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RESEARCH ARTICLE

ANTIOXIDANT AND HEPATOPROTECTIVE ACTIVITY OF *ANDROGRAPHIS PANICULATA* AGAINST ACETAMINOPHEN (PARACETAMOL) INDUCED HEPATOTOXICITY IN ALBINO RATS

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ABSTRACT

The antioxidant and hepatoprotective effect of *Andrographis paniculata* on Acetaminophen (Paracetamol) induced hepatotoxicity inalbino rats was investigated. Oral administration Acetaminophen (Paracetamol)of (250mg/kg body wt.) for 8 days resulted in a significant elevation of SGOT, SGPT,ALP and bilirubin and the levels of lipid peroxidation in liver. Acetaminophen (Paracetamol) also caused a significant reduction in the activities of Superoxide dismutase (SOD), Catalase (CAT) and reduced the Glutathione level in liver. Administration of *A. Paniculata* extract (250 mg /kg body weight and 500 mg/kg body weight.) significantly decreased the serum hepatic marker enzymes viz SGOT,SGPT,ALP and level of bilirubin along with the significant decrease in the levels of lipid peroxidation in the liver. In addition the *A.paniculata* extract significantly increased the activities of SOD, CAT and reduced GSH level in the liver of Acetaminophen (Paracetamol) intoxicated albino rats. Our results demonstrated that the ethanolic extract of *A. Paniculata* exhibited antioxidant and the hepatoprotective property. The results of the hepatoprotective activity of *Andrographis paniculata* extract is comparable to that of the commercially available silymarin preparations

INTRODUCTION

Liver the key organ of metabolism and excretion has an immense task of detoxification of xenobiotics, environmental pollutants and chemotherapeutic agents. Hence, this organ is subjected to a variety of diseases and disorders. Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can serious side effects. Inspite of tremendous advances in allopathic medicine, no effective hepatoprotective medicine is available. Herbal drugs play a vital role in the management of various liver disorders and most of these herbal drugs speed up the natural healing processes of the liver. Numerous medicinal plants and their formulations are used for liver disorders in ethno medical practice as well as in traditional system of medicine in India

The plant *Andrographis paniculata* (Family : Acanthaceae) is a herbaceous plant in the family, native to India and Sri Lanka.It is widely cultivated in Southern and Southeastern Asia, where it is used to treat infections and some diseases, often being used before antibiotics were created. Mostly the leaves and roots were used for medicinal purposes. *Andrographis paniculata* is an erect annual herb extremely bitter in taste in all parts of the plant body. The plant is known in north-eastern India as Maha-tita, literally "king of bitters", and known by various vernacular names *(Kirayat in Hind and Nilavembu, Sirunangai, Siriyanangai in Tamil).* As an Ayurveda herb it is known as *Kalmegh or Kalamegha*, meaning "dark cloud". It is also known as *Bhui-neem,* meaning "neem of the ground", since the plant, though being a small annual herb, has a similar strong bitter taste as that of the large Neem tree (Azadirachta indica. The genus *Andrographis* consists of 28 species of small annual shrubs essentially distributed in tropical Asia. Only a few species are medicinal, of which *A. paniculata* is the most popular. Keeping this in view, the present study has been undertaken to investigate hepatoprotective activity and antioxidant role of the ethanol extract of the plant *Andrographis paniculata* on Acetaminophen (Paracetamol) induced liver damage in albino rats. Specifically it aimed to compare the hepatoprotective activity (i.e.efficacy) of the *Andrographis paniculata* extract with the commercially available preparation (Silymarin capsules).

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MATERIALS AND METHODS

Plant Material

The *Andrographis paniculata* fresh plants were collected from the rural areas of Palani, Dindukal District, Tamilnadu. The plants were water washed cleaned and shade dried and milled into coarse powder by a mechanical grinder.

Preparation of Extract

The coarse powder plant material was extracted with ethanol: water (1:1) by using soxhlet apparatus. The solvent were removed under reduced pressure to get semisolid mass.

Preliminary phytochemical screening of the extract was performed to know the phytochemical constituents in the extract, it was found that the extract contains alkaloid, flavonoides, glycosides, steroid, and tannins

Animals

Healthy Albino rats, weighing between 150-200 g of both sexes were used for the study. The rats were acclimatised to laboratory conditions and fed with pellet food (M/s , Hindustan Lever Limited) and given tap water *ad libitum*. They were housed in standard metal cages.

Study Design

Five groups with 6 rats each were chosen. The period of study was for 8days. Pre-test liver function test were conducted to determine homogeneity of the groups of rat.

