


Phytochemical and Toxicological Effect of Ethanol Extract of *Heliotropium Indicum* on Liver of Male Albino Rats

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Abstract: *Heliotropium indicum* is a plant of the *Boraginaceae* family reported to have multiple beneficial health effects, including analgesic, anti-malaria, anti-microbial, anti-tumor, anti-inflammatory, amongst others. 42 Male Wistar rats were used for this study. Group A served as control and received distilled water only. Groups B, C, D, E, and F were fed with the extract of *Heliotropium Indicum* in different concentrations. (500 mg/kg, 1000 mg/kg, 1500 mg/kg 2000 mg/kg and 3000 mg/kg) respectively. This study showed that *Heliotropium indicum* contains phytochemicals such as saponins tannins, cardiac glycosides, and terpenoids. Our results revealed that *Heliotropium indicum* decreased the ALT, AST, ALP, and bilirubin levels, especially at doses from 1500 mg/kg B.W. Also, inflammatory cytokine expressions were significantly reduced at similar doses. However, the level of antioxidants (GSH, CAT, GST, SOD, GPx) were significantly increased by *Heliotropium indicum* administration at doses ranging from 1500 mg/kg to 3000 mg/kg. The antioxidative potentials of *Heliotropium indicum* are further complemented by its ability to reduce inflammatory cytokine expression, including TNF- α , IL-1 β , IL-6, COX-2 as well as NO and LPO. Ethanol extract of *Heliotropium indicum* at doses between 1500 mg/kg and 3000 mg/kg has antioxidative, anti-inflammatory effects and can counteract lipid peroxidation.

Keywords: *Heliotropium indicum*; phytochemicals; oxidative stress; inflammation; liverfunction.

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1. Introduction

Traditional herbal medicines are normally occurring, plant-sourced substances with next to zero modern handlings that have been utilized to alleviate sickness within conventional healing practices [1,2]. African populaces utilize some type of conventional home grown medication, and the overall yearly market for these items approaches US\$ 60 billion. Nature has given a huge wellspring of therapeutic agents from plants for over a thousand years. A noteworthy number of current medications have been isolated from natural sources. These plants contain several beneficial phytochemicals that potentiate their medicinal activity [3]. High convergences of phytochemicals, which may secure against free radical damage, aggregate in foods grown from the ground [4,5]. Phytochemicals explained explicitly are synthetic substances delivered by plants. Plants containing advantageous phytochemicals may enhance the requirements of the human body by exerting antioxidative capabilities [6]. The

most significant of these bioactive plant constituents are alkaloids, tannins, flavonoids, and phenolic compounds[5,7].

Heliotropium Indicum is a plant identified from the family *Boraginaceae*, the borage or “forget-me-not” family that is usually shrubs, includes different shrubs, trees, and herbs, making-up about 2,000 species in 146 genera found worldwide. The name “heliotrope” is derived from the idea that plants of this species turn their leaves to the sun [8,9]. It is likewise recognized as a typical weed which develops uncontrollably in crop fields in subtropical and tropical regions of the world. The plant is an annual, erect, branched plant about 15 to 50 cm high; it is a leafy and a flowering plant [10]. The parts of these plants used medicinally include the whole plant, leaves, flowers, seeds, and the roots. The leaves are utilized for ringworm; the juice is utilized in eye sickness; decoction is utilized in fevers and urticaria. Roots are a love potion; utilized for the fix of night visual deficiency [11].

Heliotropium indicum is accepted to be valuable in treating malaria, stomach agony, and dermatitis. The most elevated number of utilizations (22%) was accounted for its use against malaria. The mixture of the flower is taken orally for the treatment of menorrhagia in Jamaica [12]. In a concise review by[11], *Heliotropium indicum* is indicated to have antimicrobial [13], anti-tumour [14,15], anti-inflammatory [16], anti-fertility [12], anti-tuberculosis [17], Analgesic, anti-plasmodial [15] wound healing [18,19], anti-diabetic [20], anti-hepatic and anti-cataract activity [21].

