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Protective role and antioxidant activity of arabic gum against trichloroacetate-induced toxicity in liver of male rats

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ABSTRACT

Investigation of functional hepatoprotective components from plants origin is a new model for drug evolution. The study examined the protective effects of Arabic gum (AG) induction on liver serum markers, antioxidant enzymes and lipid peroxidation in liver tissue against trichloroacetate (TCA) induced hepatotoxicity in male albino rats. Also, the chemical constituents and antioxidant assay of aqueous extract of AG was determined. TCA at the dose of 50 mg/kg for 2 months produced hepatotoxicity as examined by the significant increase of serum activities of ALT, AST, ALP and conjugated bilirubin level in the TCA treated animals alone and decrease in total protein and albumin levels. Pretreatment with AG aqueous extract (0.5 g/kg/day) significantly ($P< 0.05$) lowered the serum enzyme activities and increased in total proteins and albumin level. The antioxidant studies showed that the activities of hepatic SOD, CAT and GPx were decreased in TCA induced animals with significantly elevated in MDA content which is reversed in pretreatment with aqueous extract of AG. Histopathology of the liver sections confirmed that the AG extract ameliorated hepatic damage induced by TCA. In conclusion, the study demonstrated that AG supplementation for 2 months in TCA induced toxicity in rats benefited hepatic antioxidant status and improved liver injury and damage in male albino rats exposed to TCA.

Key words: Antioxidants, Arabic gum, Biochemical parameters, Histopathology, Lipid peroxidation, Trichloroacetate.

INTRODUCTION

The liver is the key organ regulating homeostasis in the body. It is involved with almost all the biochemical pathways related to growth, fight against disease, nutrient supply, energy provision and reproduction (Ward and Daly, 1999). The liver is expected not only to perform physiological functions but also to protect against the hazards of harmful drugs and chemicals. Inspite of tremendous scientific advancement in the field of hepatology in recent years, liver problems are on the rise. Jaundice and hepatitis are two major hepatic disorders that account for a high death rate (Pang *et al*., 1992). Presently, a few hepatoprotective drugs and that too from natural sources, are available for the treatment of liver disorders. Hence, people are looking at the traditional systems of medicine for remedies to hepatic disorders.

Arabic gum is also known as gum acacia, obtained from the Acacia tree. The acacia grows in a region stretching from Senegal to Sudan in Africa. The Acacia Senegal Gum is a medium-sized tree with thorns growing on the African savannah grassland (James and Webb, 1985). Arabic gum is widely used in the pharmaceutical and food industries as an emulsifier and stabilizer. Acacia is comprised of saccharides and glycoproteins and is adapted to be consumed by humans (Verbeken and Dierckx, 2003). Scavenging of nitric oxide by arabic gum has been reported to limit the acetaminophen-induced hepatotoxicity in mice (Gamal El-din *et al*., 2003). Other studies have documented the antioxidant properties of gum arabic in a variety of animal model system (Rehman *et al.,* 2004). Antioxidant potential and free radical scavenging activity by pod extracts of Acacia Senegal and Antidiabetic activity of Acacia senegal pod extract in streptozotocin-induced diabetic rats (Hooda *et al.,* 2013).

Natural antioxidants of plants origin are known to exhibit a wide range of biological effects, including antioxidant, antibacterial, antiviral, anti-inflammatory, anti-allergic, antithrombotic and vasodilatory activity (Liyana and Shahidi, 2005) and (Zhang *et al*., 2011).

It has been recorded that free radicals are involved in causing many diseases (Ames *et al.,* 1993). Free radicals are known to be the major cause of various chronic and degenerative diseases, including aging, coronary heart disease, inflammation, stroke, diabetes mellitus and cancer (Slater, 1984; Cheng *et al*., 2003). The tissue injury caused by reactive oxygen species may include DNA damage (Halliwell and Gutteridge, 1984; Bartold *et al*., 1984), protein damage (Varani *et al*., 1985), and oxidation of important enzymes (Gamal *et al*., 2003) in the human body. These events could consequently lead to the occurrence of various free radical related diseases. Recently, natural foods

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and food derived antioxidants such as vitamins and phenolic phytochemicals have received growing attention, because they are known to function as chemopreventive agents against oxidative damage.

