Biosciences, Biotechnology Research Asia Vol. **6**(1), 341-350 (2009)

In vitro hepatoprotective and antioxidant activities of the leaf ethanolic and aqueous extracts of *Asteracantha longifolia* and *Andrographis paniculata* against lead acetate induced toxicity

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(Received: October 10, 2008; Accepted: November 15, 2008)

ABSTRACT

The present study was carried out to evaluate the *in vitro* hepatoprotective activity of 90% ethanolic and aqueous extracts of leafs of *Asteracantha longifolia and Andrographis paniculata* against lead acetate induced hepatic damage. In addition, antioxidant activity was also studied in the aqueous and ethanolic leaf extracts of *Asteracantha longifolia and Andrographis paniculata*. The hepatoprotective property of aqueous and ethanolic extracts of Asteracantha longifolia and Andrographis paniculata was investigated by using lead acetate to induce toxicity. The extent of lipid peroxidation (LPO), Superoxide dismutase (SOD), Catalase (CAT), Aspartate transaminase (AST), Alanine transaminase (ALT, Acid phosphatase (ACP), Alkaline phosphatase (ALP) and Lactate dehydrogenase (LDH) were assayed in the liver homogenate. In addition the antioxidant property of aqueous and ethanolic extract was checked by assaying the activities of enzymatic antioxidants and free radical scavenging activity. The activities of liver marker enzymes Aspartate transaminase, Alanine transaminase, Acid phospatase, Alkaline phosphatase, Lactate dehydrogenase and the levels of lipid peroxidation was found to be increased during the lead acetate toxicity. Administration of aqueous and ethanolic leaf extracts of *Asteracantha longifolia and Andrographis paniculata* to the lead acetate induced hepatotoxicity reaches to near normal. In addition, the aqueous and ethanolic leaf extracts of *Asteracantha Longifolia and Andrographis paniculata* have potent antioxidant effects against several antioxidants/ROS and capability to induce the invitro antioxidant system. The results indicate that the leafs of *Asteracantha longifolia and Andrographis paniculata* possess hepatoprotective property possibly because of its antioxidant activity.

Key words: *Asteracantha longifolia, Andrographis paniculata*, *In vitro* hepatoprotective, antioxidant, lead acetate toxicity.

INTRODUCTION

Recent times natural products derived from plants are focused in search of new drugs by indicating new modes of pharmacological action. However there is a lack of precise guidelines to study the herbal compunds and till date a very meagre portion of this tremendous potential drug-repertoire has been sientifically screened. Hence there is a real need for scientific based validation of these agents[1]. By viewing the scope and significance of the plant based medicine the present study has been focused to investingate the medicinal potentiality of the plants that belongs to the family acanthaceae.

Andrographis paniculata (family: Acanthaceae) is a much branched erect annual herb. Branches are four angled. Leaves are lance-shaped. Flowers appear in large spreading sparse branches. The constituents found in plants are kalmeghin and andrographolide. The herb is reported to possess astringent, anodyne, antipyretic and useful in dysentery, cholera, diabetes,

bronchitis, swellings and itches, piles and gonorrhea[2].

Asteracantha longifolia (family: Acanthaceae) is a robust, erect, annual herb. The stems are sub-quadrangular with thickened nodes. The leaves are oblanceolate, with a yellow spine in its axil. The flowers are pale, purple blue, densely clustered in axils. The fruits are oblong, glabrous capsules, 4-8 seeded. It grows throughout India. The chemical composition include alkaloids, phytosterol, mucilage and potassium salts. The roots, leaves and seeds have been used in Indian systems of medicine as diuretics and also employed to cure jaundice, dropsy, rheumatism, anasarca and diseases of the urinogenital tract[3,4]. However the antioxidant and invitro hepatoprotective effect of aqueous and ethanolic extracts of *Asteracantha longifolia* and *Andrographis paniculata* against lead acetate (1mm/g) induced toxicity in tissues remains unexplore. Therefore the present study was undertaken to evaluate antioxidant and protective and preventive role against lead induced toxicity.

MATERIAL AND METHODS

Plant material

The whole plant of *Asteracantha longifolia* and *Andrographis paniculata* were collected from Tamilnadu Agricultural University and Institute of Forest Genetics and Tree Breeding, Coimbatore.