Medicinal plants in traditional clinical practices are as of now not appropriately checked, resulting in an expanded danger of toxicity because of the wrong administration, particularly of those plants that are dangerous at low doses [22]. There is clear proof to implicate oxidative stress as a toxicity mechanism in various tissues. Oxidative stress mirrors a lopsidedness in the degree of body oxidants and its capacity to detoxify them [23]. Oxidative stress has likewise been implicated in several disease conditions, including malignant growth [24], neurodegenerative sicknesses such as Alzheimer’s [25], Parkinson’s [26], Huntington [27], amyotrophic lateral sclerosis [28], diabetes [29], ulcer [30], among others.

The liver is the major detoxification organ of the body; this is because phase I and phase II transformation pathways occur majorly in the liver. It is known to have significant levels of detoxifying enzymes. These enzymes are largely Cytochrome P450 oxidases (CYPs), UDP-glucuronosyltransferases (UGTs), Glutathione S-transferases (GSTs), amongst others [31]. It is worthy of note that the presence of these enzymes also makes the liver a target for oxidative-stress induced toxicity[23]. This paper is therefore focused on *Heliotropium indicum* phytochemical constituents as well as its toxicity in the liver of male Wistar rats.

2. Materials and Methods

2.1. Materials.

2.1.1. Animals.

Albino rats (*Rattus norvegicus*) weighing between 100.0g and 180.0g were used in this evaluation. These animals were obtained from Ayoola farmhouse in Ilorin, Kwara State, Nigeria. They were held under standard condition ($23 \pm 2^\circ\text{C}$) ($55 \pm 10\%$ humidity) with a 12 h light/dark cycle. Pellet diet and water were given *ad libitum* during the course of the experiment. Significantly, the creatures acclimatized to their new environment for about fourteen days before the inception of the experiment.

2.1.2. Chemicals.

Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline Phosphatase (ALP), and Total Bilirubin were obtained and are products of Randox Laboratories Limited, UK. TNF, COX 2, IL1, and IL 6 ELISA kits were obtained from Cloud-clone Intl Wuhan, China.

2.1.3. Plant source and identification.

Heliotropium indicum was obtained from the forest near Ikeji-Arakeji, Osun State. The plants were taken to the Department of Agricultural Sciences, Joseph Ayo Babalola University, for identification.

2.2. Methods.

2.2.1. Preparation and extraction of crude plant extract.

Heliotropium indicum leaves were air-dried for three weeks. It was blended into a powdery form using an industrial blender. Exactly 250g of the *Heliotropium indicum* leaf sample was measured with an electronic balance and was soaked in 700ml of ethanol at room temperature for 48 hours. The solvent was filtered using Whatman filter paper. The extract was stored in the refrigerator until required for use.

2.2.2. Phytochemical screening of plant extract.

Basic phytochemical screening is the method employed to test the presence of certain biologically active compounds in plants, e.g., tannins, saponins, anthraquinone, alkaloids, cardiac glycosides, and terpenoid. The methods are as follows [32].

2.2.2.1. Test for tannins.

About 0.2 g of each portion of plant extract was stirred with 5 ml of distilled water, filtered, and 0.1 % ferric chloride reagent was added to the filtrate. A blue-black green precipitate was taken as evidence for the presence of tannins.

2.2.2.2. Test for saponins.

The capacity of saponins to generate frothing in ethanol solution and to haemolyse red blood cells was used as a screening test for these compounds. For the frothing test, the methods described by each plant extract were shaken with water in a test tube. Frothing which persists on warming was taken as preliminary evidence for the presence of saponins.

2.2.2.3. Test for anthraquinone.

About 0.2 g of the extract was shaken with 5ml concentrated benzene, and 5 ml of 10% NH₃ solution was added to the filtrate. The mixture was shaken, and the presence of pink, red, or violet color in the ammonical phase indicates the presence of anthraquinones.

2.2.2.4. Test for alkaloids.

A quantity (1 cm³) of 1 % aqueous HCl was added to 3 cm³ of each extract in a test-tube, and the mixture heated for 20mins, cooled, and filtered. 1 cm³ portion of the filtrate was

treated with two drops of Wagner's reagent. Formation of cream or brown precipitate respectively indicated the presence of alkaloids

2.2.2.5. Test for cardiac glycosides.

About 1.0 ml of the ethanol extract of the sample plant was added to 2.0ml of chloroform and shaken. The mixture was allowed to settle down, and H₂SO₄ was added carefully to calm the solution. A reddish-brown color at the interface indicates the presence of an aglycone portion of cardiac glycoside.

