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ANTIOXIDANT AND HEPATOPROTECTIVE ACTIVITIES OF BLEPHARIS LINARIIFOLIA PERS AND GUIERA SENEGALENSIS J. F. GMEL. AGAINST CCL_4-INDUCED HEPATOTOXICITY

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ABSTRACT

Herbal medicines from folk practice have proven to be safe and have ability in alleviating the toxicity induced by xenobiotics. *Blepharis linariifolia Pers* (Acanthaceae) and *Guiera senegalensis* J. F. Gmel. (Combretaceae) are two widely used medicinal plants in Sudan. Forty two Wister Albino rats (85-140 g) were used to evaluate the protection of *B. Linariifolia* and *G. Senegalensis* extracts from hepatic injury. Rats in Group 1 were used as negative control. Acute liver injury (Group 2) was induced using subcutaneous injection of carbon tetrachloride (CCl_4). Group 4 and 5 received *B. Linariifolia* extracts at 200 and 400 mg/kg, respectively. Group 6 and 7 received *G. senegalensis* extracts at 200 and 400 mg/kg, respectively. Silymarin was used as a positive control (Group 3). Phytochemical and antioxidant properties were conducted using DPPH assay and pharmacognosic standard methods, respectively. Administration of CCl4 induced distinctive histological changes such mononuclear cell infiltration, vacuolar and hepatocellular degeneration and bile duct proliferation. Elevation in hepatic enzymes, urea and total protein was evident in Group 2. Dose dependent protective effects against these pathological lesions and indicators were observed on the groups received plants extracts and Silymarin. *B. Linariifolia* and *G. senegalensis* and propyl gallate have demonstrated

bleaching effect of 78±0.01%, 92±0.02% and 89±0.01%, respectively, on DPPH assay. Various phytochemicals have been detected in both in both extracts. The hepatoprotection of *B. Linariifolia* and *G. Senegalensis* could be attributed to their antioxidant effects.

KEYWORDS: Hepatoprotection, Blepharis Linariifolia, Guiera Senegalensis, pesticides, Environmental hazards, Medicinal plants.

INTRODUCTION

Liver, the largest organ in the body protect the body by detoxification and regulating normal physiological. It plays an important role in fat metabolism via bile acid secretion; it helps the fat soluble vitamin storage and utilization and metabolism of drugs (Gao et al., 2008). Even though liver actively detoxify many chemical compounds, the chemical compound and xenobiotics do harm to liver by various mechanisms. These drugs and chemical substances usually initiate a biochemical stress within the hepatic cells via a covalent binding or through direct mitochondrial damage. This usually induces heavy load of oxidative stress though modulating stress inducing signaling pathways and eventually leads to diminishing mitochondrial function. According to WHO, about 75% of the idiosyncratic drug reactions result in liver transplantation or death. It is one of the main reason for withdraws of many clinically used drug from the market (Martin et al., 2000; Somasundaram et al., 1997).

There are many herbal medicines from folk practice have proven to be safe and have ability in alleviating the toxicity induced by xenobiotics. *Blepharis linariifolia Pers* (Acanthaceae) and *Guiera senegalensis* J. F. Gmel. (Combretaceae) are two widely used medicinal plants in North Kordofan (Western Sudan). In the folk medicine practice of Sudan Decoction of both the plants were used against stomach pain and in Jaundice (EL-Kamali, 2009; Suleiman, 2015). *Blepharis linariifolia* is a low-growing, wiry herb with prickly bracts. To our best of the knowledge, there are no scientific reports available concerning bioactivities or phytochemical studies of this plant. Meanwhile *Guiera senegalensis* has been explored for the properties such as antileprosy (Elrahman et al., 2009), antidiabetic and hypolipidemic effects (Kamal et al., 2013). In an attempt to find out the secondary metabolite present in this plant, (Maleš et al., 1998) and (Elrahman et al., 2009) had found significant presence of alkaloids, flavonoids, saponins, and tannins in their leafs. Nonetheless there are no reports available about this plant's capacity and mechanism on how it protects the liver from external stimuli especially oxidative stress is not available.

