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Antioxidant and Hepato-renal Protective Activity of *Anogeissus leiocarpus* Bark against CCl_4–induced Hepatorenal toxicity in Rats.

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Abstract

Sahab (*Anogeissus leiocarpus*) has been used as traditional remedy in the Sudan for the treatment of a variety of diseases including liver disorders. Antioxidant and hepatorenal protective effects of *A. leiocarpus* bark ethanolic extract were investigated in rats against carbon tetrachloride (CCl_4) induced hepatorenal injury. Chemical constituents of the plant were also conducted. *In vitro* antioxidant properties of *A. liocarpus* bark was explored using DPPH radical scavenging and iron chelating assays. Rats were injected CCl_4 at a dose of 1.25ml/kg as single dose at 12 hour (h) after administration of the plant extract three times at 0h, 12h and 24h. Samples were taken after 36h for biochemical, haematological and histopatiological investigations. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (Tbil), direct bilirubin (Dbil), total protein (TP), albumin (Alb), globulin (Glo), urea and creatinine were measured as biomarkers of liver and renal damage. The tissues of liver and kidney were also isolated carefully for histopathology. Phytochemical screening of *A. leiocarpus* bark ethanolic extract indicated the presence of tannins, saponins, flavonoids, sterols, triterpenoids and cumarins. Antioxidant activity and iron chelating antioxidant assay exhibited moderate activity (43%±0.01) using DPPH radical scavenging assay compared to standard antioxidant propyl gallate (72%±0.03). However the iron chelating antioxidant assay showed low antioxidant activity against ferrous iron and ferrozine complex (23%±0.034) compared to standard antioxidant compound EDETA (98%±0.01). Oral administration of the ethanolic extract of the plant at a dose of 200 mg/kg displayed a significant ($P < 0.05$) hepatorenal protective effect against CCl_4 by lowering liver biomarkers (AST, ALT, ALP), kidney biomarkers levels (urea, creatinine) as well as haematological parameters when compared with standard drug silymarin. Histopathological investigation of liver and kidney tissues verified the protection effect of the plant extract.

Keywords: *Anogeissus liocarpus*, hepato-renal activity, Silymarin, carbon tetrachloride.

Introduction

The development of several liver diseases such as fibrosis, cirrhosis, hepatocellular carcinoma (HCC), alcoholic liver disease, and nonalcoholic fatty liver disease (NAFLD) is due to an increase of oxidative stress. Thus, Antioxidants play major role for protection of the liver from various diseases caused by oxidative stress (Muriel, 2009 and Murriel and Casas-Grajales, 2017).

Liver has crucial role in the metabolism of drugs and chemicals thus protecting body against toxic foreign materials. During this process the liver is exposed to high concentration of toxic chemicals and their metabolites which may cause liver injury. Alternative remedies of plants origin have been used successfully worldwide for the treatment of liver disorders for better efficacy and safety to replace currently used drugs (Chang *et al*., 2008).

Anogeissus leiocarpa belongs to the family *Combretaceae*. It's local and Arabic named is *sahab*. In Sudan *A. leiocarpus* tree is widespread in Kassala and Darfur States (Elamin, 1990). In Sudan the decoction of the barks is used traditionally against cough (El Ghazali *et al.,* 2003). In Africa the plant is used for parasitic diseases such as malaria, trypansomiasis, helminthasis and dysenteric syndrome (Okpekon 2004). It is also used in traditional medicine as a remedy for many ailments of livestock and man, which include schistosomiasis, leprosy, diarrhoea and psoriasis (Burkill, 1985). It is also used for the treatment of pain, asthma, fungal infections, and diabetic ulcers (Batawila, 2005 and Victor, 2013). The methanolic extract of leaves and roots of *A. leiocarpus* were found to be strongly active against malaria and trypansomiasis when tested using *in vitro* model (Okpekon 2004). Castalagin fraction that isolated from the aqueous, butanol fractions of the methanolic extract of stem bark showed significant trypanocidal and leishmanicidal activities (Shuaibu *et al.,* 2008 and Shuaibu *et al.,* 2008). The bark extract showed antibacterial properties (Arbonnier, 2004). Methanolic and ethyl acetate extracts of the plant showed antioxidant and hepatoprotective activities (Atawodi *et al.,* 2011). The aim of this study was to evaluate the antioxidant and hepato-renal protective effect of *A. leiocarpus* bark ethanolic extract against CCl_4 induced liver damage in rats.