The present study is aimed to evaluate the hepatoprotective and antioxidant activity of aqueous extract of the roots of Arabic gum against TCA-induced hepatotoxicity in rats. Also, the chemical constituents and antioxidant activity of aqueous extract of AG was determined.

MATERIALS AND METHODS

Plant: Root parts of Acacia Senegal (AG) were purchased from local market in Jeddah, Saudia Arabia and it was identified and authenticated by the Department of Botany and Microbiology, University of King Abdulaziz, Jeddah, KSA.

Extract Preparation: The roots were dried under environmental temperatures and grinded with electrical grinder to produce fine powder which was dissolved in distilled water to prepare 10 % extract solution. Afterwards, the extract was leaved for 3 days, filtered by Whatman No. 1 filter paper.

Total phenolic (TP): TP contents were estimated using the Folin–Ciocalteu reagent as described by Al-Farsi *et al.* (2005). Calculation was based on a calibration curve obtained with gallic acid. The TPC was expressed as milligrams of gallic acid equivalents (GAE) per g of dry material (DM).

Total flavonoid (TF): TF contents were determined according to the method of Zhishen *et al*. (1999). The standard curve (0.05–0.5 mg gallic acid / mL), and the results were expressed as milligrams of gallic acid equivalents (GAE)/ g DM.

Reducing power: RP capacity was determined according to the method of Julkunen-Titto (1985). Ascorbic acid was used as standard, and the results were expressed as milligrams of AAE/ g DM (0.05–1 mg/ml).

Gas chromatography – mass spectrometry (GC-MS): Gas chromatography-mass spectrometry was conducted on a Fisons GC 8000 gas chromatograph connected to mass detector of Fisons MD 800 under electron impact ionization (70 eV). The temperature of interface is 230 ^{0}C, and the MS scan range was 35-450 atomic mass units (AMU). The column used for chromatographic analysis was fused silica OV1 capillary column (25 m X 0.25 mm i.d.). The carrier gas was helium at a flow rate of 10 ml/min. Two samples were analyzed with the column start firstly at 60 ^{0}C for 2 min and increased to 170 ^{0}C with a 2 ^{0}C/ min heating ramp for 3 min. Finally, temperature was increased to 250 ^{0}C with a 3 ^{0}C/min heating ramp for 120 min. for both samples. The injection was completed in split mode at 220 ^{0}C. Peaks were recognized by computer searches in commercial reference libraries. Good spectral matches for some compounds could be found in the Wiley and National Bureau of Standards (NBS) mass spectral library

Experimental animals: Twenty-eight adult Wister albino male rats (weighting 170 - 220 g) were purchased from the Central Animal House in Jeddah, Saudi Arabia. The experiments and the protocols were approved by the Institutional Animal Ethics Committee (IAEC) of Saudi Arabia, Jeddah. All experimental procedures were completed according to the ethical guidelines of International Association for the Study of Pain (Zimmermann, 1983). Rats were housed in stainless steel cages placed in a well-ventilated rat house, maintained for two weeks as acclimatization period under standard laboratory conditions on free supply of food and water provided ad libitum, and subjected to 12 h natural light and dark cycles. After the period of acclimatization, rats were divided randomly into four groups, 7 animals in each. The animal experiments were conducted for 2 months. Group 1 was received daily 0.5 mL of saline solution (0.9%NaCl) orally for 2 months and was used as control. Group 2 was administrated orally with AG (0.5 g/kg body weight) by gavage (Daniel *et al*. 1992). Group 3 was received 0.5 mL of TCA at a dose of 50mg/kg body weight/day (Acgih, 1991). Group 4 was received the AG dose and then receive TCA.

Blood collection and tissue preparation: The rats were anesthetized and scarified after the examination period (2 months), the blood samples were collected from a cardiac puncture and put immediately into glass tubes and centrifuged at 3,000 rpm for 20 min at 4 °C to separate serum samples which stored in aliquots at -20 ^{0}C till used. The livers were immediately removed, washed with ice-cold physiologic saline solution (0.9 %, w/v), blotted, and weighted. Small pieces were fixed in 10 % neutral buffered formalin for routine histopathology and the remaining part was homogenized in ice-cold Tris-buffered saline (TBS), pH 7.4, and centrifuged at 3,000 rpm for 10 min at 4 °C. The homogenate was collected in aliquots, stored at “80 °C until use.

