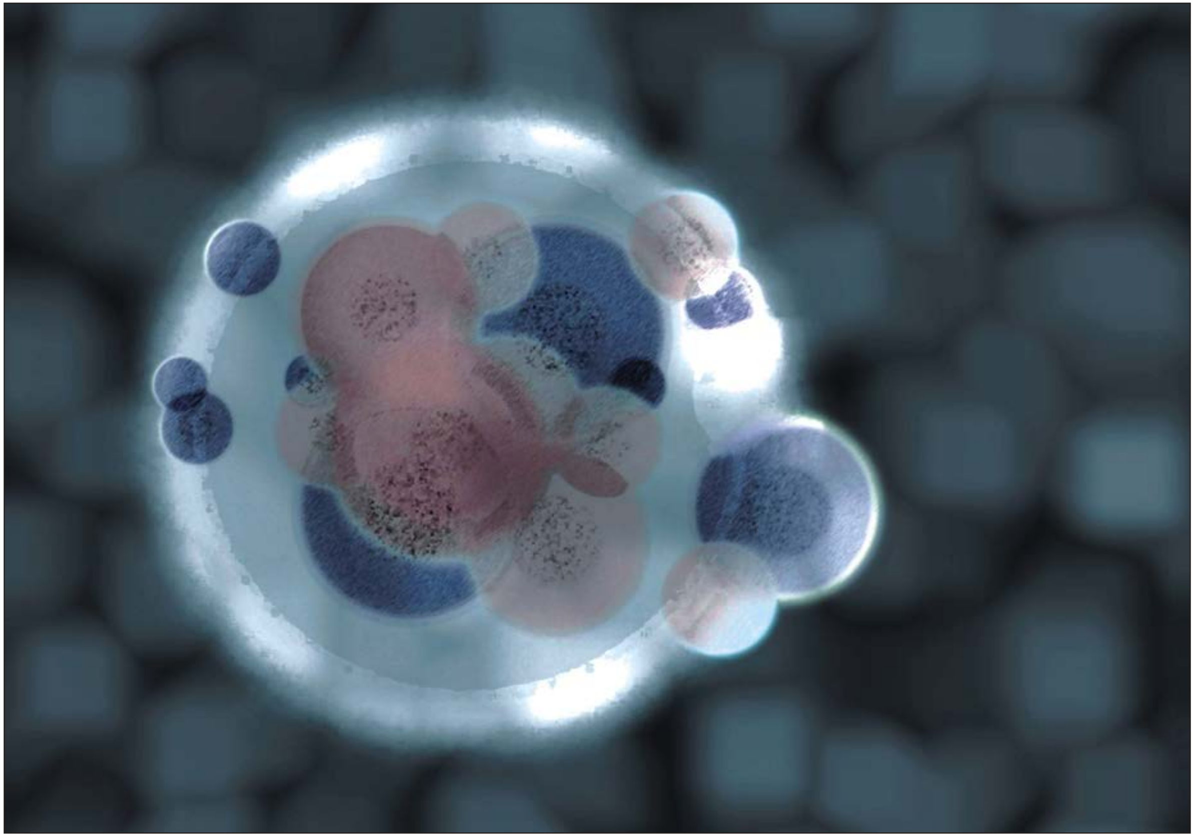

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Hepatoprotective and Antioxidant Effect of *Andrographis echioides* N. against Acetaminophen Induced Hepatotoxicity in Rats

[1]S.K. Basu, [2]M. Rupeshkumar and [3]K. Kavitha
[1]Department of Pharmaceutical Technology, Jadavpur University, Kolkatta-700 032, India
[2]Department of Pharmacology, Bharathi College of Pharmacy, Bharathi Nagara, Mandya Dist., Karnataka-571 422, India
[3]Department of Pharmaceutics, Bharathi College of Pharmacy, Bharathi Nagara, Mandya Dist., Karnataka-571 422, India

