupffer cells, Central vein (CV), compared to control group (Fig. 8).

4. Discussion

Obesity is the serious health problem, giving negative effect on body and mind, it is the increasing fatal disease with increasing prevalence of various diseases (hypertension, coronary heart disease, diabetes mellitus, hyperlipidemia, etc (Hurt et al., 2010). Due to adverse effects and high cost of modern drugs, traditional plants with secondary metabolites are under investigation to use as potential, effective and safe antiobesity drugs (de Freitas Junior and de Almeida, 2017).

The present paper emphasizes on study related to the antiobesity effect of plant extracts on 5-7week old C57Bl/6 mice. The effect of 5% (w/v) of *Acarus calamus* (AC) extract were evaluated on parameters like body weight, biochemical, liver and kidney function markers and kidney histopathology. The plant extracts exhibited good antiobesity effect without negative alterations of resulted pathological reports (Singh et al., 2017). Extracts rich in polyphenols are responsible for antiobesity activity (Boccellino and D'Angelo, 2020).

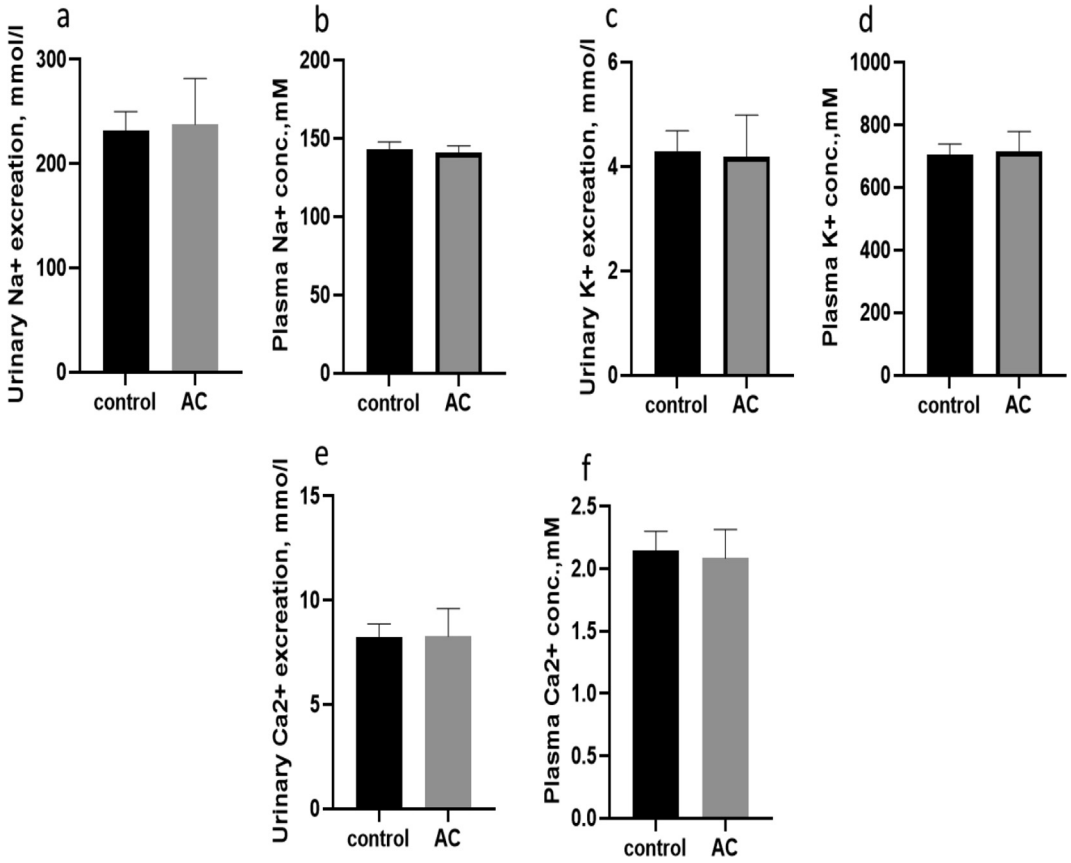

Fig. 3. Arithmetic means ± SEM of urinary Na^+ excretion (a), plasma Na^+ concentration (b), Urinary K^+ excretion (c), plasma K^+ concentration (d), urinary Ca^{2+} excretion (e), plasma Ca^{2+} concentration (f), of control and 5% *Acarus calamus* drink after 15 days.