Preparation of extracts

The leafs of the plants were taken and shade dried and made in to a coarse powder. About 25g of coarse powder was subjected to successive soxhlet extraction using 90% ethanol and distilled water according to the order of polarity for 48 hours. The solvent was removed under reduced pressure which gave a greenish black coloured sticky residue.

Antioxidant activity

Free radical Scavenging Activity

Super oxide scavenging activities of compounds were determined by the method of Liu and Chang (1997)[5], which depends on the light, induced super oxide generation by riboflavin and the corresponding reduction of Nitro blue terazoluim (NBT).

Hydroxyl radical scavenging activity was measured by studying the competition between deoxy ribose and test compounds for hydroxyl radicals generated from the Fe^{3+}/ascorbate/EDTA/H_2O_2 System of Halliwell *et al*., 1987[6].

Nitric oxide was generated from sodium nitroprusside and measured by Griess reaction. DPPH Radical Scavenging Activity was measured according to the method of Shimada *et al*., 1992[7]. Ferric reducing ability of the plant as measuring of antioxidant power was measurd. Hydrogen Peroxide-Scavenging Activity was determined by the method of Ruch *et al*., 1989[8].

The antioxidant activity of the extracts was evaluated using the β- Carotene-Linoleate Model System (Miller, 1971)[9].

Enzymatic antioxidants

Activity of catalase was determined by the method of Luck, 1974. Peroxidase activity was determined by the method of Reddy *et al*., 1995. Polyphenoloxidase was estimated by the method of Esterbaur *et al*., 1997.

Evaluation of hepatoprotective activity of plant leaf extracts

About 2.0g of goat liver was weighed and incubated with lead acetate (1.0mM/g) for one hour to induce toxicity.

Experimental Set Up

Group I : Served as control

Group II : Toxicity (Administered lead acetate 1.0mM/g for one hour and Incubated).

Group III : Curative

- Lead Toxicity + Aqueous extracts of *Asteracantha longifolia*
- Lead Toxicity + Ethanolic extracts of *Asteracantha longifolia*
- Lead Toxicity + Aqueous extracts of *Andrographis paniculata*
- Lead Toxicity + Ethanolic extracts of *Andrographis paniculata*

Group IV : Preventive

- Aqueous extracts of *Asteracantha longifolia.*
- Ethanolic extracts of *Asteracantha longifolia.*

Table 1: Free radical scavenging activity of plant leaf extracts

S. No.	Parameters	Asteracantha Longifolia		Andrographis Paniculata	
		Aqueous Extract	Ethanol Extract	Aqueous Extract	Ethanol Extract
1	DPPH	42.69	34.59	54.98	77.44
2	FRAP	400	376	475	505
3	Scavenging of Nitric Oxide radical	41.82	74.21	39.98	75.27
4	Scavenging of hydroxyl radical	76.32	38.13	77.83	47.17
5	Scavenging of Hydrogenperoxide radical	26.49	32.85	31.09	39.98
6	Scavenging of Super oxide radical	41.82	40.81	51.07	49.97
7	B carotene-linoleic acid assay	54.76	47.62	47.62	57.14

Units of free radical scavenging activities are expressed in % of radical scavenged for (DPPH, Nitricoxide radical, Hydroxyl radical, Hydrogen peroxide radical, superoxide radical, β- carotene linoleic acid assay.
FRAP is expressed in units of um.

Table 2 Enzymatic Antioxidant Activity of Plant Leaf Extracts

S. No.	Enzymatic antioxidants		Asteracantha Longifolia		Andrographis Paniculata	
			Aqueous Extract	Ethanol Extract	Aqueous Extract	Ethanol Extract
1.	Catalase		39.4	32.93	34.47	52.25
2	Polyphenol	Catechol	1.414×10^{-3}	0.6528×10^{-3}	1.694×10^{-3}	1.305×10^{-3}
	oxidase	Laccase	1.258×10^{-3}	0.5808×10^{-3}	1.904×10^{-3}	1.162×10^{-3}
3	Peroxidase		24.5	12.0	22.01	13.0

Units are expressed in %.
Values of Mean ± SD.
Treatment of groups are as follows:
Group I (Control), Group II (Tocicity), Group III (Curative), Group IV (Preventive).