Abstract: In this study, the methanolic extract of *Andrographis echioides* (MEAE) was investigated for its hepatoprotective and antioxidant effects against acetaminophen induced hepatotoxicity in Wistar albino rats. The plant extract (200 and 400 mg kg^{-1}, p.o./day for 10 days) showed a remarkable hepatoprotective and antioxidant activity. Hepatotoxicity was induced by acetaminophen at the dose of 750 mg kg^{-1} p.o. for 10 days. The serum marker enzymes such as aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphates (ALP), total bilirubin and liver gamma glutamate transpeptidase (GGTP), lipid peroxidase (LPO) were significantly increased with a reduction of liver total protein, superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glutathione-S-transferase (GST) in acetaminophen induced rats. Treatment of rats with different doses of plant extract (200 and 400 mg kg^{-1}, p.o.) significantly ($p<0.001$) altered serum marker enzymes and antioxidant levels to near normal against acetaminophen-treated rats. The activity of the extract at the different dose was comparable to the standard drug, silymarin (50 mg kg^{-1}, p.o.). Extensive vascular degenerative changes and centrilobular necrosis in hepatocytes was produced by acetaminophen. Treatment with different doses of aerial parts of methanol extract of *A. echioides* produced only mild degenerative changes and absence of centrilobular necrosis, indicating its hepatoprotective efficiency. Results indicate that *A. echioides* possesses hepatoprotective and antioxidant effects against acetaminophen induced hepatotoxicity in rats. Thus, the study substantiates the hepatoprotective and antioxidant potential of methanol extract of *A. echioides*.

Key words: *Andrographis echioides*, acetaminophen, biochemical parameters, antioxidants, lipid peroxidation, histopathology

INTRODUCTION

Antioxidants are substances that when present in foods or body at low concentrations may help the body to protect itself against various types of oxidative damage caused by reactive oxygen species (Sanchez-Moreno *et al*., 1999). Liver, the key organ of metabolism and excretion has an immense task of detoxification of xenobiotics, environmental pollutants and chemotherapeutic agents. Acute liver failure is caused by a variety of insults, including viral hepatitis, toxic liver damage by poisons or drugs and ischemia. The liver is the first line of protection against damage by ingested agents, including xenobiotics. Hepatic injury by these agents frequently results in both hepatic necrosis. Oxidative damage plays a prominent role in hepatic injury mediated by drugs and poison, whereas viral hepatitis and immune-mediated liver damage are believed to occur largely via activation of the Fas apoptotic death pathway (Rebecca, 2003).

Andrographis echioides N. (Acanthaceae) is a procumbent annual herb, distributed throughout India. The plant is stated to have properties similar to those of *Andrographis panniculata* (Anonymous, 2003). The herb is useful for the treatment of dysentery, diabetes, swellings and also used for liver diseases and jaundice (Nadkarni, 2005). However, there are no scientific reports regarding the pharmacological activities of *A. echioides*. The aim of the present study was to investigate the antioxidant and hepatoprotective properties of methanolic extract of the aerial parts of *A. echioides* against acetaminophen induced hepatotoxicity in rats and also to evaluate its medicinal value and to point to an easily accessible source of natural antioxidants and hepatoprotectives that could be used as a possible food supplement or in the pharmaceutical industry.

Corresponding Author: M. Rupeshkumar, Department of Pharmacology, Bharathi College of Pharmacy, Bharathi Nagara, Mandya Dist., Karnataka-571 422, India Tel: +91-9980002265 Fax: +91-8232-235111

MATERIALS AND METHODS

Chemicals: Silymarin was purchased from Micro Labs, India, 1-chloro-2, 4-dinitrobenzoic acid (CDNB), 5, 5-dithiobis (2-nitrobenzoic acid) (DTNB), reduced glutathione (GSH) and glutathione were purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. Thiobarbuturic acid was purchased from E-Merck, India. All other chemical used were of analytical grade.

Collection and extraction: The fresh aerial parts of *A. echioides* were collected from SKM Siddha and Ayurvedic Medicines India Pvt. Ltd., Erode Dist., Tamilnadu, India, in the month of August 2006 and identified by GVS Murthy, Botanical Survey of India, Coimbatore, India. A voucher specimen has been deposited in the laboratory for future reference (BSI/SC/5/23/06-07/TECH.835).

The aerial parts of the plant were shade dried and pulverized. The powder was defatted with petroleum ether. Later, it was subjected to continuous hot extraction with methanol in a Soxhlet apparatus. The extract was concentrated under vacuum and dried in a desiccator (yield 68 g, 6.8% w/w). Aliquot portions of the crude extract were suspended in 5% gum acacia for use on each day of present experiment. The preliminary phytochemcial analysis was performed by Wagner *et al.* (1984).

