Original Article

Ethanolic Extract From Hygrophila Auriculata (Schumach.) Heine Leaves Exhibited A Promising Protective Effect Against Drug-Induced Nephrotoxicity In Rodents

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Abstract

Hygrophila auriculata (Schumach) Heine is a tropical herb from the Acanthaceae family. Owing to its traditional and folklore medicinal properties and in numerous preclinical investigations, this plant has been shown to have a variety of pharmacological effects. Firstly, the leaves were subjected to ethanol-based extraction, followed by preliminary phytochemical screening. After calculating the safe dose of EHAE extract using the acute oral toxicity OECD 420 guideline, it was revealed that at a maximum dose of EHAE of 2000 mg/kg BW and showing no signs of toxicity or mortality, the doses of 250 and 500 mg/kg BW were chosen for the study. For each disease model, a group of six animals was allocated. Intriguingly, results from all three models indicated a significant and dose-dependent reduction of serum biomarkers upon EHAE administration. Further in vitro antioxidant activity was also carried out for the extract. The results showed a significant elevation of antioxidant markers at both doses, showing strong antioxidant activity. The present investigation identified that *EHAE extract* exhibited a promising protective effect against drug-induced nephrotoxicity in rodents. In this line, our current effort is a preliminary one to investigate the leaves of this herb against nephrotoxicity using albino Wister rats in different drug-induced animal models such as cisplatin, gentamycin, and paracetamol.

Keywords: Drug Induced-Nephrotoxicity, Hygrophila auriculata, Antioxidant activity, Cisplatin, Paracetamol, Gentamycin

INTRODUCTION

Nephrotoxicity is the impairment of normal renal function caused by chronic exposure to specific toxins and chemical agents [1]. Numerous studies have shown that chronic exposure to certain pharmacological agents, such as Cisplatin, aminoglycosides (Gentamycin), and over-the-counter pain relievers, can cause nephrotoxicity [2]. Since the discovery of these deleterious effects, these compounds are now widely used as chemical inducers for *in vivo* nephrotoxicity testing. A variety of molecular mechanisms contribute to drug-induced nephrotoxicity, including glomerular hemodynamic changes, glomerulonephritis, and interstitial nephritis, crystalline nephropathies, rhabdomyolysis, and thrombotic microangiopathy [3]. Notably, cisplatin is the most used chemical inducer for modelling drug-induced nephrotoxicity (DIN) in rodents. Cisplatin is a platinum-based drug with a wide range of anticancer effects and nephropathy as a common adverse systemic response [4], where it exerts its nephrotoxicity by damaging nuclear DNA and mitochondrial DNA, resulting in oxidative stress, which is then associated with apoptosis and/or necrosis in a proximal tubular cell line architecture [5]. Gentamycin (GM) is an important member of the aminoglycoside class used to treat gram-negative bacterial infections, even though all aminoglycosides are known to cause nephrotoxicity [6]. The nephrotoxicity of GM is comparably more severe and dose-dependent than that of any other antibiotic in this class [7]. GM accumulation in proximal tubules provokes tubular cell apoptosis, which is a widely implicated mechanism in gentamycin-induced nephrotoxicity. In addition, a recent study has identified a novel necroptotic pathway in mediating collecting duct epithelial cell death, interstitial inflammation, and fibrosis in a mouse model, which may be an ideal target to attenuate gentamycin-induced toxicity [8]. Paracetamol is one of the drugs that is most prescribed all over the world. It is highly valued for its analgesic and antipyretic properties. However, nephrotoxicity and hepatotoxicity are the most common side effects, which may limit its use to small doses and short periods of time. The underlying mechanism of paracetamol's nephrotoxic potential is the induction of apoptosis via ER stress in tubular cells [9]. There is currently no treatment for

drug-induced nephrotoxicity. Palliative care, which involves withdrawing the toxic drug and stabilizing the patient, is the sole recommended method of care.