Materials and Methods

Plant material and extraction

The bark of *A. leiocarpus* was collected from natural habitat and shade dried at room temperature. The plant was identified and authenticated by the botanists in the Medicinal and Aromatic Plants, Traditional Medicine and Research Institute, National Center of Research, Khartoum, Sudan. The powder bark of the plant was extracted by maceration in ethanol 80% for one week. The plant extract was evaporated to dryness under 40ºC by a rotary vacuum evaporator and the yield was calculated (Harborne. 1984). The residue obtained was kept in dry clean bottles till used for pharmacological study.

Phytochemical screening:

Phytochemical screening was carried out according to the method of Harbone, (1984) to identify the chemical constituents of the plant material. 10 g of the powdered part of the plant was refluxed with100 ml of 80% ethanol for four hours. The cool solution was filtered and screened for the phytoconstituents.

Antioxidant activity:

The antioxidant properties of *A. liocarpus* bark was investigated using DPPH radical scavenging and iron chelating assays (Adedapo *et al*., 2008 Olutayo *et al.,* 2013 and Nabti and Belhattab 2016). The ethanolic extract was dissolved in Dimethyl sulphoxide (DMSO) (5µg/ml). Propayl gallate, and Ethylene diamine tetra acetic

(EDTA), were used as comparative antioxidant standards.

Experimental Animals

Healthy adult Wistar albino rats of both sex weighing 120 – 130 g were obtained from the Animal Laboratory House, Faculty of Veterinary Medicine, University of Khartoum, Sudan. They were housed in specific standard laboratory conditions in temperature control environment in the Medicinal and Aromatic Plants Research Institute (MAPRI), natural Centre for Research (NCR), Khartoum, Sudan. Rats were fed with standard rats chow diet and water *ad libitum* and were left for seven days to acclimatize to laboratory environment.

Hepato-renal protective activity

Twenty four rats were allotted to four groups of six rats each. They were treated as follows; Group I: the normal control rats, received three doses of 5% a*cacia mucilage* (vehicle) at a dose of 1ml/Kg orally at 12 hour intervals (0, 12 and 24 hours). Group II: carbon tetrachloride group, administered three doses of vehicle at 12 hour intervals and injected subcutaneously a single dose of carbon tetrachloride (1.25ml/kg) diluted in liquid paraffin (1:1) 30 minutes after the administration of the first dose of vehicle. Group III: the test extract group administrated orally three dose of *A. leiocarpus* extract at a dose of 200mg /kg at 0, 12 and 24 hours; carbon tetrachloride was injected subcutaneously (1.25ml/kg) 30 minutes after the administration of the first dose of the extract. Group (IV): a hepato-renal protective drug control; rats were given three doses of silymarin at a dose of (100mg/kg) at 0, 12 and 24 hours; carbon tetrachloride was injected subcutaneous a single dose (1.25ml/kg) 30 minutes after the administration of the first dose of silymarin.

Blood samples

Blood was collected under anaesthesia by puncturing retro-orbital plexus using capillary tubes for haematological and serobiochemical parameters at 36 hour after sacrificed the animals. EDTA was used as an anticoagulant in blood samples for haematological studies and analyzed immediately while the serum was stored at -20°C until analyzed.

Biochemical analysis

The biochemical parameters were measured using standard kits (Stanbio laboratory Inc., San Antonio, TX, USA). The parameters included the determination of Aspartate transaminase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP), total protein, albumin, and bilirubin. While the globulin was obtained by subtracting albumin concentration from that of total protein. Serum urea and creatinine concentrations were also measured by an enzymatic method using a commercial kit.

Haematolgical studies

Packed cell volume (PCV), haemoglobin concentration (Hb), red blood cells count (RBC), white blood cells count (WBC), Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were analyzed using automatic analyser (Humacount plus- Human GmbH Max- Planck-Ring21, D-65205 Wlesbaden, Germany).

Histopathological studies:

The rats were sacrificed after 36 hour and the livers were carefully isolated and fixed in 10% formalin and embedded in paraffin wax. Sections of 4-5 microns thickness were made and stained with haematoxyline-eosin (Drury and Wallington, 1980).