2.2.2.6. Test for terpenoid.

1ml of chloroform was added to 2 ml of each extract, followed by a few drops of concentrated sulphuric acid. A reddish-brown precipitate produced immediately showed the presence of terpenoids.

2.2.3. Proximate analyses.

The parameters determined for proximate analyses include ash, moisture, crude protein, fat, fiber, ether extract, and nitrogen-free extract. All of these were carried out using the methods described by [32].

2.2.4. Animal treatments and Experimental design.

The experimental animals were divided into six groups of seven animals, each according to their body weight. Group A served as the control and received distilled water only. Groups B to F was fed with the extract of *Heliotropium Indicum* in different concentrations. (Group B received 500 mg/kg *Heliotropium Indicum*, Group C received 1000 mg/kg *Heliotropium Indicum*, Group D received 1500 mg/kg *Heliotropium Indicum*, Group E received 2000 mg/kg *Heliotropium Indicum*, Group F received 3000 mg/kg *Heliotropium Indicum*). At the end of the treatment, the rats were sacrificed, and the liver samples were collected in a clean, dry heparinized bottle, weighed, and homogenized phosphate Buffer solution and used for biochemical tests.

2.2.5. Determination of oxidative stress and antioxidant indices.

The liver of each rat was removed, weighed, and washed with ice-cold saline and subsequently homogenized in cold potassium phosphate buffer (0.05 M, pH 7.4). Subsequently, the homogenate was centrifuged at 10,000 g for 15 min at 4°C, and the supernatant was used for the determination of oxidant/antioxidant markers. Protein concentration was determined according to the method of Bradford *et al.* [33] using bovine serum albumin as standard. Catalase (CAT) activity was determined using H₂O₂ as a substrate according to the method described by Clairborne [34]. The inhibition of the autoxidation of epinephrine was used to determine the activity of superoxide dismutase using the method of Misra and Fridovich [35] GSH was evaluated according to the method described by Jollow *et al.* [36]. Glutathione S-transferase activity was determined according to the method of Habig *et al.* [37]. Glutathione peroxidase activity was quantified according to the method of Rotruck *et al.* [38]. Lipid peroxidation was determined as malondialdehyde levels (MDA).

2.2.6. Concentrations of pro-inflammatory biomarkers.

Liver nitrites content as stable end-products of NO was obtained using a sodium nitrite curve as standard and expressed as micromolar of nitrites per milligram of protein according to the method described by Green *et al.* [39]. TNF α , IL 1, IL6, and COX 2 were carried out according to the standard operative procedure of the respective assays.

2.2.7. Statistical Analysis.

All results were presented as mean \pm Standard Deviation (SD). Data were analyzed by using Microsoft Excel 2007 (Redmond, Washington, USA) and Graph Pad Prism 6 software (Graph Pad Software Inc., USA). All the information on treatment groups was analyzed and compared with the control group by utilizing one-way ANOVA accompanied by Dunnett's multiple comparison tests. In all the groups, differences were considered statistically significant among groups when $p < 0.05$.

3. Results and Discussion

3.1. Results.

3.1.1. Phytochemical Screening.

The table below shows the result of the phytochemical screening of ethanol extract of *Heliotropium indicum* leaf.

Table 1. Results of phytochemical screening.

S/N	Phytochemical components	<i>Heliotropium indicum</i> ethanol extract
1.	Tannin	+
2.	Saponin	+
3.	Anthraquinone	-
4.	Alkaloids	+
5.	Cardiac Glycoside	-
6.	Terpenoid	+

+ = present
- = absent

3.1.2. Proximate Analysis.

The table below shows the result obtained for the proximate analysis of dried leaf of *Heliotropium indicum*.

Table 2. Proximate analysis of dried leaf of *Heliotropium indicum*.

Plant Sample	% Moisture	% Ash	% Protein	% Fat	% Fibre	% Carbohydrate
<i>H. indicum</i>	18.29 \pm 2.10	1.42 \pm 0.12	3.93 \pm 0.08	1.24 \pm 1.00	13.10 \pm 0.02	62.44 \pm 3.04

The mean value of three determinations Mean \pm SD

3.1.3. Result of body weight.

The table shows the weight of rats used for the experiment before the treatment and after the treatment with ethanol extract of *Heliotropium indicum*.