Most modern medicines are derived from plant sources and are used to treat a variety of diseases. The plant kingdom never ceases to amaze us with its rich and diverse secondary metabolites. It has continued to provide us with inexhaustible leads or serve as a chemical scaffold for the development of new therapeutic compounds and may be an adjuvant therapy [10]. Plant extracts are currently reviving the once-glory of plant-based medicine owing to its multimechanistic approach to preventing, treating, or relieving the symptoms of a disease or abnormal state with comparably fewer side effects and off-target consequences than synthetic drugs.
Hygrophila auriculata (Schumacher) Heine (also known as *Asteracantha auriculata* Nees, *Asteracantha longifolia* Neesis), is a typical annual herb in the family of Acanthaceae found in marshes and wetland areas of subtropical India, Sri Lanka, and the Indo-Malay region. It is referred to as marsh barbel in English, enugu palleru, kokilaksi, and vana-sringatamu in Telugu, kokilaksah in Sanskrit, and nir-mulli in Tamil [11]. The two primary ancient medical systems in India, Siddha, and Ayurveda have recognized the therapeutic use of various components of this plant, including the roots, leaves, seeds, flowers, fruits, and even the whole plant and plant ashes, to treat a range of ailments. Aviltholadi Bhasmam, Yakrut Shula, Vinashini Vatika, and Panaviraladi Bhasmam are some of the most well-known Ayurvedic preparations developed from this plant for the treatment of liver, spleen, and edema disorders [12]. Many studies have investigated various parts of *H. auriculata* and reported different pharmacological activities. Leaf extracts have been shown to have analgesic and antimotility [13], anthelmintic and antibacterial activities [14], anti-inflammatory and antipyretic activities [15], hematopoietic potential [16], and erythropoietic activity [17]. Treatment with aerial parts exhibited significant anti-diabetic activity [18] and alleviated diabetic peripheral neuropathy [19] in experimental animal models. Furthermore, an ethanolic extract of the aerial parts appears to have hematinic properties in anemic male albino rats [20]. In addition, methanolic extracts of aerial parts exhibited the strongest protection against ethylene glycol-induced nephrolithiasis in rats [21]. In studies conducted independently, terpenoid-rich fractions from the whole plant demonstrated an anti-endotoxin effect in lipopolysaccharide-induced septic shock in rats [22], and n-butanol fractions exhibited diuretic potential [23]. Crude extracts from different parts have been extensively studied for their hepatoprotective effects in different models [24, 25, 26, 27, 28, 29, 30]. Terpenoid fractions have been shown to have potent neuroprotection against transient global cerebral ischemia-induced oxidative stress [31], and seed extracts have been demonstrated to have aphrodisiac and spermatogenic effects [32, 33]. Methanolic extract from aerial parts and ethanolic extract from the whole plant were investigated against gentamycin and cisplatin-induced renal nephrotoxicity models, respectively [34,35]. However, the nephroprotective effects of this plant's leaf extracts have not yet been investigated.

Thus, we designed this study to assess the nephroprotective activity of ethanolic extract of *H. auriculata* (Schumach.) Heine leaves against Gentamicin, Paracetamol, and Cisplatin-induced nephrotoxicity in animal models by evaluating traditional biochemical parameters like serum parameters and tissue parameters.

MATERIAL AND METHODS

Chemicals and reagents

Cisplatin, gentamycin, and paracetamol, the chemical inducers used to model DIN in rats, were provided as free samples by the Indian pharmaceutical company Mylan Pvt. Ltd. The AR Grade ethanol required for extraction is bought from Merck, Inc. All the reagents were purchased from Sigma-Aldrich Co. in St. Louis, Missouri, in the United States.

Plant collection and authentication

Fresh leaves of H. auriculata Linn. were obtained in the rainy season from subtropical swamplands close to Tirupati, Chittoor, India. The plant material was identified and authenticated by Dr. Madhava chetty, S.V. University, Tirupati, India, where the specimen was deposited in the form of an herbarium with voucher number

Preparation of Ethanolic extract

Leaves were washed under cold running water to wash away any dirt and debris, then shade-dried before being pulverized into a coarse powder. About 1000 gm of leaf powder was weighed and loaded into a porous thimble and placed into the main chamber of the Soxhlet apparatus attached to a 250 ml round-bottomed flask of 200 ml of ethanol. In the reflux setup, extraction cycles were repeated until the solvent became colorless by refluxing the solvent through the thimble using a condenser and siphon side arm. Later, the solvent was filtered using Whatman No-1 filter paper, and the filtrates were concentrated using a vacuum drier at 30–45 °C for 20 min. The yield (%) of extracts was calculated.