A. Aqueous extract of *Asteracantha longifolia.*
B. Ethanol extract of *Asteracantha longifolia.*
C. Aqueous extract of *Andrographis paniculata.*
D. Ethanol extract of *Andrographis paniculata.*

Enzyme Units are expressed as: ACP – Micromole of phenol; ALP – Micro molar of phenol, LDH – Micro moles of pyruvate, Aminotransferase-micro moles of pyruvate liberated/min/mg protein at 37^{0}C.
Comparisons are made between groups are as follows:

a. Between group I and group II
b. Between group II and group III
c. Between group I and IV.

The symbols represent the statistical significance: * $p < 0.001$ $ pNS

- Aqueous extracts of *Andrographis paniculata.*
- Ethanolic extracts of *Andrographis paniculata.*

At the end of the experimental setup liver was removed and washed with saline two to three times and the liver homogenate was prepared for further enzymatic analysis.

Preparation of Liver Homogenate

A 10% homogenate of the liver tissues were prepared in 0.01M Tris HC1 buffer, pH 7.4 using Teflon homogenizer. The homogenate was centrifuged at 12000g for 30 minutes in a refrigerator high speed centrifuge. The supernatant was used for the assay of enzymes such as ACP[10], AST, ALP[11], LDH[10], SOD[12], CAT[13], LPO[14] and protein[15]. Results of biochemical estimations were reported as mean± DF in each group. The data were subjected by student's t test. $p<0.001$ was considered as statistically significant.

RESULTS AND DISCUSSION

Antioxidant Activity

Free radical scavenging activity

The aqueous and ethanolic extract of *Asteracantha longifolia and Andrographis paniculata* were subjected to screening for their possible antioxidant activity. The complementary test systems namely DPPH free radical scavenging, FRAP, β- Carotene linoleic acid system, Nitric oxide radical scavenging, Hydrogen peroxide radical scavenging system, Hydroxyl radical scavenging systems and superoxide anion scavenging system were used for the analysis of the antioxidant capacity of the aqueous and ethanolic extract extracts of *Asteracantha longifolia and Andrographis paniculata* have potent antioxidant effects against ROS and have the capability to induce the invitro antioxidant systems. The results are tabulated in Table 1.

Enzymes as antioxidants

Table 2 illustrates the percentage inhibition of enzymatic antioxidants generated by aqueous and ethanolic extracts of *Asteracantha longifolia and Andrographis paniculata*.

Table 3: Activities of AST, ALT, ALP, ACP, LDH in the Normal and Experimental Groups

S. No.	Parameters	Group I (Induced	Group II	Group III (Curative)				Group IV (Preventive)			
			Toxicity) (Control)	A	B	C	D	A	B	C	D
1	AST	0.79±0.04	0.99±0.02^{a*}	0.85±0.03	0.86±0.03^{b1*}	0.84±0.03^{b1*}	0.89±0.03^{b1*}	0.71±0.04$^{c1\$}$	0.73±0.04$^{c2\$}$	0.74±0.05$^{c3\$}$	0.73±0.07$^{c4\$}$
2	ALT	0.95±0.07	1.27±0.42^{a*}	1.13±0.04^{b1*}	1.15±0.04^{b2*}	1.14±0.05^{b3*}	1.13±0.05^{b4*}	0.90±0.07$^{c1\$}$	0.89±0.07$^{c2\$}$	0.87±0.05$^{c3\$}$	0.91±0.07$^{c4\$}$
3	ACP	0.97±0.07	1.29±0.02^{a*}	1.14±0.04^{b1*}	1.15±0.054^{b2*}	1.17±0.04^{b3*}	1.19±0.04^{b4*}	0.92±0.07$^{c1\$}$	0.89±0.05$^{c2\$}$	0.87±0.05$^{c3\$}$	0.92±0.08$^{c4\$}$
4	ALP	1.13±0.07	1.41±0.04^{a*}	1.25±0.05^{b1*}	1.23±0.05^{b2*}	1.24±0.05^{b3*}	1.27±0.05^{b4*}	0.99±0.07$^{c1\$}$	1.02±0.07$^{c2\$}$	1.07±0.08$^{c3\$}$	1.10±0.08$^{c4\$}$
5	LDH	0.98±0.07	1.32±0.03^{a*}	1.10±0.05^{b1*}	1.13±0.05^{b2*}	1.18±0.05^{b3*}	1.16±0.07^{b4*}	0.93±0.06$^{c1\$}$	0.89±0.07$^{c2\$}$	0.92±0.07$^{c3\$}$	0.90±0.07$^{c4\$}$