Table 3. Bodyweight change induced by *Heliotropium indicum*.

Groups	Body weight (g)		
	Final	Initial	% Change
Group A (Control)	145.42 \pm 1.46	145.20 \pm 1.67	0.22
Group B (500 mg/Kg)	142.52 \pm 4.01	141.82 \pm 2.93	0.70
Group C (1000 mg/Kg)	110.66 \pm 4.91	109.65 \pm 6.44	1.01

Groups	Body weight (g)		
Group D (1500 mg /Kg)	180.05 ± 1.65	178.76 ± 1.34	1.29
Group E (2000 mg/Kg)	117.36±1.05	116.35±2.41	1.01
Group F (3000mg /Kg)	148.94 ±3.02	147.56 ± 3.75	1.38

3.1.4. *Heliotropium indicum* elevated liver function enzymes and bilirubin levels.

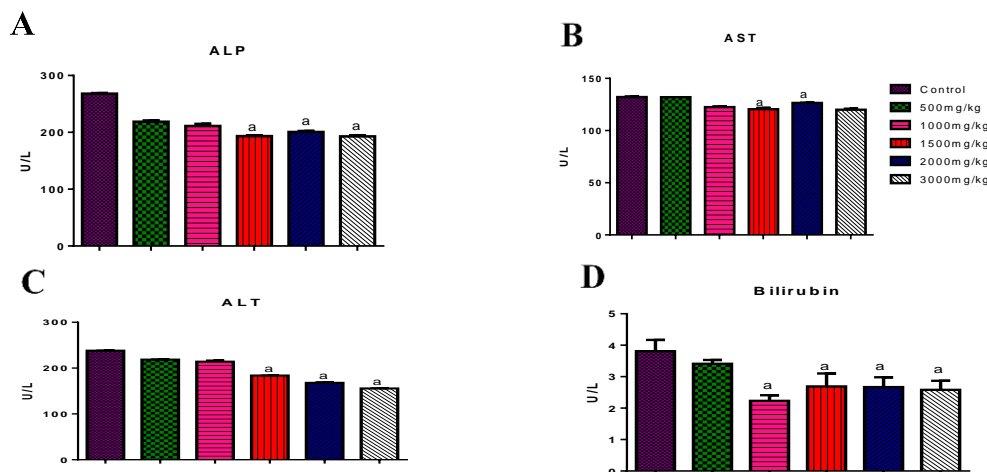


Figure 1. *Heliotropium indicum* effect on liver function A) ALP B) AST C) ALT and D) BIL. Results are represented as mean ± SD, n= 5. a- Significantly different from control (p< 0.05).

The effect of *Heliotropium indicum* on liver function enzymes was investigated. Results from figure 1 showed that liver function enzymes (ALT, AST, and ALP) and bilirubin are significantly decreased from doses from 1500 mg/kg and above (p < 0.05); however, doses below 1500 mg/kg were not significantly elevated.