Phytochemical screening

The resulting extract was subjected to a battery of identification tests, as described in earlier standard methods [36], to determine the presence of different classes of phytoconstituents (alkaloids, glycosides, tannins, resins, steroids, saponins, flavonoids, carbohydrates, and proteins).

Experimental animals

Adult healthy male Wistar albino rats (weighing 200–250 g) were procured from the central animal house of Sigma Institute of Clinical Research & Administration Pvt. Ltd., Hyderabad. Animals were housed at a temperature of 24±2°C and a relative humidity of 30–70%. A 12:12 hr light-to-dark cycle was followed. All animals had free access to water and a standard laboratory animal diet. Animals were acclimatized to laboratory conditions one week prior to the experiment procedure, which was approved by the Institutional Animal Ethical Committee of SICRA labs with CPCSEA registration number 541/02/C/CPCSEA.

Acute oral toxicity

According to OECD Test Guidelines No. 420 (Acute Oral Toxicity-Fixed Dose Procedure), female Wistar rats (180-200 g), which are nulliparous and non-pregnant, were chosen randomly. After the Sighting Study was dosed using the fixed dosages of 5, 50, 300, and 2000 mg/kg in a stepwise approach, 2000 mg/kg (p.o.) was chosen as the initial dose. The test chemical was given orally to one rat, starting at 5 mg/kg BW. The rat was examined for toxic effects every eight hours for the first 24 hours after the initial 30 minutes. We dosed another group of rats with the subsequent dose (50 mg/kg BW) and followed the same procedure if the first rat showed no signs of toxicity or mortality within 24 hours. The procedure was carried out step by step until the maximum dose of 2000 mg/kg BW was reached. If all the rats survived, they were monitored and observed once daily for the next 13 days.

Experimental protocol

There were a total of 12 groups, each with six albino wistar rats (n = 6) of both sexes. Each of the disease-induced models had four groups allocated to it (Cisplatin, Gentamycin, and paracetamol).

Cisplatin-induced nephrotoxicity

Group I (Normal control) received only normal saline for 14 days via oral gaveling, while Group II (Disease/Negative control) received Cisplatin 12 mg/kg i.p. on day 7. Groups III and IV (Test groups) received EHAE doses of 250 and 500 mg/kg p.o., respectively, for 10 days in addition to cisplatin treatment (12 mg/kg (i.p.).

Gentamycin induced nephrotoxicity

Group NC received normal saline (10 mL/kg b. wt.) as a control, Group GM received GM 120 mg/kg/day (i. p.) for 14 days as a disease control, and Group T1 and T2 (Test Extracts): Animals received 250 and 500 mg/kg b. wt. EHAE orally, respectively, along with GM 120 mg/kg/day (i.p

Paracetamol-induced nephrotoxicity

Group A was given distilled water by oral gavage for 7 days and was used as a normal control. Group B received a single dose of PCM at 1g/kg and distilled water for 7 days. For 7 days, Groups E1 and E2 received a single dose of PCM-1 at a dose of 1 g/kg and EHAE at doses of 250 mg/kg (p.o.) and 500 mg/kg (p.o.) respectively.

Blood analysis

Quantitative diagnostic kits were used in this study to measure the serum levels of blood urea nitrogen, creatinine, uric acid, SGOT, SGPT, and alkaline phosphate.

Estimation of enzymic antioxidant parameters

A tissue sample of a kidney was homogenized in 0.9% saline and spun for 15 minutes. The translucent supernatant layer was taken and used to analyze oxidative stress markers such as superoxide dismutase (SOD) [37], catalase (CAT) [38], reduced glutathione (GSH) [39], glutathione transferases (GSTs) [40] and glutathione Peroxidase (GPx) [41].