Table 4: Activities of protein, SOD, Catalase, Lipid Peroxidation in the Normal and Experimental Groups

S. No.	Parameters	Group I (Induced Toxicity) (Control)	Group II	Group III (Curative)				Group IV (Preventive)			
				A	B	C	D	A	B	C	D
1	Protein	1.57±0.06	0.67±0.05^{a*}	0.98±0.04^{b1*}	0.89±0.08^{b2*}	0.99±0.05^{b1*}	1.02±0.08^{b1*}	0.47±0.08$^{c1\$}$	1.46±0.07$^{c2\$}$	1.47±0.05$^{c3\$}$	1.51±0.07$^{c4\$}$
2	SOD	03.92±0.12	1.28±0.11^{a*}	2.04±0.13^{b1*}	2.42±0.14^{b2*}	2.51±0.13^{b3*}	2.41±0.17^{b4*}	3.79±0.1$^{c1\$}$	3.83±0.12$^{c2\$}$	3.82±0.13$^{c3\$}$	3.81±0.12$^{c4\$}$
3	Catalase	25.82±0.91	10.18±0.86^{a*}	17.05±0.87^{b1*}	16.61±0.82^{b2*}	15.59±0.82^{b3*}	18.28±0.81^{b4*}	23.5±0.61$^{c1\$}$	24.10±0.62$^{c2\$}$	23.780±0.63$^{c3\$}$	24.120±0.64$^{c4\$}$
4	LPO	2.85±0.13	4.27±0.21^{a*}	3.22±0.18^{b1*}	3.18±0.17^{b2*}	3.25±0.17^{b3*}	3.21±0.21^{b4*}	2.77±0.21$^{c1\$}$	2.75±0.12$^{c2\$}$	2.79±0.12$^{c3\$}$	2.75±0.12$^{c4\$}$

Values of mean ± SD

Treatment of group are as follows:

Group I (Control), Group II (Toxicity), Group III (Curative), Group IV (Preventive)

A. Aqueous extract of *Asteracantha longifolia.*

B. Ethanol extract of *Asteracantha longifolia.*

C. Aqueous extract of *Andrographis paniculata.*

D. Ethanol extract of *Andrographis paniculata.*

Enzyme Units are expressed as: SOD: Units/mg Protein, Catalase: µ moles of the H_2O_2 consumed/min/mg protein, LPO-Nanomoles of MDA formed / mg protein at 37^{0}C.

Protein – mg/g tissue

Comparisons are made between groups are as follows:

a. Between group I and group II

b. Between group II and group III

c. Between group I and IV.

The symbols represent the statistical significance:

* p <0.001 $ pNS.