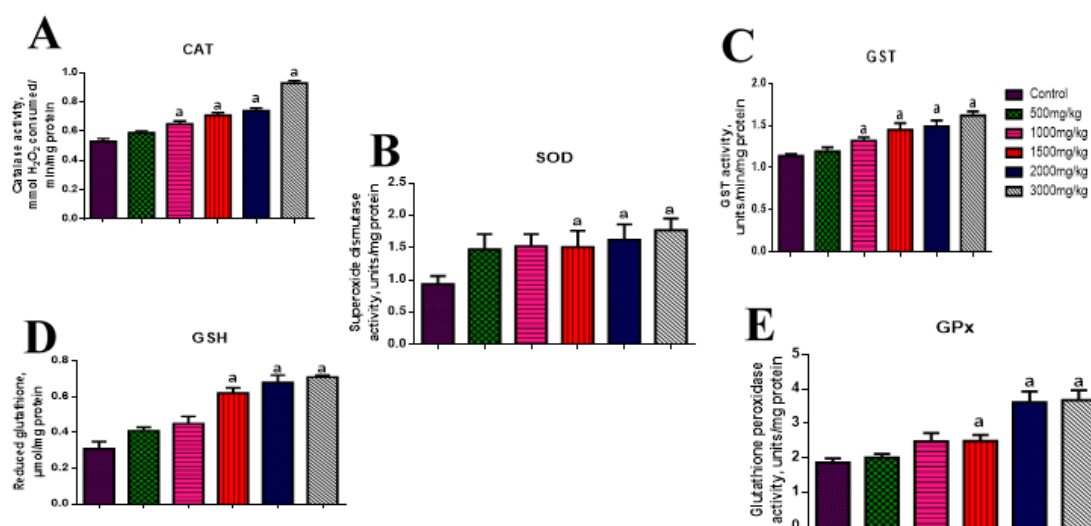


Figure 2. *Heliotropium indicum* effect on enzymatic and non-enzymatic antioxidants A) CAT B) SOD C) GST D) GSH E) GPx. Results are represented as mean ± SD, n= 5. a- Significantly different from control (p< 0.05).

3.1.4. *Heliotropium indicum* elevated levels of enzymatic and non-enzymatic antioxidants.

The effect of *Heliotropium indicum* on enzymatic and non-enzymatic antioxidants was investigated. Figure 2 shows a significant increase in antioxidant enzymes (CAT, SOD, GST, GPx) and Glutathione, especially at doses higher than 1500 mg/kg (p < 0.05); however, doses below 1500 mg/kg were not significantly elevated.

3.1.5. *Heliotropium indicum* reduced Lipid peroxidation and Inflammatory Markers.

Heliotropium indicum effect on lipid peroxidation and inflammatory markers were assessed. Figure 3 shows a significant decrease in lipid peroxidation at all doses of *Heliotropium indicum*. Inflammatory markers levels, including TNF- α , COX-2, IL-1 β , IL-6, and NO, was also significantly decreased, especially at doses from 1500 mg/kg ($p < 0.05$).

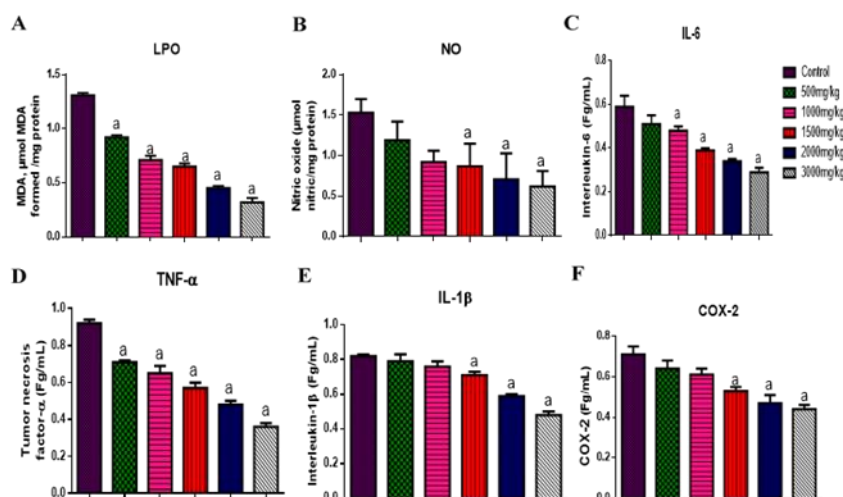


Figure 3. *Heliotropium indicum* on Lipid peroxidation and Inflammatory markers A) LPO B) NO C) IL-6 D) TNF- α E) IL-1 β F) COX-2. Results are represented as mean \pm SD, n= 5. a- Significantly different from control ($p < 0.05$).

3.2. Discussion.

Medicinal plants are important contributors to the pharmaceutical, agricultural, and food industries [40,41]. WHO [42] defines herbal medicines as normally occurring, plant-sourced substances with next to zero modern handlings that have been utilized to alleviate sickness within conventional healing practices. *Heliotropium indicum* is a medicinal plant whose beneficial effects have been shown by various researchers. Results from table 1 showed that *Heliotropium indicum* is rich in phytochemicals, including saponins, alkaloids, tannins, and terpenoids but anthraquinones and cardiac glycosides are absent in the leaf extract of this plant. These results correlate with that of Basak *et al.* [43], who reported that *Heliotropium indicum* ethanol extract contained alkaloids, saponins, flavonoids, amongst others. The presence of these phytochemicals may be indicative of its numerous beneficial health effects that have been reported by several studies. Proximate analysis of the extract showed significant carbohydrate content and fiber content but lowered protein and fat content. Also, the moisture content of the extract is higher than the ash content. Santhosha *et al.* [44] also reported that ethanol extract of *Heliotropium indicum* is rich in phenols and flavonoids, although this was not shown by our study. The results of this study show that it increases body weight even when administered at doses as high as 3000 mg/kg