Biological assays: Serum aspartate (AST) and alanine aminotransferase (ALT) activities were determined according to the method of Reitman and Frankel, 1957. Serum alkaline phosphatase (ALP) activity and conjugated Bilirubin (CB) levels were determined according to Kind and King, 1954 and Malloy and Evelyn, 1937, respectively. Serum total protein (TP) was determined by Biuret method (Treitz, 1970) while that of serum albumin (ALB) was determined by bromocresol green according to Waterborg, 2002. The lipid peroxidation end product, MDA, was measured as thiobarbituric acid reactive substance. Also, the activities of antioxidant enzymes including the catalase enzyme (CAT; EC 1.11.1.6), superoxide dismutase (SOD, EC.1.15.1.1), and glutathione peroxidase (GPx; EC. 1.1.1.9) were assayed in

liver tissue using commercial assay kits according to the manufacturer's instructions.

Histopathology of the rat livers: The liver was immersed in tap water and different alcohols (methyl, ethyl and absolute ethyl) were used in serial dilutions for dehydration. Samples were cleared in xylene and immersed in paraffin at 56 ^{0}C in hot air oven for 24 h. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns by sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin and eosin stains (Carleton, 1979) for histopathological examination through the electric light microscope.

Statistical analysis: The results were expressed as mean ± standard deviation (SD). The data were subjected to one-way analyses of variance (ANOVA) and student's t-tests using the statistical analysis program. P-value ≤ 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Antioxidant activities and phenolic compounds of the aqueous AG extract: Standard curves prepared were used for the determination of total phenolic, total flavonoids and reducing power capacity using gallic acid and ascorbic acid. The results of the in vitro antioxidant indices of the AG are as follows: TPC (28 ± 0.026 mg GAE/ g DM), TFC (16 ± 0.01 mg GAE/ g DM, and reducing power content (4.14±0.05 mg AAE/ g DM), Table (1).

In the present study, qualitative analysis of the phenolic compounds of root extract from AG was performed by GC-MS analysis (Table 2). A total of nine phenolic compounds (Chlorogenic acid, Caffeic acid, Quinic acid, p-Coumaric acid, Ferulic acid, Gallic acid, Gallic acid methyl ester, Naringenin and Catechin) were characterized by comparison to the retention times and UV spectra of authentic standards analyzed under identical analytical conditions. Under the optimized condition, Gallic acid and gallic acid methyl ester were present in high levels (22 % and 18.0 %, respectively). Traces of Chlorogenic, Caffeic, p-Coumaric and ferulic acids were also detected, but they were about one-tenth or even just one-hundredth to that of gallic and gallic acid methyl ester. Therefore, they were the main components of the root extract (Table 2), which were evaluated as phenolic controls in successive antioxidant tests.

Table 1: The total phenolic, flavonoids contents and reducing power capacity of the aqueous extract of AG.

Parameters	Amounts
Total Phenolic content (mg of GAE/g DM)	28.0 ± 0.026
Total Flavonoids content (mg GAE/g DM)	16.0 ± 0.01
Reducing power capacity(mg AAE / g DM)	4.14 ± 0.05

Values are expressed as mean ±SD of three replicates.

Table 2: Profile of phenolic compounds in AG

Phenolic compounds	% of total phenolic compounds
Chlorogenic acid	1.34
Caffeic acid	2.35
Quinic acid	4.5
p-Coumaric acid	0.89
Ferulic acid	1.14
Gallic acid	22
Gallic acid methyl ester	18
Naringenin	4.5
Catechin	11

Effects of TCA on biochemical liver markers: The results obtained in this study (Table 3) revealed that the treatment with TCA at 50 mg/kg BW induced a significant ($p<0.05$) elevation in serum enzyme activities of ALT, AST and ALP as compared with the control rats. Treatment with AG allowed these parameters to decrease and come near the control group values. In another way, the administration of TCA increased the conjugated bilirubin level and decreased the total protein and the albumin level when compared to control rats. However, treatment with AG attenuated these modifications and restored it near the normal values of control rats or those treated with AG only. The AG was showed its ability to restore the normal functional status of the intoxicated liver and also to protect against subsequent TCA hepatotoxicity.