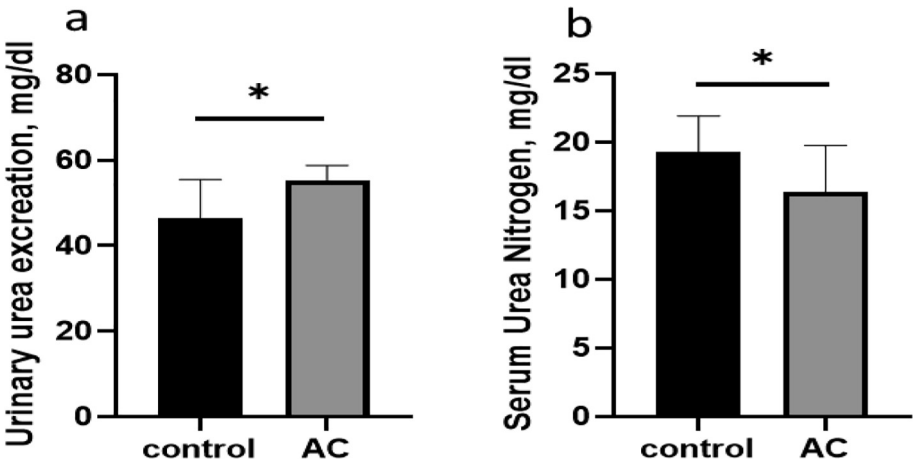

Fig. 4. Arithmetic means ± SEM of urinary urea excretion (a), and serum urea concentration (b),*indicates significant difference (P < 0.05) between control and 5% *Acarus calamus* drink after 15 days.

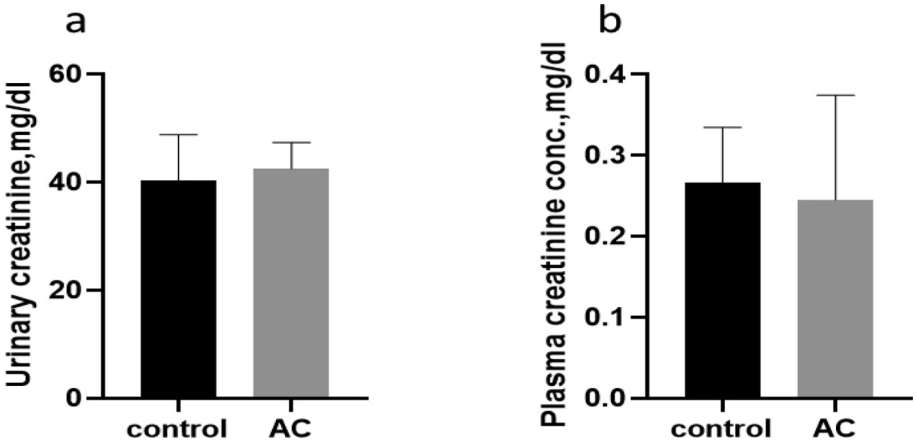

Fig. 5. Arithmetic means ± SEM of Urinary creatinine excretion (a), and plasma creatinine concentration (b), of control and 5% *Acarus calamus* drink after 15 days.