Statistical analysis:

The data were expressed as mean ± standard error (SE). The Significance of differences among the group was assessed using T-test (Mendenhall, 1971).

Results

Clinical finding and necropsy:

After injection of CCl_4, all treated groups suffered slight convulsion and depression. At necropsy, the livers of CCl_4 group showed fatty changes and slight enlargement in liver size compared to the control groups. These changes were less noticed or disappeared in the rats treated with the bark extract of *A. leiocarpus* and silymarin group.

Phytochemical screening:

The results of phytochemical screening of the powdered material of plant revealed the presence of tannins, saponins, flavonoids, sterols, triterpenoids and cumarins.

Antioxidant activity:

The DPPH radical scavenging assay demonstrated that *Anogeissus leiocarpus* at the concentration of 500 µg/ml exhibited moderate scavenging activity (43%±0.01) compared to standard antioxidant propyl gallate (PG) that scored up to 72%±0.03. Whereas the iron chelating antioxidant assay showed low antioxidant activity against ferrous iron and ferrozine complex (23%±0.034) compared to standard antioxidant compound EDETA (98%±0.01)

Effect of *Anogeissus leiocarpus* on liver and renal enzymes

The serobiochemical parameters were significantly increased in CCl_4 intoxicated group 1 compared to the normal values of the control rats group II. There were significant decrease ($P<0.05$) in the activities of AST, ALT. ALP, Tbill, Dbill, urea, creatinine levels and the concentration of total protein, albumin and globulin ($P<0.05$) in *A. leiocarpus* ethanolic bark extract treated group III, and silymarin group IV (standard drug), when compared to CCl_4 intoxicated group 1. These values were comparable to the normal value of control rats group II (Fig.1,2 and 3).

Haematological findings

In CCl_4 group there was significant decreased in the values of Hb, RBC, MCH and WBC compared to the standard drug silymarin. There was significant increase in Hb and MCHC values in silymarin group and ethanolic extract of *A. leiocarpus* compared to control group (Table 1).

Histopathological findings:

Liver sections of normal control rats showed no abnormalities cellular changes. Rats received CCl_4 intoxicated rats, showed the lay centerilobular vacuolations and necrotic hepatocytes (Fig.4). Liver sections from the rats that received CCl_4 and *A. leiocarpus* (Fig.6) demonstrated signs of protection evident by the less vacuolated hepatocytes and cellular regeneration. While rats given silymarin and CCl_4 revealed clear protection of hepatic tissue from CCl_4 (Fig.5). Kidney sections of CCl_4 showed dilation of tubules with degenerative changes in tubular epithelium and these changes were less noticed in rats given silymarin and *A. leiocarpus* extract.

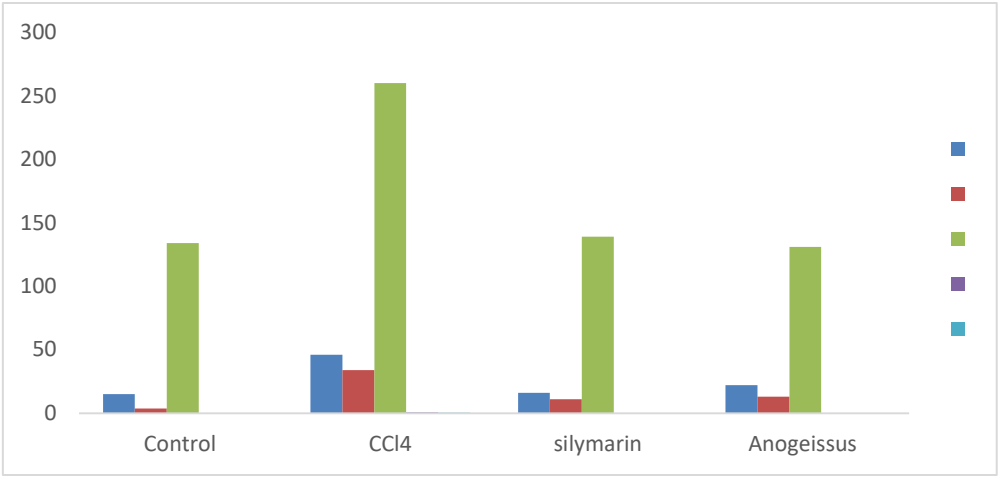

Fig.1 the protective effect of *A. leiocarpus* bark ethanolic extract against CCl_4 – induced hepatorenal toxicity in rats on liver function test