Group I	Water only
Group II	Acetaminophen treated (250mg / Kg bodyweight)
Group III	Acetaminophen (250mg/kg) + *Andrographis paniculata* ethanol *extract* (APEE)250 mg/kg body weight of animal
Group IV	Acetaminophen (250mg/kg) + *Andrographis paniculata* ethanol *(APEE)* 500 mg/kg body weight of animal
Group V	Acetaminophen (250mg/kg) + Silymarin 100 mg/kg body weight of animal

All treatments were given orally by means of oral gavage. On the 8th day all animals were sacrificed for experimental studies.

Biochemical studies

The blood was obtained from all animals by puncturing retro-orbital plexus. The blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500rpm at 30^{0}C for 15 min and the estimation of various biochemical parameters were performed. After collection of blood samples the rats in different groups were sacrificed and their livers were excised immediately and washed in ice cold normal saline, followed by 0.15 M Tris-Hcl (pH 7.4) blotted dry and weighed. A 10%w/v of homogenate was prepared in 0.15 M Tris-Hcl buffer and processed for the estimation of Lipid peroxidation. A part of homogenate after precipitating proteins with Trichloroacetic acid (TCA) was used for estimation of Glutathione. The rest of the homogenate was centrifuged at 1500 rpm for 15 min at 40 ^{C}. The supernatant thus obtained was used for estimation of SOD and CAT activities

Serum hepatospecific markers

SGOT and SGPT

Activities of Serum Glutamate Oxaloacetate Transaminase (SGOT) and Serum Glutamate Pyruvate Transaminase (SGPT) were estimated by following the Method of Reitman, *et al.,* 1957. 0.05 ml of serum with 0.25 ml of substrate (aspartate and α-ketoglutarate for SGOT; alanine and α - keto glutarate for SGPT, in phosphate buffer pH 7.4) was incubated for an hour and 30 min in case of SGOT and SGPT respectively. 0.25 ml of DNPH solution was added to arrest the reaction and kept for 20 min in room temperature. After incubation 1 ml of 0.4N Sodium hydroxide was added and absorbance was read at 505 nm in uv-vis spectrophotometer. Activities were expressed as IU/L.

Alkaline phosphatase

Based on the method King and Armstrong 1934..Alkaline Phosphatase activity was assayed with disodium phenyl phosphate as substrate. The colour developed was read at 510 nm in uv-vis spectrophotometer after 10 min. Activities of ALP was expressed as IU/L.

Serum total bilirubin

Serum total bilirubin level was estimated by Diazotised sulphonilic acid - method of Malloy and Evelyn1937. 0.1 ml serum + 0.9 ml distilled water allowed to react and forms purple colored azobilirubin, which was measured at 540 nm in uv-vis spectrophotometer. Activities of total bilirubin were expressed as mg/dl.

Serum Total Protein

Serum total protein level was estimated based on the method of Lowry's *et al* 1951 1.0 ml the reagent was allowed to reacts with serum (10 µL) and the colour developed was read at 600 nm in uv-vis spectrophotometer. Activities of total protein was expressed as mg/dl.

Determination of Thiobarbituric Acid Reactive Substances (TBARS)

Lipid peroxidations in liver tissues were estimated colorimetrically by measuring thiobarbituric acid reactive substances (TBARS) by the method of Ohkawa *et al.*,1979. To 0.2ml of sample, 0.2ml of 8.1% Sodium dodecyl sulfate, 1.5 ml of 20% acetic acid and 1.5 ml of 0.8% TBA were added. The volume of the mixture was made up to 4ml with distilled water and then heated at 95^{0}C in a water bath for 60 min. After incubation the tubes were cooled to room temperature and the final volume was made upto 5 ml in each tube. Then 5 ml of n-butanol: Pyridine mixture was added and the contents were vortexed thoroughly for 2 min. After centrifugation at 3000 rpm for 10 min the upper organic layer was taken and its OD was read at 532 nm against an appropriate blank without the sample. The levels of lipid peroxides were expressed as milimoles of thiobarbituric acid reactive substances (TBARS)/100gram of liver tissue using an extinction co-efficient of 1.56x10 5M-1cm-1.