Animals: Swiss albino mice (20-25 g) and male Wister rats (150-200 g) were procured from Venkateshwara Enterprises, Bangalore, Karnataka, India and used throughout the study. They were housed in microlon boxes in a controlled environment (temperature 25±2°C and 12 h dark/light cycle) with standard laboratory diet and water *ad libitum*. The study was conducted after obtaining Institutional Animal Ethics Committee Clearance.

Acute toxicity studies: Acute toxicity studies were performed according to OECD-423 guidelines (Ecobichon, 1997). Male Swiss mice selected by random sampling technique were employed in this study. The animals were fasted for 4 h with free access to water only. The plant extract was administered orally at a dose of 5 mg kg^{-1} initially and mortality if any was observed for three days. If mortality was observed in two out of three animals, then the dose administered was considered as toxic dose. However, if the mortality was observed in only one animal out of three animals then the same dose was repeated again to confirm the toxic effect. If no mortality was observed, then higher (50, 300 and 2000 mg kg^{-1}) doses of extract were employed for further toxicity studies.

Hepatoprotective activity: Rats were divided into 5 groups, each group consisting of six animals.

- **Group I:** Untreated control received the vehicle (normal saline, 2 mL kg^{-1}, p.o.)
- **Group II:** Received acetaminophen (750 mg kg^{-1} p.o.) at every 72 h for a period of 10 days (Araya *et al.*, 1987)
- **Group III:** Received silymarin at a dose of 50 mg kg^{-1} p.o. at every 72 h for 10 days and simultaneously administered acetaminophen 750 mg kg^{-1} body wt.
- **Group IV:** Received methanol extract of *A. echioides* 200 mg kg^{-1} p.o. at every 72 h for 10 days and simultaneously administered acetaminophen 750 mg kg^{-1} body wt.
- **Group V:** Received methanol extract of *A. echioides* 400 mg kg^{-1} p.o. at every 72 h for 10 days and simultaneously administered acetaminophen 750 mg kg^{-1} body wt.

At the end of experimental period, all the animals were sacrificed by cervical decapitation. Blood samples were collected by retro-orbital puncture allowed to clot. Serum was separated by centrifuge at 2500 rpm for 15 min and analyzed for various biochemical parameters such as aspartate amino transferase (AST), alanine amino transferase (ALT) (Reitman and Frankel, 1957), alkaline phosphatase (ALP) (Kind and King, 1954), γ-glutamate transpeptidase (GGTP) (Szaszi, 1969), total bilirubin (Malloy and Evelyn, 1937) and total protein (Lowery *et al.*, 1951).

Assessment of liver function: The liver was removed, rinsed in ice cold normal saline followed by 0.15 M Tris-HCl buffer (pH 7.4) blotted dry and weighed. A 10% of liver homogenate was prepared in 0.15 M Tris-HCl buffer and used for antioxidant studies such as lipid peroxidation (LPO) (Devasagayam and Tarachand, 1987), superoxide dismutase (SOD) (Marklund and Marklund, 1974), catalase (Sinha, 1972), glutathione peroxidase (GPx) (Rotruck *et al.*, 1973) and glutathione-S-transferase (GST) (Habig *et al.*, 1974). A portion of liver was fixed in 10% formalin for histopathological studies.

Histopathological studies: After draining the blood and liver samples were excised, washed with normal saline and processed separately for histopathological observation. Initially the materials were fixed in 10% buffered neutral formalin for 48 h and then with bovine solution for 6 h. Paraffin sections were taken at 5 mm thickness processed in alcohol-xylene series and was stained with alum hematoxylin and eosin. The sections were examined microscopically for histopathology changes.

Statistical analysis: The values were expressed as Mean±SEM. Statistical analysis was performed by

one way analysis of variance (ANOVA) followed by Tukey multiple comparison tests and $p<0.05$ were considered as significant.

RESULTS AND DISCUSSION

Preliminary phytochemical studies revealed the presence of alkaloids, steroids, saponins, triterpenes flavonoids and polyphenolic compounds. For the acute toxicity studies, the extract treated rats were observed for mortality up to 72 h. Based on the results the extract did not produce any mortality up to 2000 mg kg^{-1} b.wt. The animals did not showed any CNS depressant activity.

The levels of serum AST, ALT, ALP, total bilirubin and GGTP were markedly elevated and that of protein decreased in acetaminophen treated animals, indicating liver damage (Table 1). Administration of *A. echioides* extract at the doses of 200 and 400 mg kg^{-1} remarkably prevented acetaminophen-induced hepatotoxicity in a dose dependent manner.