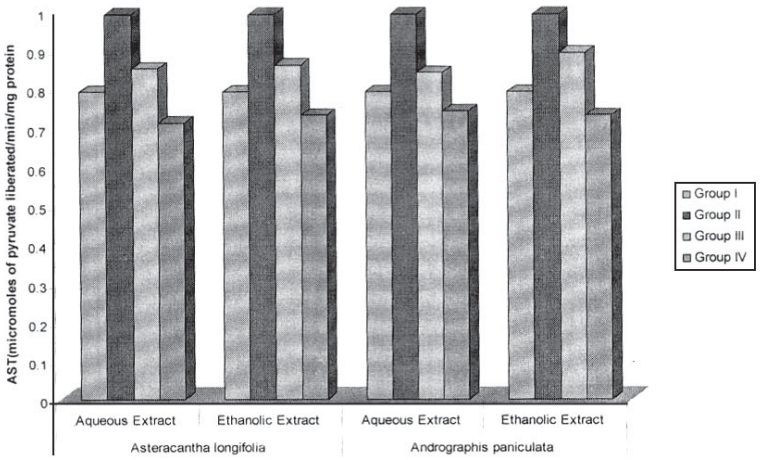

Fig. 1: Levels of AST in the normal and experimental groups

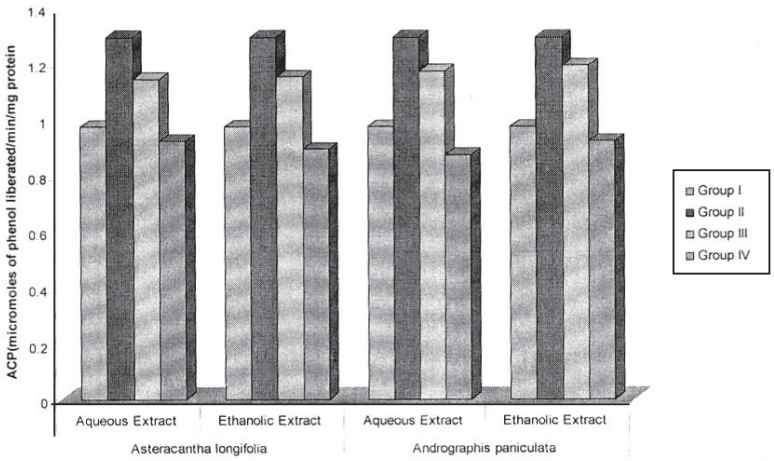

Fig. 2: Levels of ASP in the normal and experimental groups

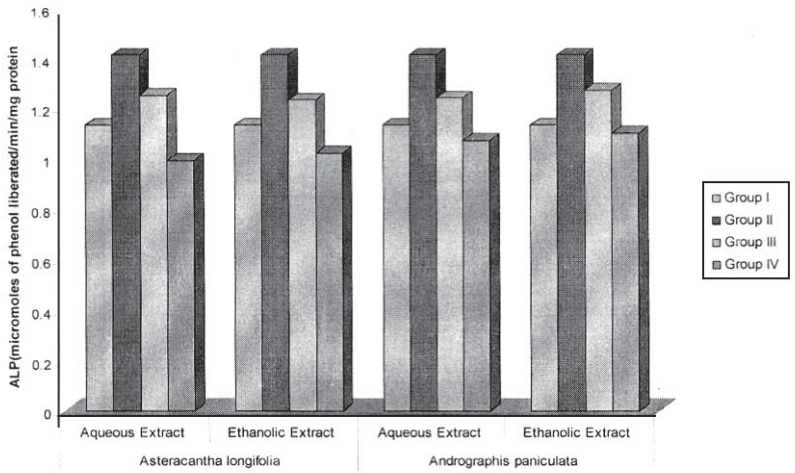

Fig. 3: Levels of ASL in the normal and experimental groups

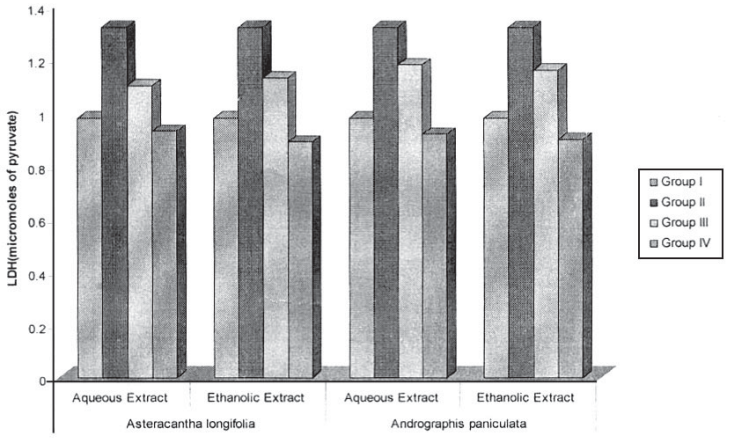

Fig. 4: Levels of LDGH in the normal and experimental groups

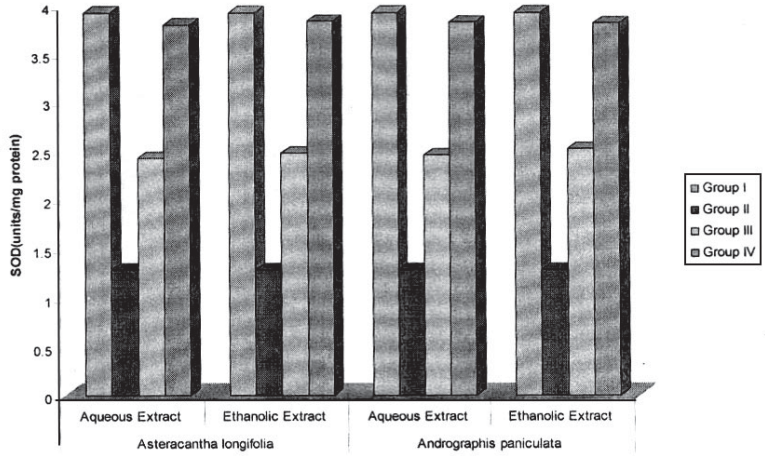

Fig. 5: Levels of SOD in the normal and experimental groups

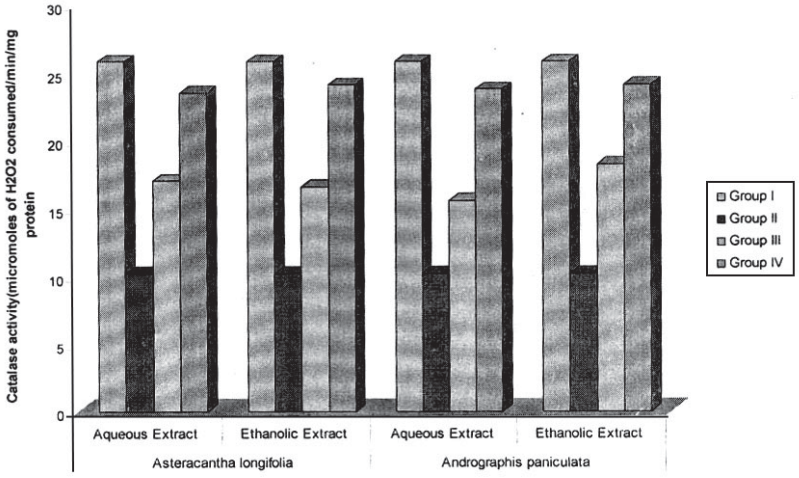

Fig. 6: Levels of Catalases in the normal and experimental groups

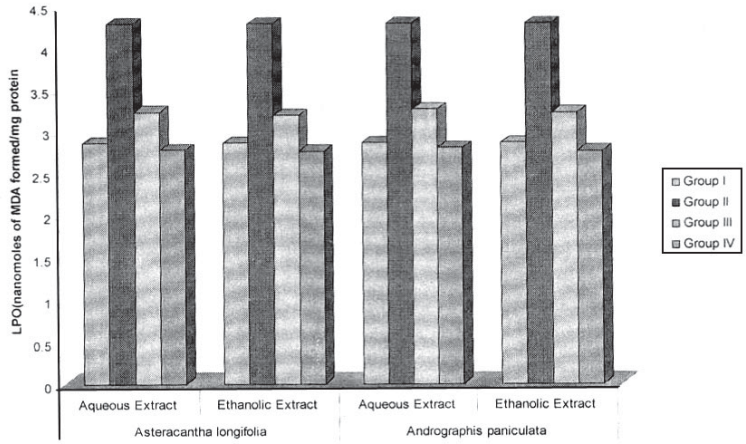

Fig. 7: Levels of LPO in the normal and experimental groups

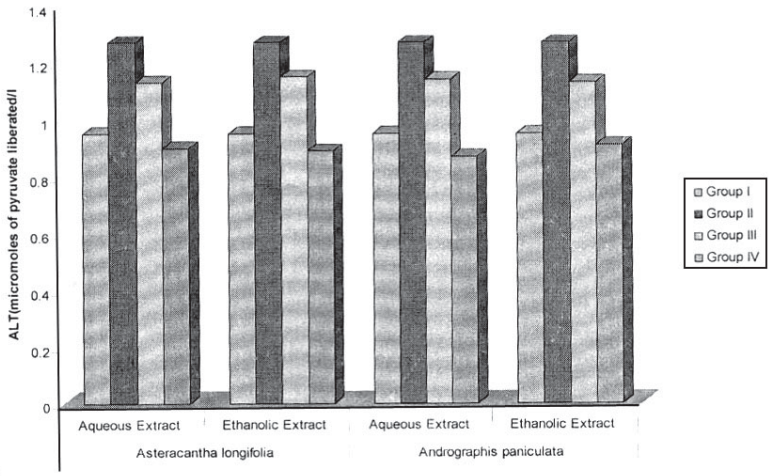

Fig. 8: Levels of ALT in the normal and experimental groups

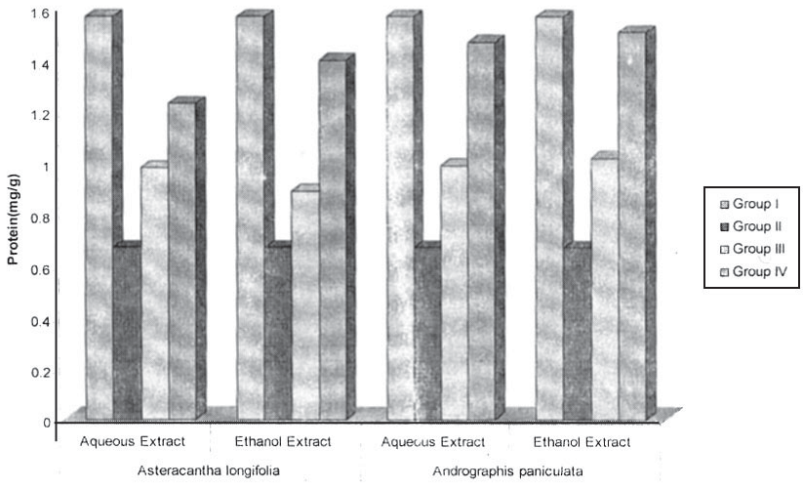

Fig. 9: Levels of protein in the normal and experimental groups