Effects of TCA on biochemical antioxidant status: Table 4 showed the effects of oral supplementation with 0.5 g/kg/ day of AG and subsequent orally TCA treatment on the antioxidant markers (SOD, CAT and GPx) in the treated rats. TCA treatment caused significant ($p < 0.05$) decreases in the hepatic tissue SOD, CAT and GPx activities while causing

Table 3: Effect of AG aqueous extract on serum marker enzymes activities, total protein, albumin, and conjugated bilirubin levels affected by TCA.

Parameters	Control	AG Extract	TCA	AG Extract + TCA
ALT (U/L)	18 ± 2^a	20 ± 2^a	41 ± 6^b	22 ± 3^c
AST (U/L)	35 ± 5^a	33 ±4^a	54 ± 4^b	42 ± 5^c
ALP (U/L)	74 ± 7^a	70 ± 5^a	126 ± 6^b	80 ± 8^c
TP(g/dl)	6.1 ± 0.14^a	6 ± 0.67^a	4.01 ± 0.48^b	6.08 ± 0.6^a
Albumin (g/dl)	4.12 ± 0.13^a	4 ± 0.23^a	2.98 ± 0.19^b	3.99 ± 0.21 ^{a}
Conjugated Bilirubin (mg/dl)	0.23 ± 0.02^a	0.24± 0.022^a	0.45 ± 0.016^b	0.28 ± 0.02^a

Data are expressed as means ± SD (n=7 rats per group). Comparison between groups was made using Duncan test. Values in the same columns not sharing a common letter (a–c) differ significantly at $p<0.05$.

Table 4: Effect of AG aqueous extract pretreatment on liver tissue SOD, CAT, GPx activities and MDA level in experimental rats treated with TCA

Parameters	Control	AG Extract	TCA	AG Extract + TCA
SOD (U/mg protein)	20 ± 1[a]	22 ±1.5[a]	12 ± 2.3[b]	20 ± 2.4[a]
CAT (U/mg protein)	25 ± 1[a]	23 ± 3[a]	15 ± 2[b]	25 ± 2.4[a]
GPx (U/mg protein)	40 ± 1.5[a]	41 ±1.1[a]	29 ± 3.2[b]	38 ± 3[a]
MDA (nmol / g tissue)	45 ± 3.5[a]	44 ± 4.0[a]	60 ± 4[b]	48 ± 3[a]

Data are expressed as means ± SD (n=7 rats per group). Comparison between groups was made using Duncan test. Values in the same columns not sharing a common letter (a–b) differ significantly at $p<0.05$.

significant ($p < 0.001$) increases in the hepatic tissue MDA level (Table 4). Oral administration of AG significantly ($p < 0.05$) attenuated decreases in the hepatic tissue activities of SOD, CAT and GPx while significantly attenuated increases in the hepatic tissue MDA levels (Table 4).

Histopathology of rat liver tissue: Fig (1) is the photomicrographs of the lesions induced by TCA treatment oral supplementation with 0.5 g/kg/ day of AG on the treated rat livers. TCA injection was marked by hepatic centrilobular vacuolation and vascular congestion indicative of early hepatic necrosis when compared to normal hepatic architecture (Fig. 1). However, oral treatments with 0.5 g/ kg/day of AG for 60 days caused amelioration of the TCA-induced histological lesions of hepatic necrosis. With repeated daily oral pretreatments with 0.5 g / kg/day of AG,