The role of carbon tetrachloride (CCl_4) in inducing liver toxicity is inevitable in hepatoprotective animal models. It typically mimics the biochemical changes and cellular injury as other xenobiotics made in liver. This halogenated alkane explicates the mechanisms of action of hepatotoxic properties such as fatty degeneration, fibrosis, hepatocellular death, and carcinogenicity (Weber et al., 2003). It is well known that upon ingestion of CCL4, it will be rapidly metabolized to CCl_3 (trichloromethyl radical) due to the activation of cytochrome enzymes in the liver. The formed free radicals then can react with the glutathione and thiol groups of protein and initiate lipid peroxidation in the hepatic cells (Nurrochmad et al., 2013). Hence the use of CCl_4 in making liver injury is best available method which can be used for finding out the capacity of herbal preparation's antioxidant ability. Thus, in the current study we used this model of liver toxicity to find out the action and mechanism of *Blepharis linariifolia Pers* and *Guiera senegalensis* as mentioned in the folk practices.

MATERIALS AND METHODS

Plants Materials

The plants materials used in this study namely, *Blepharis Linariifolia* (whole plan) and *Guiera senegalensis* (leaves) were collected by researchers from Medicinal and Aromatic Plants Research Institute (MAPRI), National Center for Research (NCR), Sudan during scientific trips to Nuba mountains and Ingassana mountains, respectively (December 2015). The whole plant of *B. linariifolia* and leaves of *G. senegalensis* were cleaned, shade dried, ground coarsely by mechanical grinder and kept in special plastic containers. the plants were identified and authenticated by botanist from the Department of Chemistry, Production and Classification, MAPRI, NCR, Khartoum, Sudan. Herbarium's specimens were prepared and deposited in the Herbarium of the Institute.

Extraction Procedure

Extraction was carried out according to method described earlier (Sukhdev et al., 2008). Specific weight of each sample was extracted by soaking in 2000 mL ethanol (80%) for seventy two hours with daily filtration and evaporation. Solvent was evaporated under reduced pressure to dryness using rotary evaporator apparatus and the extract of each plants were combined together. The yield percentages for *B. linariifolia* and *G. senegalensis* were 14.13% and 26.82%, respectively.

Phytochemical analyses: Phytochemical screening for the active constituents was carried out using the methods described earlier (Balick, 1990; Martinez and Valencia, 2003; Ramesh et al., 2001; Sofowora, 1982; Wall et al., 1952) with many few modifications. These well-established methods were conducted for tannins, sterols, triterpenes, alkaloids, flavonoids, saponins, cumarins and anthraquinone glycoside, respectively.

DPPH radical scavenging assay

The DPPH radical scavenging was determined according to the modified method of Shimada (Shimada et al., 1992). In a 96-wells plate, the test samples were allowed to react with 2.2-Di (4-tert-octyl Phenyl1)-1-Picry1-hydrazyl1 stable free radical (DPPH) for 30 min at 37^{o}C. The concentration of DPPH was kept as 300µM. The test samples were prepared in ethanol. After incubation of DPPH and test samples, decrease in absorbance was measured at 517 nm using multiple reader spectrophotometer (Thermo Fisher Scientific 1500, USA). Percentage of radical scavenging activity induced by the samples were determined in comparison with DMSO as a control. All tests and controls were run in triplicates.

Animals and Experimental model: Forty two Wister Albino rats (85-140 g) were used. They were fed with a standard pellet diet and water *ad libitum*. Before their use the experiment, the rats were kept in standard environmental conditions, (temperature 25-28 ^{o}c and 12 h light / dark cycle). Animals were procured from the Animal House, MAPRI, NCR, Khartoum, Sudan. All Procedures regarding ethical clearance to conduct the study were obtained from the institutional appropriate committee. Animals were divided into seven groups, each one contained six animals. Group 1 represent normal control which received distilled water for 5 days. Group 2 represent positive control which received carbon tetra chloride [CCL_4; 3 mL/Kg, subcutaneously (S.C.)] on the third day. Group 3 received silymarin as a positive control. Group 4 and 5 received *B. Linariifolia* extracts at 200 and 400 mg /kg, respectively. Group 6 and 7 received *G. senegalensis* extracts at 200 and 400 mg /kg, respectively. CCL_4 was given subcutaneously on the third day for groups 3 to 7.

Clinical signs and biochemical parameters: Clinical signs were reported for each group. On the 6th day, animals were killed under diethyl ether anesthesia and blood samples for serobiochemical parameters were collected. The serobiochemical parameters considered are serum glutamic oxalacitic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP),urea and total protein (TP) and they were measured according to the standard methods (Ahmed et al., 2003).