***In vitro* Hepatoprotective Activity**

The activities of liver marker enzymes AST, ALT, ACP, ALP and LDH, and the levels of lipid peroxidation was found to be increased during the lead acetate toxicity p>0.001. The levels of antioxidant enzymes (SOD, Catalase) and protein were found to be decreased during lead acetate toxicity p>0.001. Administration of aqueous and ethanolic leaf extracts of *Asteracantha longifolia and Andrographis paniculata* to the lead acetate induced hepatotoxicity reaches to near normal [Table 3,4]. The statistical analysis indicates that aqueous and ethanolic leaf extracts of *Asteracantha longifolia* and *Andrographis paniculata* possess curative as well as preventive activity against lead induced toxicity t\in liver.

Endogenous antioxidant enzymes (SOD & CAT) are responsible for preventing and neutralizing the free radical induced damages on tissues[16]. Lead induced decrease in SOD and CAT might be due to the formation of reactive oxygen species by lead[17].

A significant increase in the levels of TBARS in animals treated with lead showed the induction of lipid peroxidation by lead. This might be due to the release of free radicals and membrane damage by lead. Lead is reported to release free radicals there by stimulating the process of lipid peroxidation.

Lipid peroxides have been shown to impair tissue membranes which is a risk factor in variety of diseases[18].

The site specific oxidative damage of some of the susceptible amino acids of proteins is now regarded as the major cause of metabolic dysfunction during pathogenesis[19]. The capacity of liver to synthesize albumin is adversely affected by hepatotoxins[20]. The lowered level of total proteins recorded in the present study can be attributed to these features.

Protective agents from plant origin with antiperoxidative and antioxidant properties play an important role in protecting the liver against toxicity[21].

CONCLUSION

The present study demonstrates that the aqueous and ethanolic lead extracts of *Asteracantha longifolia and Andrographis paniculata* possess hepatoprotective property possibly because of its antioxidant activity.

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