Statistical analysis

The experimental data obtained were statistically analyzed by one way ANOVA followed by Tukey's multiple comparisons test employing the trial version of Graph Pad Prism, San Diego version (Prism graph pad version 8.0.2 (263, GraphPad Software, Inc. La Jolla, CA USA).

RESULTS

Phytochemical analysis

The yield (%) of ethanolic extract of H. auriculata leaf was found to be 4.0% w/w. Preliminary phytochemical analysis revealed the presence of alkaloids, proteins, saponins, triterpenoids, steroid flavonoids, and cardiac glycosides.

Acute oral toxicity

The experimental animals survived the EHAE 2000 mg/kg BW dose and showed no signs of physical or behavioral symptoms of acute toxicity during the 14-day observation period. Therefore, doses of 250 and 500 mg/kg BW are safe.

Effects of Crude Extract on Cisplatin-Induced nephrotoxicity in Rats.

The kidney function markers in rat serum and the enzyme antioxidant parameters are affected by EHAE treatment, as demonstrated in (Table 1). Administration of cisplatin caused renal injury as evidenced by higher levels of BUN (mg/dL),

creatinine (mg/dL), uric acid (mg/dL), SGOT (IU/dL), SGPT (IU/dL), and alkaline phosphatase (IU/dL) in the experimental animals of Group II (disease control), and these changes were considered statistically significant compared to the normal group I ($p < 0.001$). Groups III and IV were pretreated with EHAE extracts at 250 and 500 mg/kg p.o. along with cisplatin (12 mg/kg i p.) treatment exhibited a significant reduction in levels of serum biomarkers compared to disease control ($p < 0.01$ and 0.05). However, the decreased serum biomarker levels were dose dependent. The kidney function markers significantly decreased at the dose of 500 mg/kg p.o. test extract when compared to the 250 mg/kg p.o. test extract.

In comparison to the group I (normal control), levels of SOD, catalase, reduced glutathione, glutathione transferases, and glutathione peroxidase were significantly lower in group II (disease control). A dose-dependent increase in antioxidant levels was observed in group III and group VI test extracts at 250 and 500 mg/kg p.o. compared to disease control ($p < 0.01$ and 0.05). These findings show that EEHA has a potent nephroprotective effect against cisplatin-induced rat nephrotoxicity.

Effects of Crude Extract on Gentamycin-Induced nephrotoxicity in Rats.

The serum marker levels of all the groups showed a statistically slight increase after GM administration in the disease control group. After 7 days, all values significantly decreased in both the treatment groups (250 and 500 mg/kg p.o. in group T1 and T2, respectively ($P < 0.01$) (Table 2).

The levels of SOD, catalase, GSH, GSTs, and Gpx showed a significant decrease in group GM compared to group NC. Treatment with plant extract doses of 250 and 500 mg/kg p.o. showed a significant increase in the level of antioxidants near normal levels ($p < 0.05$) ($p < 0.01$).

Effects of Crude Extract on paracetamol-Induced nephrotoxicity in Rats.

Paracetamol-induced nephrotoxicity in rats showed significant elevations in BUN, creatinine, uric acid, SGOT, SGPT, and alkaline phosphatase as compared with normal control. Group E1 and Group E2 treated with plant extracts at 250 and 500 mg/kg p.o., respectively, showed a significant reduction in serum levels ($p < 0.05$) ($p < 0.01$). A significant depletion in the antioxidant SOD, catalase, GSH, and MDA levels of disease control rats was observed. Treatment with EHAE extract significantly upraised these values ($p < 0.01$) (Table 3).