Histopathological examination: Liver pieces were preserved in 10% formaldehyde solution. The pieces of liver processed and embedded in paraffin wax. Section of about 4-6 microns were made and stained with hematoxylin and eosin and photographed. All sections were assessed by an independent histopathologist.

Statistical analysis

The results were expressed as mean ±SEM and analyzed for statistical difference using one-way ANOVA followed by multiple comparison *post hock* test. The data were analyzed using SPSS software version 22. The difference showing a level of $p<0.05$ was considered to be statistically significant.

RESULTS

Phytochemical investigations and antioxidant activity

Both extracts were screened for various phytochemical contents using standard procedures. *B. Linariifolia* and *G. senegalensis* showed the presence of alkaloid, tannins, sterols, triterpenes, alkaloids, flavonoids, saponins and cumarins. Tannins and flavonoids were more abundant in these two plants. Anthraquinone glycoside was not conducted in both plants while the triterpenoids was only detected on *B. Linariifolia*. As shown in Figure 1, the antioxidant activity of *B. Linariifolia* and *G. senegalensis* was assessed using DPPH bleaching assay with propyl gallate as a standard antioxidant control. The decrease in DPPH chromogenic reading caused by antioxidant action of both plants was expressed as a percentage. *B. Linariifolia, G. senegalensis* and propyl gallate have demonstrated bleaching effect of 78±0.01%, 92±0.02% and 89±0.01%, respectively.

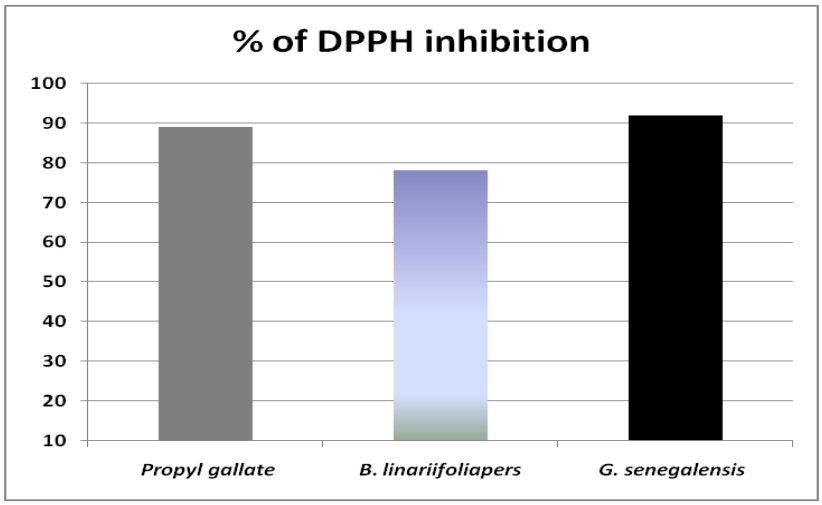

Figure. 1. DPPH radical scavenging assay. Findings are expressed as percentages.

Clinical findings: Rats in group 2 (CCl_4) showed severe lack of appetence, intermittent diarrhea, emaciation, erection of hair and weakness of the fore and hind limbs. These signs appear on days 3, 4 and 5 after CCL_4, however, these signs were remarkably less in rats received B. *Linariifolia*, *G. senegalensis* and silymarin. Postmortem evaluation of the liver and kidney showed some macroscopic lesions related to CCl_4 dosing. However, these macroscopic observations were not observed in rats of groups of B. *Linariifolia*, *G. senegalensis* and silymarin. There are no lesions in the control-undosed rats (Group 1)**.**

Serobiochemicals findings: These parameters are shown in Table 1. In CCl_4-administered rat (Group 2), the activities of AST, ALT, ALP, urea and total protein were significantly ($P<0.01$) modulated when compared with the untreated group (Group 1) and the standard drug group (Silymarin), during the period of the experiment. A dose-dependent effect was observed on the inhibition of CCl_4 intoxication by B. *Linariifolia* and *G. senegalensis.*