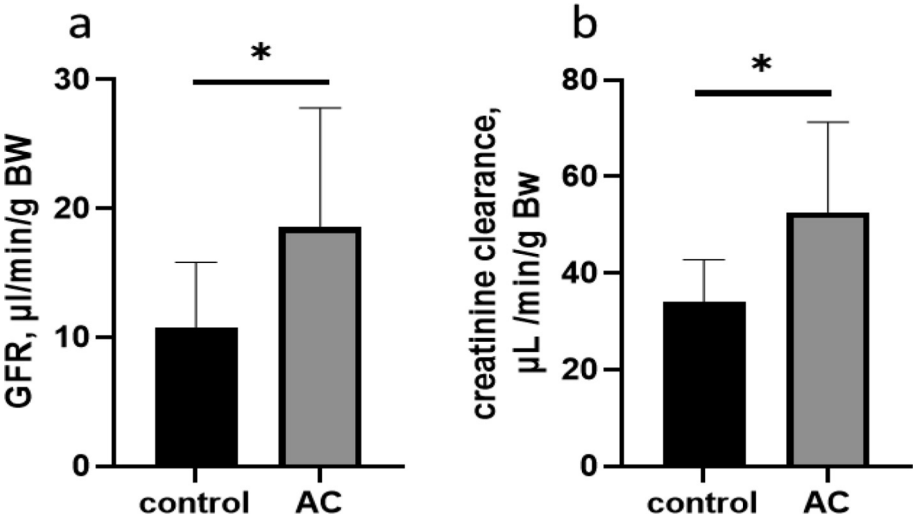

Fig. 6. Arithmetic means ± SEM of glomerular filtration rate, GFR (a), and normalized 24-h creatinine clearance (b), *indicates significant difference (P < 0.05) between control and 5% *Acarus calamus* drink after 15 days.

Table 5
Liver marker test.

	Control	*Acarus calamus*	P-value
Random Blood Glucose level, (mg/dl)	99.30 ± 2.49	80.60 ± 4.91**	0.0032
Alkaline Phosphatase (ALP), U/L	64.70 ± 9.27	68.28 ± 9.82	0.7939
Alanine Aminotransferase (ALT), U/L	44.67 ± 8.96	36.84 ± 8.19	0.5275
Aspartate Aminotransferase (AST), U/L	46.82 ± 4.83	40.22 ± 8.68	0.5152
Bilirubin, (mg/dl)	0.24 ± 0.02	0.25 ± 0.04	0.8223
Cholesterol, (mg/dl)	26.60 ± 1.63	25.80 ± 2.24	0.7763
High-density lipoproteins (HDL), mg/dl	20.60 ± 1.26	29.20 ± 6.18	0.1893
Triglycerides, (mg/dl)	36.20 ± 8.03	35.20 ± 6.62	0.9245

Arithmetic means ± SEM (n = 10 each), **indicates highly significant (P < 0.001) difference between control and 5% *Acarus calamus* drink after 15 days.

Table 6
Complete Blood Count (CBC) analysis.

	Control	Sweet flag	P-value
White Blood Cells(WBC), 10^3/ml	6.77 ± 0.22	6.69 ± 0.41	0.8661
Lymphocyte, 10^3/ml	5.30 ± 0.41	5.19 ± 0.60	0.8813
Lymphocyte (LYM), %	87.63 ± 1.15	88.82 ± 1.37	0.5134
Granulocytes (GRA), 10^3/ml	0.35 ± 0.05	0.32 ± 0.06	0.6800
Granulocytes (GRA), %	3.12 ± 0.26	2.67 ± 0.16	0.1505
MID,10^3/ml	0.43 ± 0.05	0.46 ± 0.08	0.7809
MID, %	5.20 ± 0.16	5.30 ± 0.19	0.6966
Hemoglobin (Hb), g/l	13.63 ± 0.25	14.01 ± 0.20	0.2538
Mean Corpuscular Volume (MPV), fl	7.37 ± 0.39	7.71 ± 0.35	0.5295

Arithmetic means ± SEM (n = 10 each), for control and 5% *Acarus calamus* drink after 15 days.
MID, indicates the combined value of the other types of white blood cells not classified as lymphocytes or granulocytes.

In this study enzymes of hepato marker such as alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), were decreased when compared with control, the decreasing levels in the drug- treated animals suggested amelioration of fatty liver. The improvement of liver functions shows the outcome of the improved obesity condition due to the inhibitory effect of AC on pancreatic lipase activity, A pancreatic lipase inhibitor, orlistat, has been clinically proven to prevent obesity and hyperlipidemia by increasing fat excretion into the faeces and inhibiting pancreatic lipase (Heck et al., 2000). Bilirubin levels with not much change was noted hence no hepatocellular damage was noted.

It was reported the presence of Tannins in AC, Tannins (polyphenols) are known to have antioxidant efficacy, and proven antioxidants, such as β-carotene, vitamin E and astaxanthin, can result in a decrease in plasma transaminase levels as a result of oxidative damage (Chen et al., 2015). Therefore, the antioxidant efficacy of the AC plant drug may also lead to the decrease in elevated plasma transaminase levels (Subathraa and Poonguzhali, 2012).