DISCUSSION

Synthetic drugs possess a range of drawbacks, with nephrotoxicity standing out as one of the most serious. Drug-induced nephrotoxicity, which not only affects health but occasionally results in death, sadly has no specific cure. The damage can be brought on by the kidneys being directly exposed to medications, such as certain non-steroidal anti-inflammatory drugs, aminoglycoside antibiotics, and antipyretics like paracetamol. Natural remedies are more frequently used to treat a wide range of illnesses in the twenty-first century, not only because they are affordable and have few side effects, but also because they have multiple mechanisms of action that help manage the disease. Plants like *H. auriculata* have shown robust activities with applications in traditional medicinal systems. In this study, the effect of *H. auriculata* has been investigated to study its reno-protective effect induced by certain synthetic drugs.

Frequently used to treat carcinoma, lymphoma, and germ-cell malignancies, cisplatin is a well-known anti-neoplastic medication made from platinum. Acute kidney injuries (AKI), which make up around 26% of all kidney injuries, have been linked to cisplatin. It was established that Cis-induced nephrotoxicity affected renal hemodynamics due to changes in the renal vasculature. Cis has been connected to kidney damage caused by oxidative stress leading to the death of renal tubular cells, although the precise process is uncertain. The popular aminoglycoside gentamicin damages tubules by causing the necrosis of tubular epithelial cells, mainly in the proximal segment. The primary cellular elements involved in the transfer of water and solutes also undergo changes because of GM. Tubular cytotoxicity is the main GM nephrotoxic factor. On the other hand, paracetamol is the over-the-counter analgesic and antipyretic medicine that is most widely used globally. Limitations of PCM include nephrotic toxicity and liver damage, which frequently accompany chronic use and/or overdose. The poisonous metabolite N-acetyl-p-benzoquinone imine (NAPQI), which is damaging to the kidneys, is produced by the primary enzyme system of cytochrome P450. In an overdose situation, the conjugation routes become saturated, and as a result, the accumulation causes the reduced glutathione (GSH) to be depleted faster than it can be produced. NAPQI binds covalently to the cysteine groups of cytosolic and mitochondrial proteins, resulting in cell death and renal impairment. Renal cortex necrosis also develops from the deacetylation of PCM in the kidneys into the nephrotoxic metabolite para-aminophenol.

All three medications have the potential to cause nephrotoxicity, which is supported by the study's measurements of blood urea nitrogen, uric acid, and creatinine levels, which are renal diagnostic indicators. Additionally, it was clear that all three models showed reduced antioxidant enzymes and increased oxidative stress. Following the administration of GM, PCM, and Cis, the ethanolic and ethyl acetate fractions of the leaf extract were chosen as the treatment against the nephrotoxicity that was created since they showed higher phytochemical contents. Following the completion of acute toxicity investigations, one low dose of 200 mg/kg and one high dose of 500 mg/kg were selected for treatment.

The rate of glomerular filtration and tubular reabsorption determine the level of blood urea nitrogen (BUN), a nitrogenous by-product of protein metabolism. BUN is so essential for diagnosing and evaluating renal function. In Cis, GM, and PCM-induced nephrotoxicity models, a high dose of EEHA (500 mg/kg) dramatically reduced the levels of BUN. Like this, increased creatinine levels signify deteriorated kidney health. The blood level of creatinine increases when the kidneys are damaged for any reason because the kidneys are unable to adequately remove the creatinine from the body. As a result, in traditional blood tests, the level of creatinine in the blood is commonly tested. The rate of glomerular filtration and tubular reabsorption determine the level of blood urea nitrogen (BUN), a nitrogenous by-product of protein metabolism. BUN is so essential for diagnosing and evaluating renal function. In Cis, GM, and PCM-induced nephrotoxicity models, high EEHA doses (500 mg/kg) dramatically reduced the levels of BUN. Similar to this, increased creatinine levels signify deteriorated kidney health. The blood level of creatinine increases when the kidneys are damaged for any reason because the kidneys are unable to adequately remove the creatinine from the body. As a result, in traditional blood tests, the level of creatinine in the blood is commonly tested. In all three animals, this was reversed when EEHA was used as a treatment. The levels of serum uric acid decreased in a dose-dependent manner in the treatment groups. The fact that these three measures decreased after receiving EEHA treatment suggests that H. auriculata may be able to protect the kidney against toxicity brought on by drugs.