The localization of radical formation resulting in lipid peroxidation, measured as Malondialdehyde (MDA) contents in rat liver homogenate. MDA were increased in acetaminophen control group. MEAE 200 and 400 mg kg^{-1} were significantly inhibited MDA level when compared to acetaminophen induced hepatic damage. The effect of *A. echioides* was comparable with that of standard drug silymarin.

Acetaminophen treatment caused a significant ($p<0.001$) decrease in the level of SOD, catalase, GPx and GST in liver tissue when compared with normal group (Table 2). The treatment of *A. echioides* at the doses of 200 and 400 mg kg^{-1} resulted in a significant increase of SOD, Catalase, GPx and GST when compared to. acetaminophen treated rats. The liver of silymarin treated animals also showed a significant increase in antioxidant enzymes levels compared to acetaminophen treated rats. Morphological observations showed an increased size and enlargement of the liver in acetaminophen treated groups. These changes were reversed by treatment with silymarin and also *A. echioides* at the doses tested (Fig. 1). Histopathological studies, showed acetaminophen to produce extensive vascular degenerative changes and centrilobular necrosis in hepatocytes. Treatment with different doses of *A. echioides* extract produced mild degenerative changes and absence of centrilobular necrosis when compared with control (Fig. 2). All these results indicate a hepatoprotective potential of the extract.

The present study documented the hepatoprotective activity for the methanolic extract of *A. echioides* against liver injury induced by a toxic dose of acetaminophen. It is established that following an oral therapeutic dose, a fraction of acetaminophen is converted via the cytochrome P450 to a highly toxic metabolite, N-acetyl-p-benzoquinone-imine (NAPQI) (Dahlin *et al*., 1984) which is normally conjugated with glutathione and excreted in the urine as conjugates. Overdose of acetaminophen deplete glutathione stores, leading to accumulation of NAPQI, mitochondrial dysfunction (Parmar *et al*., 1995) and the development of acute hepatic necrosis. Also depletion of glutathione enhances the expression of Tumor Necrosis Factor alpha (TNFα) (Agarwal and Piersco, 1994). TNFα primes phagocytic NADPH oxidase to the enhanced production of oxygen free radicals and contributes to liver damage (Gupta *et al*., 1992). Studies demonstrated that

Table 1: Effect of *A. echioides* on biochemical parameters in Acetaminophen-induced hepatotoxicity in rats

Treatment	Dose (mg kg^{-1})	AST (U L^{-1})	ALT (U L^{-1})	ALP (U L^{-1})	Total bilirubin mg (%)	Total protein mg (%)	GGTP (U L^{-1})
Untreated control	-	157.7±4.60	74.20±2.92	188.4±3.16	0.80±0.05	8.13±1.40	26.01±1.10
Acetaminophen alone	750	227.5±6.80*	176.00±4.7*	578.0±8.90*	1.10±0.08**	6.35±0.35	62.10±2.48*
Silymarin+acetaminophen	50	151.4±6.63*	89.20±3.6*	228.4±5.42***,*	0.72±0.03*	8.12±0.56	35.30±1.78*
A. echioides+acetaminophen	200	217.5±4.64*	122.00±2.5*,*	414.0±12.89*,*	0.65±0.06*	9.22±0.31	39.60±1.08**,*
A. echioides+acetaminophen	400	184.3±8.37**	96.70±55.1**,*	381.8±8.76*,*	0.70±0.04*	8.90±0.60	31.50±3.61*

N = 6 in each group. Values are expressed as Mean±SEM. *p<0.001; **p<0.05; ***p<0.01 vs. normal; *p<0.001 vs. Acetaminophen treated group

Table 2: Effect of *A.echioides* on antioxidants level in Acetaminophen-induced hepatotoxicity in rat

Treatment	Dose (mg kg^{-1})	LPO	SOD	Catalase	GPx	GST
Untreated control	-	7.85±0.92	24.61±1.68	51.29±1.67	38.75±1.96	0.38±0.05
Acetaminophen alone	750	17.17±1.14*	238.05±7.61*	66.17±2.10*	46.82±2.10	0.09±0.02*
Silymarin+acetaminophen	50	11.01±0.87*	25.36±1.58*	48.29±1.92*	33.14±1.45*	0.25±0.03*
A. echioides+acetaminophen	200	5.63±0.70*	134.24±7.3*,*	36.24±2.10**,*	28.52±2.45*	0.10±0.01*
A. echioides+acetaminophen	400	7.51±1.38*	23.48±1.04	46.85±1.97**,*	37.79±3.88	0.39±0.01*