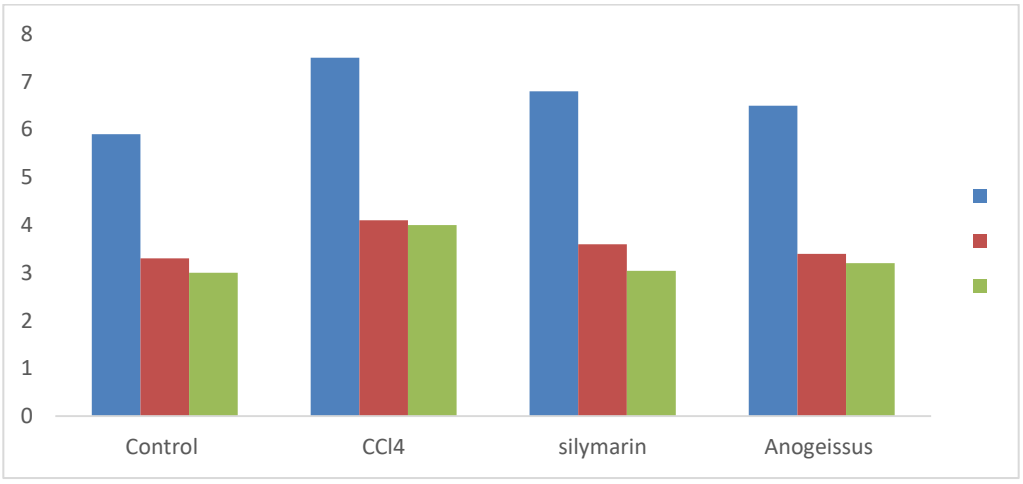

Fig.2. The protective effect of *A. leiocarpus* bark ethanolic extract against CCl_4 – induced hepatorenal toxicity in rats on proteins

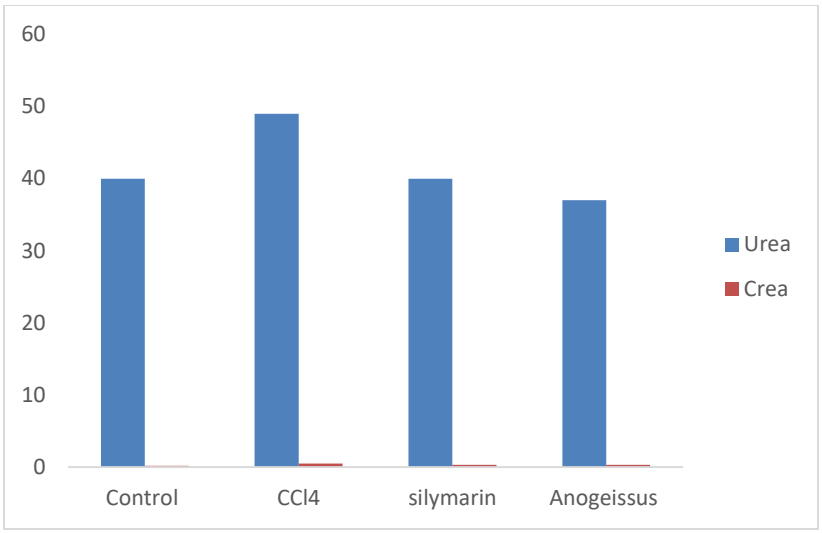

Fig.3 The protective effect of *A. leiocarpus* bark ethanolic extract against CCl_4 – induced hepatorenal toxicity in rats on kidney function test.

Table (1): Effect of *A. leiocarpus* bark extract against CCL_4 – induced liver damage in rats on heamatological values (mean ± S.E)

Parameters	CCl_4*	Control	*Anogeissus*	Silymarin
PCV%	62.7±8.0	70.0±2.0	70.0±1.0	73.0±2.0
HB(g/dl)	14.0±1.0	14.0±1.0	16.0±1.0**	17.0±1.0**
WBC($\times 10^3$ µl)	5.0±1.0	7.0±1.0	9.0±1.4	11.0±1.0*
RBC(10^6 cells/µl)	9.7±1.0	10.0±0.3	11.0±0.4*	11.0±0.4*
MCV (fl)	67.0±3.0	76.0±3.0	68.0±2.0	72.0±5.0
MCH (pg)	12.0±1.0	15.0±0.3	15.0±0.3*	15.0±0.3*
MCHC (g/l)	19.0±19.0	19.4±3.0	22.2±9.0	21.0±8.0

Statistical analysis T- test * ($P<0.05$) as compared to CCl_4 group. Values are expressed as mean±SE.