On the other hand, the typical AST, ALT, and ASP serum biomarkers, which are often estimated to assess liver function, are also looked at. In all three of the used nephrotoxicity models, the drug-induced groups showed elevated levels of AST, ALT, and ASP. Aspartate and alpha-ketoglutarate are converted to oxaloacetate and glutamate by the transaminase enzyme aspartate aminotransferase (AST), formerly known as serum glutamate oxalate transaminase (SGOT). An elevated AST is frequently combined with other tests to support a diagnosis and may be a sign of kidney, cardiac, pancreatic, anaemia, renal, and musculoskeletal problems. Another enzyme that is used in the identification of many diseases is ALT. One of the important characteristics that can be approximated to gauge renal function is a high ALT, which research suggests is linked to CKD. Furthermore, patients with CKD who had high ALP levels had higher mortality rates. ALP measurement is recommended as an additional test for CKD patients. In the current study, AST, ALT, and ASP levels were dose-dependently downregulated after EEHA administration. In Cis-induced nephrotoxicity models, high dose EEHA (500 mg/kg) dramatically normalised the levels of ALT better than the other two models. The effectiveness of EEHA therapy to reverse increased levels of AST, ALT, and ALP points to H. auriculata's potential for protection against drug-induced liver and kidney damage.

Additionally, the biochemical assessments, such as enzymatic antioxidant levels, were evaluated in all three disease models. Our current research revealed that GM and PCM-induced nephrotoxicity was followed by a significant downregulation of the enzymatic antioxidants. It indicated that there had been significant oxidative stress because of the imbalance of endogenous antioxidants and the production of free radicals by GM and PCM. Malondialdehyde (MDA) was assessed in the current study together with enzymatic antioxidants including superoxide dismutase (SOD), catalase, and reduced glutathione to assess the level of lipid peroxidation brought on by oxidative stress. Following treatment with EEHA, there was a dose-dependent rise in the levels of antioxidant enzymes as well as a dose-dependent fall in the levels of MDA.

CONCLUSION

EHAE seems to be both safe and therapeutically effective. Numerous phytochemicals were shown to have a significant impact on reversing drug-induced kidney damage, showing a significant potential for the extract to treat renal damage. Most parameters returned to normal following the administration of EHAE. It has excellent therapeutic potential for nephrotoxicity and antioxidants. To better understand the precise molecular process behind its nephroprotective activity, further study is needed.

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Table 1.-Effect of H. auriculata leaf extract on kidney function markers in serum and enzyme antioxidant parameters of the cisplatin-induced model										
Group	Kidney function markers in rat serum						Kidney tissue enzyme antioxidant parameters			
	BUN	Creatinine	uric acid	SGOT	SGPT	Alkaline phosphatase	SOD	Catalase	GSH	MDA
Normal	16.53+ 1.9	1.57 + 0.27	7.3+ 0.88	38.17 + 2.61	40.17 + 4.06	53.3+ 5.99	123.5±3.1	7.2±0.6	38.6±1.1	130.0±5.2
Diseased	39.25+ 2.07	2.25 + 0.4	15.18+ 1.63	85.83 + 8.76	67.3 + 7.16	88.5+ 6.21	66.0±2.7	3.4±0.9	17.4±2.2	308.2±4.5
EHAE 250 mg/kg	36.3+ 2.24	2.2 + 0.35	12.77+ 1.05	87.3 + 4.96	47.8 + 6.59	69.67+ 5.19	88.4±3.4	4.8±0.4	24.2±1.6	248.4±5.3
EHAE 500 mg/kg	33.23 + 3.19	1.55 + 0.23	6.08+ 0.91	70.5 + 4.31	28.0 + 3.79	55.33+ 5.04	112.8±4.0	6.4±0.6	37.5±2.6	169.3±6.7
BUN: Serum blood urea nitrogen (mg/dl); SC: Serum creatinine (mg/dl); uric acid (mg/dL); SGOT (IU/dL); SGPT (IU/dL); and alkaline phosphatase (IU/dL) * P < 0.05, ** P < 0.01, ***P < 0.001. Values are expressed as mean ± SEM (n=6) for six animals in the group; a: considered statistically significant as compared to the control group; b: considered statistically significant as compared to the cisplatin group.										