N = 6 in each group. Values are expressed as Mean±SEM. *p<0.001; **p<0.05; ***p<0.01 vs. normal; *p<0.001 vs. Acetaminophen LPO : µmoles of MDA/min/mg protein SOD: Units/min/mg protein; CAT: µmole of H202 consumed/min/mg protein; GPx: Moles of GSH oxidized/min/mg protein; GST: µmoles of CDNB conjugation formed/min/mg protein

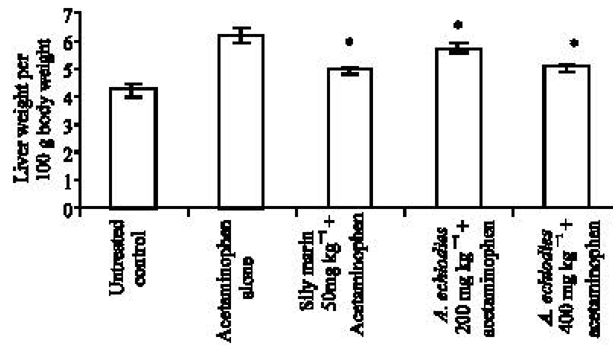

Fig. 1: Effect of *A. echioides* on liver weight variation of acetominophen induced hepatotoxicity in rats N = 6. Values are expressed as Mean±SEM. $*p \le 0.001$ vs. Acetaminophen

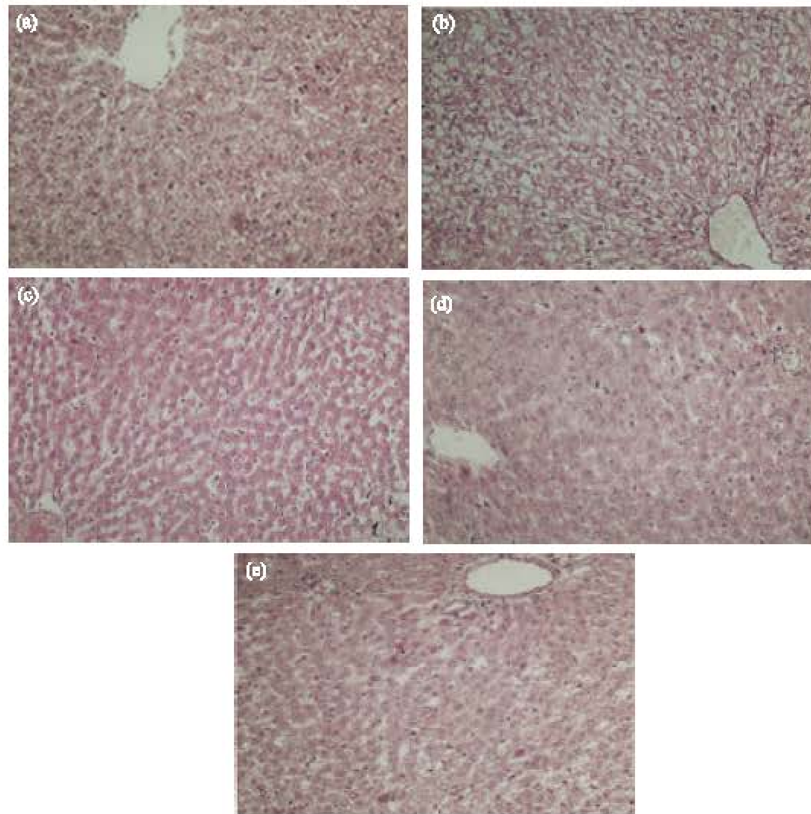

Fig. 2: Effect of *A.echioides* on acetaminophen induced liver damage in rat. (a) Liver from rat treated with saline shows normal cellular architecture with distinct hepatic cells, sinusoidal space and a central vein. (b) Liver from rat treated with acetaminophen exhibited severe hepatocyte degeneration and necrosis. (c) Liver treated with silymarin (50 mg kg^{-1}, p.o.) plus acetaminophen shows normal architecture with mild hepatocyte degeneration. (d) Liver treated with *A. echioides* (200 mg kg^{-1}, p.o.) plus acetaminophen shows mild hepatocyte degeneration. (e) Liver treated with *A. echioides* (400 mg kg^{-1}, p.o.) plus acetaminophen shows hepatocyte degeneration

acetaminophen induced hepatotoxicity can be modulated by substances that influence cytochrome P450 activity (Mitchell *et al*., 1973). This is evidenced by an elevation in the serum marker enzymes namely AST, ALT, ALP, total bilirubin, GGTP and decrease in protein.