Histopathological examination

Histopathological sections of the experiment are presented in Figure 2. In Group 2, administration of CCl_4 caused significant microscopic pathological changes such as diffuse areas of vacuolar degeneration, mainly centrilobular as well as swelling of cells and congestion of hepatic veins and sinusoids (Figure 2B). Mononuclear cell infiltration in parenchyma and around the portal area and dilation of sinusoids were also seen. In some areas hepatic cords were disturbed and hepatocyte degeneration was evident. Affected cells showed pyknotic nuclei. The interlobular bounders appear to be distinct with slight proliferation of fibrous tissues and the lobular structure was not regular, there was bile duct proliferation in the portal areas. Most these degenerative changes appeared to be more pronounced around the central veins saving the peripheral areas (Figure 2B). Dose dependent protective effects against these pathological lesions were observed on the groups received plants extracts and silymarin. Mild to moderate changes were noticed, vacuolar degeneration which appear to be peripherally in the lobules. Some hepatocytes contained small single or multiple cytoplasmic vacuoles. Hepatocyte swelling and congestion were also observed.

Table 1. Biochemical analysis of serum obtained from rats treated with hepatotoxic carbon tetrachloride and plant extracts.

Groups*	Treatment	AST	ALT	ALP	Urea	Protein
1	Normal rats	$194.48^{a\pm}10.55$	$52.41^{a}\pm5.59$	$347.52^{a}\pm56.51$	$53.01^{ab}\pm3.29$	$6.11^{ab}\pm0.09$
2	Carbon tetrachloride (CCl_4)	$366.75^{c}\pm39.26$	$111.45^{b}\pm25.15$	$224.50^{a}\pm23.27$	$73.97^{b}\pm4.61$	$6.58^{b}\pm0.27$
3	Silymarin + (CCl_4)	$313.17^{abc}\pm44.87$	$36.18^{a}\pm5.75$	$202.84^{a}\pm29.74$	$53.28^{ab}\pm7.00$	$6.62^{b}\pm0.33$
4	*B. linariifolia pers* 200mg + (CCl_4)	$336.69^{bc}\pm.81$	$53.93^{a}\pm2.97$	$233.25^{a}\pm22.93$	$43.73^{a}\pm5.95$	$5.47^{a}\pm0.13$
5	*B. linariifolia pers* 400mg+ (CCl_4)	$213.88^{ab}\pm16.07$	$43.65^{a}\pm8.58$	$224.49^{a}\pm20.05$	$45.79^{a}\pm4.27$	$5.90^{ab}\pm0.17$
6	*G. senegalensis* 200mg+ (CCl_4)	$346.19^{bc}\pm41.17$	$82.85^{ab}\pm17.72$	$330.91^{a}\pm33.93$	$52.48^{ab}\pm2.35$	$6.57^{b}\pm0.19$
7	*G. senegalensis* 400mg+ (CCl_4)	$175.23^{a}\pm33.73$	$38.10^{a}5.03$	$257.81^{a}\pm42.64$	$55.590^{ab}\pm5.22$	$6.39^{ab}\pm0.17$