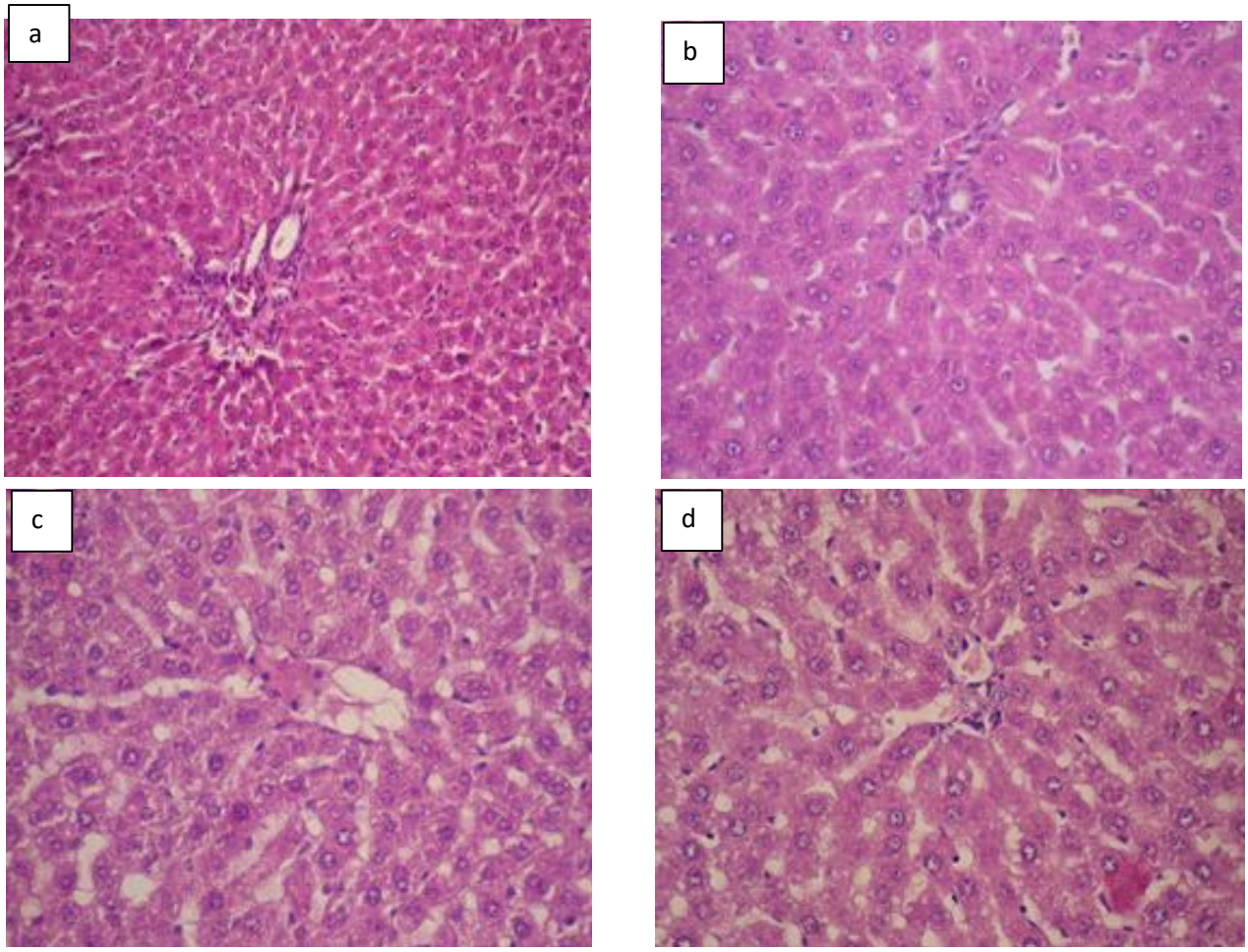

Fig 1: Liver histopathology of control and experimental rats. (a) Control rats showed normal liver, hepatic lobules showing central veins surrounded by columns of normal hepatocytes having abundunt esinophilic cytoplasm ,central rounded nuclei seperated by blood sinsuids; (b) Group II: animals treated with AG only showed normal histological structure, mild hydropic degeneration, hepatocytes were rounded with granular esinophilic cytoplasm with central rounded nuclei ; (c) Group III: a liver section of TCA-treated rats showing Central Vein congestion, hepatocytes were rounded with granular esinophilic cytoplasm with central rounded nuclei (vacculation of hepatocytes with hydropic degeneration; (d) Group IV: a liver section of AG + TCA-treated rats showing mild tubular necrosis with regenerated tubular cells (HEx40).

these histological changes were ameliorated in a dose-related fashion.