In the assessment of liver damage by acetaminophen the determination of enzyme levels such as AST, ALT is largely used. Necrosis or membrane damage releases the enzyme in to circulation and hence, it can be measured in serum. High levels of AST indicates liver damage, such

as that caused by viral hepatitis as well as cardiac infarction and muscle injury. AST catalyses the conversion of alanine to pyruvate and glutamate and is released in a similar manner. Therefore ALT is more specific to the liver and is thus a better parameter for detecting liver injury. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver (Drotman and Lawhan, 1978). Serum ALP, Bilirubin and total protein levels on other hand are related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis, in presence of increasing biliary pressure (Muriel *et al*., 1992).

Administration of acetaminophen caused a significant ($p<0.001$) elevation of serum marker enzyme levels such as AST, ALT, GGTP, total bilirubin and decrease in total protein when compared to control. *A. echioides* was significantly ($p<0.001$) restored these biomarker enzyme levels in a dose dependent manner. The reversal of increased serum enzymes in acetaminophen induced liver damage by the extract may be due to the prevention of the leakage of intracellular enzymes by stabilizing structural integrity of the membrane or regeneration of damaged liver cells. This an agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes (Thabrew and Joice, 1987). Effective control of ALP, bilirubin and total protein levels points towards an early improvement in the secretary mechanism of the hepatic cells.

The increase in LPO level in liver induced by acetaminophen suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive free radicals. Treatment with *A. echioides* significantly reverses these changes. Decrease in enzyme activity of superoxide dismutase (SOD) is a sensitive index in hepatocellular damage and is the most sensitive enzymatic index in liver injury (Curtis *et al*., 1972). SOD has been reported as one of the most important enzymes in the enzymatic antioxidant defense system. It scavenges the superoxide anion to form hydrogen peroxide and thus diminishing the toxic effect caused by this radical. In *A. echioides* causes a significant increase in hepatic SOD activity and thus reduces reactive free radical induced oxidative damage to liver.

Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues, and the highest activity is found in the red cells and liver. CAT decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals (Chance *et al*., 1952). Therefore, reduction in the activity of CAT may result in a number of deleterious effects due to the assimilation of superoxide radical and hydrogen peroxide. A higher dose (400 mg kg^{-1}) increases the level of CAT as produced by silymarin, the standard hepatoprotective drug.

Glutathione is one of the most abundant tripeptide, non-enzymatic biological antioxidant present in the liver. It removes free radical species such as hydrogen peroxide, superoxide radicals and maintains membrane protein thiols. Also it is substrate for glutathione peroxidase (Gpx) (Prakash *et al*., 2001). Decreased level of GSH is associated with an enhanced lipid peroxidation in acetaminophen treated rats. Administration of *A. echioides* significantly ($p<0.001$) increased the level of GPx and GST in a dose dependent manner.

Extensive vascular degenerative changes and centrilobular necrosis in hepatocytes was produced by acetaminophen. Treatment with different doses of aerial parts of methanol extract of *A. echioides* produced only mild degenerative changes and absence of centrilobular necrosis, indicating its hepatoprotective efficiency.

Free radical mediated process has been implicated in pathogenesis of most of the diseases. The protective effect of *A. echioides* on acetaminophen induced hepatotoxicity in rats appears to be related to inhibition of lipid peroxidation and enhancement of antioxidant enzyme levels in addition to free radicals scavenging action. Preliminary phytochemical studies reveal the presence of flavanoids in methanolic extract of *A. echioides*. Flavanoids were found to be a good hepatoprotective agents (Wegner and Fintelmann, 1999). From these studies it can be concluded that *A. echioides* possesses good hepatoprotective activity with antioxidant effect and it may be due to the presence of flavanoids. Further studies are necessary to isolate and characterize the active principles and to find out the exact mechanism.

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