Drug- induced nephrotoxicity is investigated by biochemical parameters such as blood urea, serum creatinine, creatinine clearance. Nephrotoxicity in renal function is due to increased levels in serum creatinine, blood urea nitrogen, urine creatinine and serum urea levels (Kakalij et al., 2014). In the earlier phase of the renal damage, increase in serum creatinine level is more significant than the increase in serum urea level, Experimental mice showed reduced weight loss with treated AC extract, (Table 1 and Fig. 1). Due to decreased Food intake, fecal wet and dry weight was reduced (Table 2 and Fig. 2). Animal showed more fluid intake with gradual urinary output (Table 2 and Fig. 3) due to more concentration of electrolytes in urine, concentration of electrolytes in plasma were reduced (Table 2). Hence all shows normalcy with no adverse effects on experimented mice.

It is necessary to demonstrate the toxicity of the extract before proposing it as possible therapeutic medicine. Hepatotoxicity and nephrotoxicity effects are observed with the levels of ALT, BUN, plasma creatinine, Urinary urea, Glomerular Filtration Rate GFR, Urinary creatinine, Normalized 24-h. creatinine clearance (Table 3). The extract had no measurable effects on these variables of the pathology, as they show similar levels of these mediators in treated

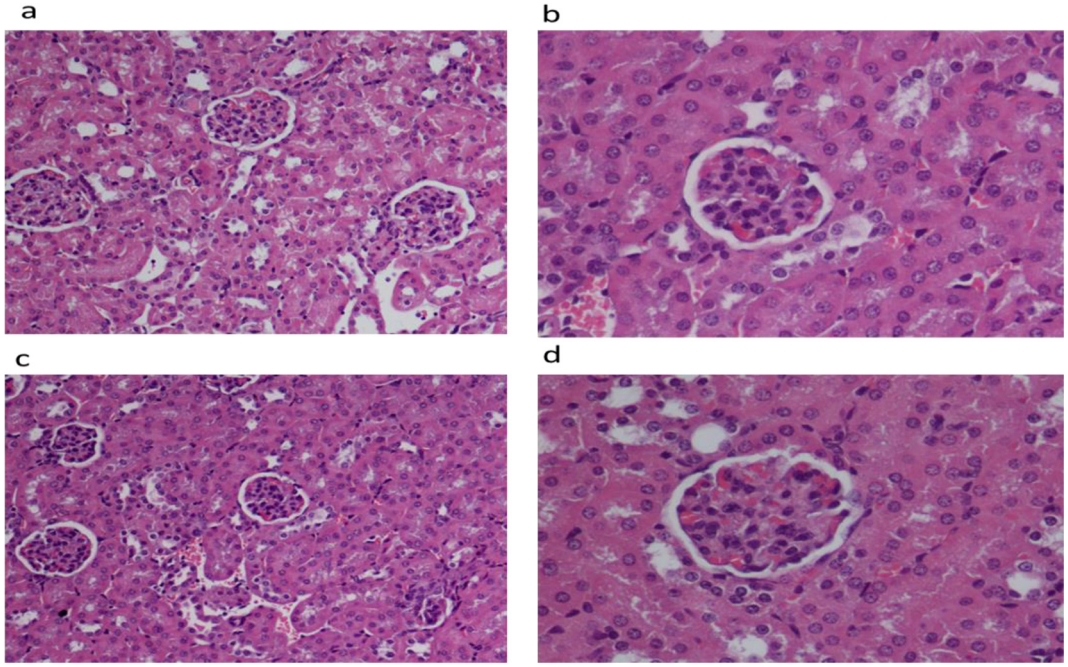

Fig. 7. Section of kidney showing glomerulus, renal tubules (a, b), of control group, (c, d), after 5% of *Acarus calamus* drink after 15 days. (H & E stain), magnification, (200× and 400×).