*Groups with different alphabets are statistically different

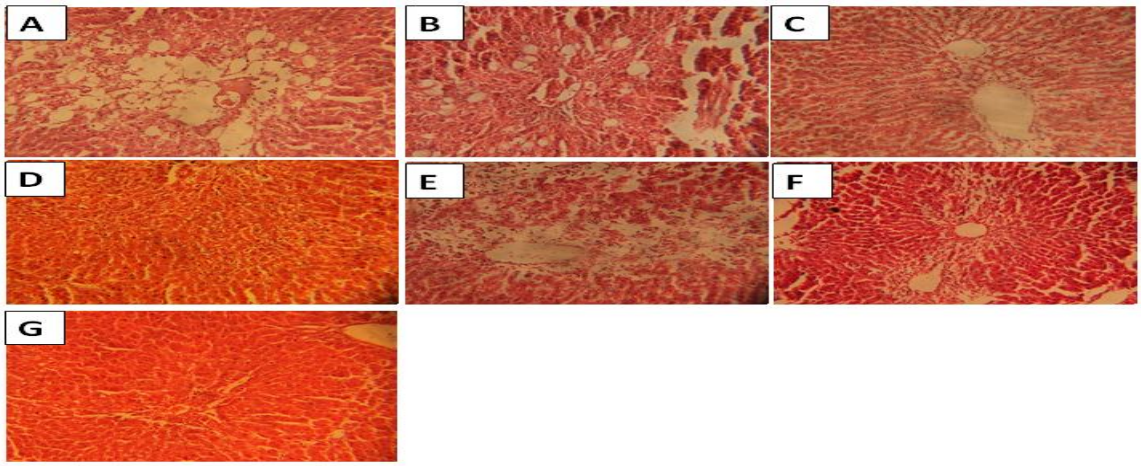

Figure. 2: Hepatoprotective activities of *Blepharis Linariifoliapers* and *Guiera Senegalensis* on CCL_4 induced hepatotoxicity. A: Normal hepatocytes, with some infiltration of neutrophils, normal un-treated rats. B: Centrilobular necrosis with sever macro-vesicular steatosis (fatty degeneration), CCL_4un-treated control group. C: Dilatation of sinusoids and infiltration of neutrophils (inflammatory cells), Silymarin group. D: Show severe congestion and haemorrhage, 200 mg/kg of *B. Linariifoliapers* extract. E: Centrilobular necrosis with sever macro-vesicular steatosis (fatty degeneration), 400 mg/kg of *B. Linariifoliapers* extract. F: Show severe diffused evacuation of hepatocytes according to diffused necrosis, severe congestion and haemorrhage and infiltration of neutrophils (inflammatory cells), 200 mg/kg of *G. senegalensis* extract. G: Show severe congestion, hemorrhage and infiltration of neutrophils (inflammatory cells), 400 mg/kg of *G. senegalensis* extract.

DISCUSSION

The present work has been an attempt to elucidate likely hepatoprotective, antioxidant and phytochemical properties of some indigenous plants commonly reputed to achieve these ends. Insufficiency of treatment with conventional drugs and potential hazards linked with their use provoked our search for better and safer hepatoprotectives of herbal origin. Our choice of the two plants (*Blepharis Linariifolia Pers* and *Guiera Senegalensis*) has been based on the widely held folkloric belief that these plants possess effect against jaundice and they are component of many polyherbal formulations (EL-Kamali, 2009; Suleiman, 2015).

Mechanism of carbon tetrachloride-induced hepatotoxicity has been established. Hepatocellular damage by reactive carbon tetrachloride metabolites induced lipid peroxidation, covalent binding, inhibition of lipoprotein secretion and steatosis (Boll et al., 2001). The efficacy of CCl_4 as inducer of animal model of liver injury depends on the measurability of its harmful effects. In this study, CCl_4 intoxication noticeably modulated the serum levels of ALT, AST and ALP in the rats, a marker of cellular leakage and failure in activities of cell membrane in liver (Sadeghi et al., 2016). These results are in fine harmony with earlier studies (Mohamed et al., 2016; Sadeghi et al., 2016). A dose-dependent effect on these hepatic markers was observed on the inhibition of CCl_4 intoxication by B. *Linariifolia* and *G. senegalensis.*

Mononuclear cell infiltration, vacuolar and hepatocellular degeneration and bile duct proliferation in the portal areas were evident in CCl_4 treated group (Al-Seeni et al., 2016) (Figure 2B). Histological changes supported the protective effects of *B. Linariifolia* (400mg/Kg) and *G. senegalensis* (400mg/Kg) against CCl_4 intoxication and significantly improved the liver tissues and nearly restored them to the normal. These results are consistent with previous findings which showed hepatoprotective role of plant extracts against pathological changes induced by CCl_4 (Al-Seeni et al., 2016).

Plant extracts contain many phytochemicals that are responsible for their biological activities. Using standard methods, the current study investigated the presence of the main phytochemical constituents in plant extracts. *B. Linariifolia* and *G. senegalensis* showed the presence of alkaloid, tannins, sterols, triterpenes, alkaloids, flavonoids, saponins and cumarins. Tannins and flavonoids were more abundant in these two plants. Previous results showed that plants with high contents of tannins and flavonoids are able to protect against CCl_4 induced liver injury (Chu et al., 2016; Mehmetçik et al., 2008). Moreover, the current

study used DPPH assay to investigate the antioxidant activities of *B. Linariifolia* and *G. senegalensis*. The findings demonstrated very strong antioxidant activity for both plants as shown in Figure 1.

It has been previously demonstrated that diminishing the metabolic pathway of CCl_4, the antioxidant effect, suppression of reactive oxygen species or by synergistic effects of these which causes tissue damage are important mechanisms in the protection against CCl_4-triggered liver damage. The findings acquired in this paper propose that the extracts possess hepatoprotective properties on the harmful effect of CCl_4 through antioxidant activity.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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