Different methods were used to evaluate the antioxidant activity of plant extract. Our work therefore has used three different methods to evaluate the antioxidant activity of Arabic gum (AG), namely, total phenolic compounds, total flavonoids and reducing power contents. Our results showed that the aqueous extract of AG contained significantly higher levels of phenols which was agreed with (Verbeken and Dierckx, 2003), which indicated that AG is a good source phenolic compounds, which can be a powerful antioxidant source in the food system .To Measure the antioxidant capacity of AG, we suggested that reducing power is a suitable method. In this study, however, the high antioxidant capacity of AG aqueous extract may be due to the majority of the active compounds dissolved in the water (Kaur and Kapoor. 2001). In general, phenolic compounds may be related directly to antioxidant action, thanks to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers (Negri *et al*., 2011). However, interactions between the different antioxidative compounds in AG might be responsible for the prevention of different diseases and therefore could protect the liver from TCA toxicity (Panuganti *et al*., 2006).

The release of internal hepatocyte enzymes from liver tissues in the circulation after exposure to TCA is one of indicator markers of liver injury. The enhanced activities of these enzymes are indicative of cellular leakage and loss of the functional integrity of the cell membranes in the liver (Rajesh and Latha, 2004).

In the present study, the hepatoprotective effects of AG against TCA-induced hepatotoxicity in rats were evaluated. Orally supplementation of TCA reported significant increases in the serum ALT, AST, ALP activities, and CB level with concomitant significant decreases in the TP, ALB levels. Significant serum elevations in these hepatic biochemical markers have been attributed to the compromised structural and functional integrity of the hepatocytes. TCA considered as a potent chemical hepatotoxin which is widely used for the induction of experimental hepatotoxicity in animal models (Richardson *et al*., 2008).

Oral treatments with 0.5 mg/kg/day of AG for 60 days significantly reversed the altered serum liver function parameters in the TCA-treated rats. Results of the present study on the hepatoprotective activity of AG is similar with that previous reported of Lotito and Frei, (2004) who reported that gum arabic has been claimed to act as an antioxidant and protective against experimental hepatic-, renal-and toxicities heart in rats.

However, the elevated level of conjugated bilirubin and the decreased albumin level showed hepatic injury in the TCA-induced groups. Administration of AG ameliorates the elevation Bilirubin level in plasma of rats suggested that the biliary dysfunction of the rats' livers during subchronic treatment with TCA has been settled.

The liver function tests corroborated the histopathological damages observed in the present work. These results indicated marker alterations in the overall histoarchitecture of the liver related to TCA. It might be related to its toxic effects firstly by the production of reactive oxygen species causing damage to the various membrane components of the cell. The histopathological changes observed in the liver consistent with previous studies reported on TCA toxicity (Acharya *et al*., 1997).

In measuring free radicals-induced oxidative stress, enzyme markers such superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and for lipid peroxidation, catabolite such as malondialdehyde (MDA), are widely accepted and used (Celik, 2007). In order to evaluate the effect of AG on TCA-induced oxidative stress, and activities of antioxidant enzymes CAT, SOD, GPx and MDA level were measured. Administration of TCA by profoundly decreased the tissue activities of these antioxidant enzymes while significantly increasing the hepatic tissue MDA concentration. Accompanying these biochemical changes are histological changes of hepatocyte vacuolation and hepatic centrilobular necrosis. These biochemical and histopathological results are in strong similarity with the earlier findings of Brattin *et al*., (1985). However, daily oral treatment with 0.5 g/kg/day of AG over 60 days afforded hepatoprotection dose-dependently with better protection offered by AG administered curatively than that offered prophylactically. In addition, AG administered post-TCA treatment produced marked dose-related reductions in the hepatic MDA level, MDA being a reliable index of lipid peroxidation. Thus, this results strongly further suggests anti lipoperoxidation activity of AG. The antioxidant and antilipoperoxidative properties of AG could be attributed to its constituent flavonoids and other polyphenolics as these phytocomponents have been widely reported to possess antioxidant and anti-lipoperoxidative activities (Kassem, 2015).

CONCLUSION

The present study suggests that AG has a potent hepatoprotective activity in TCA-induced hepatic injury in rats. AG may possess both antioxidant and anti-lipoperoxidative activities by inhibiting and scavenging free radicals generated by TCA. These findings provide biochemical and histological data supporting folkloric use of AG in the local treatment of some hepatic disorders.

AUTHORS' CONTRIBUTION

All authors have read and agreed to the content and the publication of this paper.

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