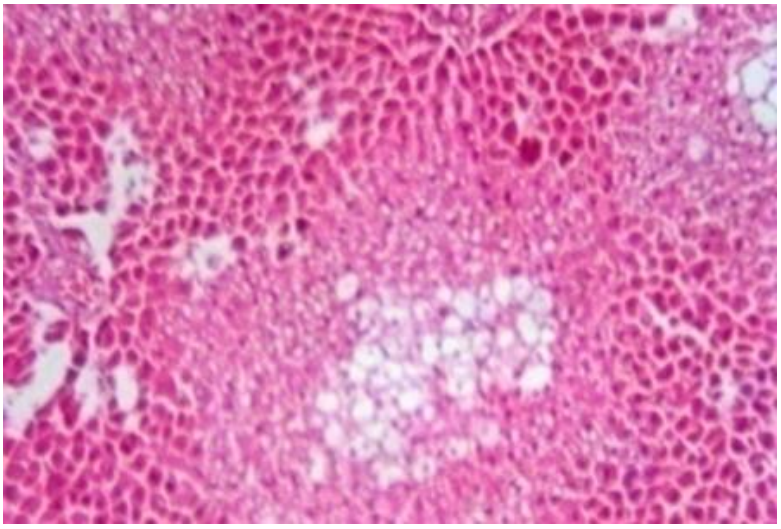

Fig.4. Liver section of CCl4 showed diffuse centerilubular vaculation with hepatic necrosis

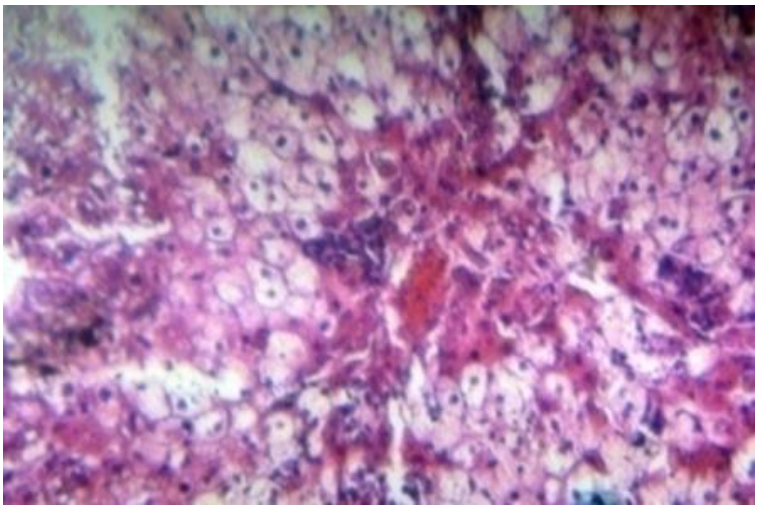

Fig.5. Liver section of silymarin showed less vacuolated areas of. hepatocytes and disorganization

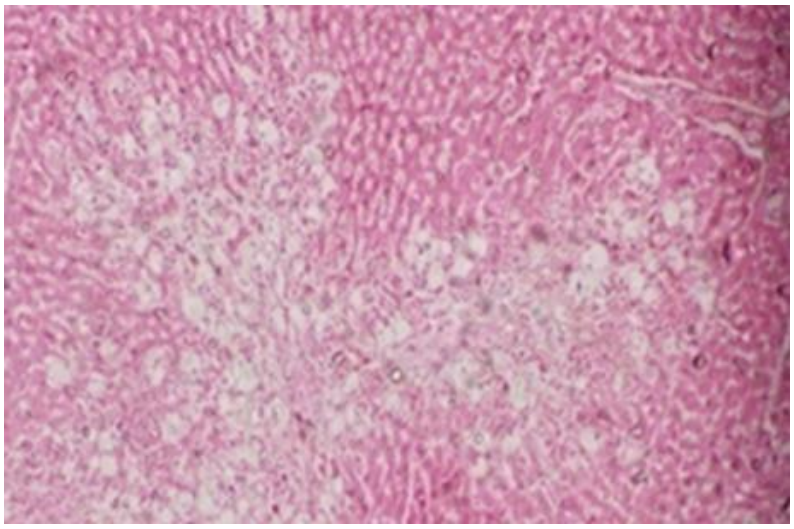

Fig.6. Liver section of *A. leiocarpus* less vacuolated. Hepatocytes and cellular regeneration.