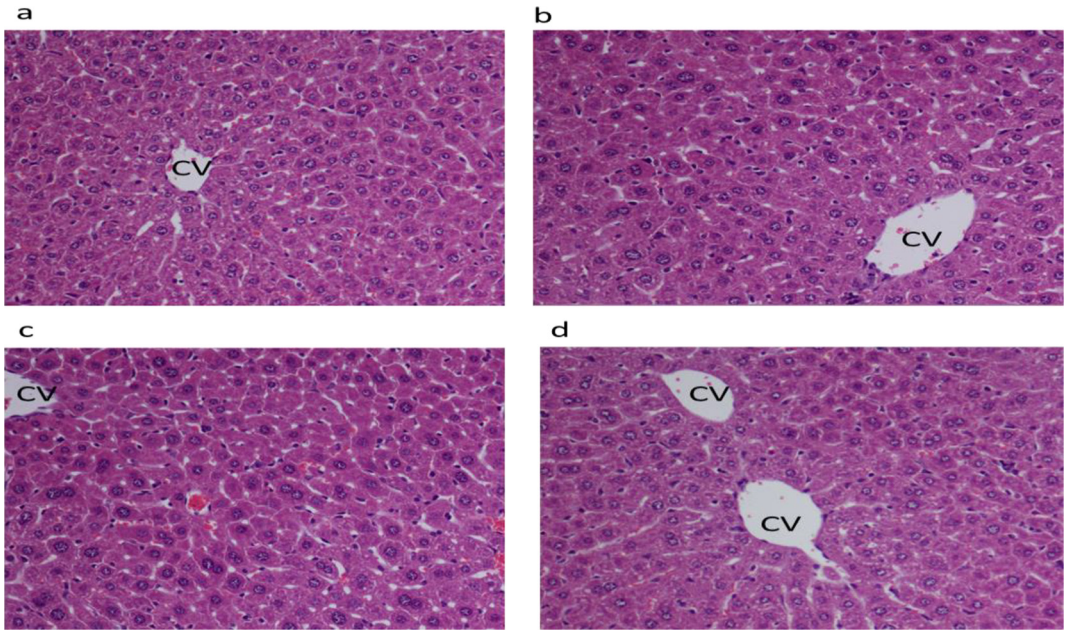

Fig. 8. Sections of liver showing normal hepatic tissues. Hepatocytes, Kupffer cells, Central vein (a, b), of control group (c, d), after 5% of *Acarus calamus* drink after 15 days, (H & E stain), magnification, 200×.

and control. This agrees the extract has no cytotoxic effect, also agrees with the findings of the other investigations that AC has no cytotoxic effect, proven with medicinal proprties (KUMAR, 2013). The results show an increase in serum concentrations of BUN and creatinine, leads to inflammatory response which directly affects renal function. Some plants containing antioxidant activity have been documented to reduce secondary damage caused which is generated by kidney oxidative stress. In this study, we found that the AC possesses nephro- protective effect, which may be related to its antioxidant activity (Mehdi and Ahmad, 2018).

The extracts showed reduced blood sugar level and body weight as compared to control group. The decreased levels of Random Blood Glucose level, (mg/dl) obtained on treatment due to less food intake, which could be normalised with efficient food. Plant extract may be responsible for their antiobesity activity (Athesh and Jothi, 2017). Increased High-density lipoproteins supposed to be a good cholesterol, suggest the improve quality of the treatment with *Acarus calamus* (AC) (Mukherjee et al., 2007). Mean Corpuscular Volume, which is measurement of the average size of red blood cells was increased with noted increased result of Hemoglobin. This is because bigger red blood cells generally contain more haemoglobin while smaller red blood cells tend to have less haemoglobin.

Generally drugs get accumulated in renal cortex leads to nephrotoxicity such as inflammation, cell necrosis tubulonephritis etc, drug deposition depends on affinity of drugs towards kidneys and on kinetics of drug trapping process. *Acarus calamus* (AC) extract showed nephroprotective activity with normal renal structure, no inflammation and no haemolysis (Ghelani et al., 2016). Renal histopathology showed hematoxylin (H) and eosin (E) stained tissue sections with clear, normal renal parenchyma, tubules and glomeruli.

5. Conclusion

It was observed that the *Acorus calamus* (AC) conferred weight reducing drink, as traditional theurepeutic medicine with hepatoprotective and nephroprotective activity by biochemical and histopathological observations. Thus, the use of *Acorus calamus* (AC) may be recommended for lifestyle-related diseases including hepatic diseases and also to improve the general health condition in animals including human beings. Before the clinical application of *Acorus calamus* (AC) as an antioxidant agent, comprehensive studies on target species should be carried out. Furthermore, the data of the results with all clinical parameters observed in AC treated mice can be consumed without any adverse effect on health. In this present study, inhibition of pancreatic lipase activity was observed, the anti-obesity ability of this selected plant drug could mediate delayed intestinal absorption of dietary fat.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

All work have been done by the principle author, Omaima Nasir.

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