The liver is the major detoxification organ of the body; its ability to detoxify many substances is attributable to the detoxification enzymes it contains; therefore, it is a significant organ in the body [31]. Results from figure 1 showed that liver function enzymes (ALT, AST, and ALP) and bilirubin are significantly decreased from doses from 1500 mg/kg and above. The aminotransferases, specifically AST and ALT, are refrained to the periportal hepatocytes, where they play a central role in transamination reactions during amino acid metabolism. The

marked increase in the level of transaminases is indicative of liver injury; this is as shown in lead-induced toxicity [45]. A significant decrease in these enzymes shows that *Heliotropium indicum* can reduce toxicity in the liver, thereby conferring protective effects on the liver. These results correlate with the study of Owolabi *et al.* [46] conducted a 5-month study on *Heliotropium indicum* extract, however, on Sprague-Dawley rats. They demonstrated that the lethal dose (LD₅₀) of *Heliotropium indicum* is about 508.78 g/kg, which is higher than all doses used in this study.

Oxidative stress is summarily explained as a lopsidedness in the level of antioxidant enzymes in the body and reactive species the body is exposed to. Oxidative stress is responsible for a host of disease conditions, including cancer and neurodegenerative diseases. An antioxidant is any molecule which in low quantities and in the presence of an oxidizable species, can mitigate the activities of reactive oxygen species. Figure 2 shows a significant increase in antioxidant enzymes (CAT, SOD, GST, GPx) and Glutathione, especially at doses higher than 1500 mg/kg. These enzymes help to maintain redox balance in the system. Their activity can be used to estimate the oxidative defense mechanism strength. [47]. Glutathione is an excellent antioxidant that helps to balance the redox state of the system. GSH is an essential cellular component of the antioxidant defense mechanism that can function as a direct or indirect free-radical scavenger [48]. SOD, CAT, and GPx remove ROS, such as superoxide free radicals, by converting them to water and molecular oxygen. The elevated levels of glutathione and other antioxidant enzymes in the liver would likely have hepatoprotective effects.

Lipid peroxidation and inflammation are markers of toxicity [49]. Lipid peroxidation, as assessed by malondialdehyde (MDA) formation in this study (Figure 3), is significantly reduced by all doses of *Heliotropium indicum*. This implied that the consumption of this medicinal plant reduced the amount of lipid peroxidation products in the liver of Wistar rats. Cytokines are little secreted proteins (<40 kDa), which are created by almost all cells to control and impact immune reactions [50]. The secretion of pro-inflammatory cytokines will prompt immune cell activation and production with concurrent further cytokines release [51]. Figure 3 shows a significant decrease in inflammatory markers, including TNF- α , COX-2, IL-1 β , IL-6, and NO. All doses of *Heliotropium indicum* significantly reduced the levels of TNF- α . TNF- α mediates cell survival and pro-inflammatory response by TNFR-I via NF- κ B and activator protein (AP)-1[52]. Additionally, TNF- α instigates signaling pathways of cell apoptosis via Fas and Caspases. This indicates that *Heliotropium indicum* may downregulate cell-death and inflammation via TNF- α decreased expression.

The essential IL-1 β sources are hematopoietic cells like macrophages, monocytes, including dendritic cells, and microglia or Kupffer cells [53]. IL-1 β is a prototypic pro-inflammatory cytokine that has pleiotropic impacts on different cells and assumes key functions in acute and chronic inflammatory and autoimmune disorders [54]. Figure 3C and 3E reveal that IL-1 β and IL-6 expressions are significantly reduced at doses from 1500 mg/kg and above. This indicates that *Heliotropium indicum* may downregulate inflammation via interleukin mediation.

4. Conclusions

Ethanol extract of *Heliotropium indicum* at doses between 1500 mg/kg and 3000 mg/kg has antioxidative, anti-inflammatory effects and can counteract lipid peroxidation. *Heliotropium indicum* can be said to be hepatoprotective at these doses.

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Conflicts of Interest

The authors declare no conflict of interest.

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