Dsicussion

Liver and kidney are the main target organs for xenobiotic metabolism (Liman and Atawodi, 2015). Carbon tetrachloride cause a significant toxicity in different mammalian tissues including kidney and liver due to the production of highly reactive oxygen radicals (CCl3°) as a result of extensive biotransformation in the livers P-450 system, which lead to peroxidation of proteins, alteration of enzymes and DNA (Melin *et al.*, 2000; Abdel-Kader *et al.*, 2016; Okolo *et al.*, 2017 and Ojiako *et al.*, 2017). Impairment of liver and kidney function is reflected as the elevation of the serum levels ALT, AST, ALP, urea and creatinine (Adelman *et al.*, 1981; Pocock and Richards, 2006 and Ojiako *et al.*, 2017). Assessment of hepatic and renal function tests in serum of animal models using carbon tetrachloride induced hepatorenal toxicity, have been used extensively as basis for discovering promising hepato-renal remedies of plants origin (Ottu *et al.*, 2013; Ojiako *et al.*, 2017; Okolo *et al.*, 2017). The efficacy of these plants based on the reduction of the activities of liver and renal enzymes to standard levels (Ottu *et al.*, 2013 and Liman and Atawodi, 2015).

In this study, acute hepatotoxic and nephrotoxic effects of CCl_4 injection were supported biochemically and histopathologically. This proved by significant elevation of serum enzymes ALT, AST, ALP, Tbill, Dbill, total protein, albumin, globulin, urea and creatinine. Extensive degenerative changes in hepatocytes, necrosis, fatty changes, inflammatory cell infiltration, sinusoidal dilatation and congestion were also observed in CCl_4. Nephrotoxic effects of CCl_4 injection were also confirmed by disrupting of the normal architecture of the kidney cell which evidence by degenerative changes in the tubules and glomerulus these findings are in agreement of Liman and Atawodi, (2015) and Abdel-Kader *et al.*, (2016). In addition, the values of Hb, RBC, MCH and WBC were significantly decreased compared with normal and other groups.

Investigation of the ethanolic extract of *A. leiocarpus* bark as hepatoprotective and nephroprotective plant against CCl_4 induced hepatorenal toxicity in rats at a dose of 200 mg /kg showed significant decrease in the levels of serum enzymes ALT, AST, ALP, Tbill, Dbill as well as significant decrease in the concentration of total protein, albumin, globulin, urea and creatinine compared to the CCl_4 group**.** The effect of the extract of *A. leiocarpus* on the hepatic enzymes (ALT, AST and ALP) and renal indices was comparable to that of silymarin a known hepatoprotective agent. Silymarin (*Silybum marianum*) is used as antioxidants and antihepatoxic agent for many liver diseases (Lin *et al*., 2004).

The protective effect of the extract may be related mainly to the antioxidant property of its high content of flavonoids, tanins, sterols and triterpens (Latha *et al*., 2003, Barku *et al.,* 2013 and Victor and Grace, 2013). In addition similar to silymarin the plant extract significantly increased Hb and MCH values compared with the normal control.

Moreover, *A. leiocarpus* ethanolic extract protected the liver and renal cells from harmful effect of CCl_4. This is seen clearly by less degenerative changes in hepatocytes and renal cells in *A. leiocarpus* ethanolic extract group. These evidences confirmed the hepato-renal protective effect of the plant.

Conclusion

In conclusion, the ethanolic extract of *A. leiocarpus* showed a possible hepato-renal protective activity against carbon tetrachloride induced hepato-renal toxicity. This also confirmed the traditional use of this plant in the treatment of jaundice and kidney problems. Further studies should be done to identify the bioactive component(s) of *A. leiocarpus* and support the protective effect of this plant through clinical trials.

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