Table 2.-Effect of H. auriculata leaf extract on kidney function markers in serum and enzyme antioxidant parameters of the gentamycin-induced model

Group	Kidney function markers in rat serum						Kidney tissue enzyme antioxidant parameters			
	BUN	Creatinine	uric acid	SGOT	SGPT	Alkaline phosphatase	SOD	Catalase	GSH	MDA
Normal	16.05 ± **0.84**	1.47 ± 0.28	43.5± 3.59	41.1 ± 4.02	190 ± 4.9	191.1± 5.5	114.4±3.8	6.6±0.2	38.0±2.9	130.0±5.2
Diseased	43.7 ± **3.59**	2.82 ± 0.65	94.17± 6.0	68.5 ± 5.65	581.6 ± 38.8	581.5± 33.3	64.9 ±2.4	2.9±0.4	20.6±1.1	308.2±4.5
EHAE 250 mg/kg	38.06 ± **2.53**	2.2 ± 0.47	90.0± 5.51	64.0 ± 5.23	472.1 ± 50.2	455.1± 43.9	67.9±3.1	3.8±0.6	24.9±0.7	248.4±5.3
EHAE 500 mg/kg	21.43 ± **2.6**	1.65 ± 0.19	70.33± 5.4	55.0 ± 6.11	254.8 ± 24.9	247.0± **16**.2	95.4±2.1	5.6±0.4	34.7±1.0	169.3±6.7

BUN: Serum blood urea nitrogen (mg/dl); SC: Serum creatinine (mg/dl); uric acid (mg/dL); SGOT (IU/dL); SGPT (IU/dL); and alkaline phosphatase (IU/dL) * P < 0.05, ** P < 0.01, ***P < 0.001. Values are expressed as mean ± SEM (n=6) for six animals in the group; a: considered statistically significant as compared to the control group; b: considered statistically significant as compared to the gentamycin group.

Table 3.-Effect of H. auriculata leaf extract on kidney function markers in serum and enzyme antioxidant parameters of the paracetamol-induced model

Group	Kidney function markers in rat serum						Kidney tissue enzyme antioxidant parameters			
	BUN	Creatinine	uric acid	SGOT	SGPT	Alkaline phosphatase	SOD	Catalase	GSH	MDA
Normal	15.8 + 0.7	1.3 + 0.4	7.05 + 0.4	44.0 + 4.5	42.3 + 4.8	188.8 + 5.1	143.8±3.0	6.6±0.2	38.0±2.9	130.0±5.2
Diseased	44.52 ± 3.6	1.3 ± 0.4	20.2 ± 4.2	95.3 ± 3.4	69.0 ± 5.9	589.0 ± 34.7	64.9±2.4	2.4±0.6	16.3±0.7	280.7±3.7
EHAE 250 mg/kg	38.1 ± 2.7	2.27 ± 0.35	18.1 ± 1.4	92.1 ± 4.9	62.5 ± 4.8	472 ± 49.0	73.3±3.2	4.4±0.4	20.8±0.9	211.7±4.1
EHAE 500 mg/kg	24.0 ± 3.5	1.55 ± 0.24	9.5 ± 1.37	72.5 ± 4.8	55.1 ± 7.9	264 ± 23.0	108.9±4.0	6.7±0.4	31.2±0.7	171.3±5.8

BUN: Serum blood urea nitrogen (mg/dl); SC: Serum creatinine (mg/dl); uric acid (mg/dL); SGOT (IU/dL); SGPT (IU/dL); and alkaline phosphatase (IU/dL) * P < 0.05, ** P < 0.01, ***P < 0.001. Values are expressed as mean ± SEM (n=6) for six animals in the group; a: considered statistically significant as compared to the control group; b: considered statistically significant as compared to the paracetamol group.