Determination of reduced glutathione (GSH)

Reduced glutathione (GSH) was determined by the method of Ellman 1959. To 0.1 ml of different tissue homogenate 2.4 ml of 0.02M EDTA solution was added and kept on ice bath for 10 min. Then 2 ml of distilled water and 0.5 ml of 50 % TCA were added. This mixture was kept on ice for 10-15 min and then centrifuged at 3000 rpm for 15 min. 1 ml of supernatant was taken and 2ml of Tris-Hcl buffer was added. Then 0.05 ml

of DTNB solution (Ellman's reagent) was added and vortexed thoroughly. OD was read (within 2-3min after the addition of DTNB) at 412 nm against a reagent blank. Absorbance values were compared with a standard curve generated from known GSH.

Super oxide dismutase activity (SOD)

Superoxide dismutase (SOD) activity was determined by the method of Kakkar *et al., 1984*. The assay mixture contained 0.1ml of sample, 1.2ml of sodium pyrophosphate buffer (pH 8.3, 0.052 M), 0.1 ml of phenazine methosulphate (186µm), 0.3 ml of nitro blue tetrazolium (300µ m), 0.2 ml of NADH (750 µm). Reaction was started by addition of NADH. After incubation at 300 C for 90 sec, the reaction was stopped by the addition of 0.1 ml of glacial acetic acid. The reaction mixture was stirred vigorously with 4.0 ml of nbutanol. The mixture was allowed to stand for 10 min, centrifuged and butanol layer was separated. The colour intensity of the chromogen in butanol layer was measured at 560 nm against n-butanol and concentration of SOD was expressed as units/g of liver tissue. Absorbance values were compared with a standard curve generated from known SOD.

Assay of Catalase activity (CAT)

Catalase was assayed according to the method of Aebi*1974*. The estimation was done spectrophotometrically following the decrease in absorbance at 230 nm. The liver tissue was homogenized in M/150 phosphate buffer (pH 7.0) at 1-40 C and centrifuged at 5000 rpm. The reaction mixture contained 0.01 M phosphate buffer (pH 7.0), 2 mM H_2O_2 and the enzyme extract. The specific activity of catalase was expressed in terms of units/gram of liver tissue. Absorbance values were compared with a standard curve generated from known CAT.

Statistical Analysis

The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnet's 't' - test. P values <0.05 were considered significant.

RESULTS

The Preliminary phytochemical studies revealed the presence of phenolic compound and flavonoids in ethanolic extracts of *Andrographis paniculata*. (Table 1).

Table 1. Phytochemical Qualitative Analysis

Constituents	Qualitative Detection
1. Alkaloid	Present
2. Flavanoids	Present
3. Glycosides	Present
4. Steroid	Present
5. Tannin	Present

The results of hepatoprotective activity of ethanolic extract of *Andrographis paniculata* on Acetaminophen treated rats are shown in Table 2 and 3. The hepatic enzymes SGOT,SGPT, ALP and bilirubin in serum was significantly (P <0.001) increased in Acetaminophen treated animals when compared to control. The Ethanol extract of *Andrographis paniculata* treatments significantly (P < 0.01) reversed the levels of SGPT, ALP and bilirubin, (P < 0.01) and ALT(P<0.001) when compared to Acetaminophen alone treated rats. Silymarin (100 mg/kg) treated animals also showed significant decrease in SGOT (P<0.01), SGOT, ALP and bilirubin (P<0.001) levels when compared Acetaminophen alone treated rats. The toxic effect of Acetaminophen was controlled in the animals treated with ethanol extracts of *Andrographis paniculata* (250 mg/kg) by way of restoration of the levels of the liver function, whereas at the concentration of 500mg/kg, drug effectively prevented the Acetaminophen induced liver damage. Acetaminophen treatment significantly increased the serum liver enzyme levels, viz., SGOT,SGPT and ALP. The activity of SGOT (199 ±0.62IU/l), SGPT (241±0.46± IU/l) and ALP (368±0.6IU/l) was significantly higher (P<0.05) in Acetaminophen treated group in Comparison to normal control (SGOT 23.9 ±0.61; SGPT 32.5±7.5; ALP 153.7±12IU/l) indicating a marked hepatocellular injury (Table2).

Acetaminophen treatment group resulted in an increase in the lipid peroxide levels in liver homogenates. Administration of ethanol extract of of *Andrographis paniculata* prevented the accumulation of lipid peroxides. Administration of ethanolic extract of *Andrographis paniculata* (250mg/kg) there was a marginal effect in the lipid peroxide level where as at higher dose (500mg/kg) the drug effectively prevented Acetaminophen – induced elevation of lipid peroxides in liver (Table2).Histological studies also confirmed the hepatoprotective effect of ethanolic extract of of *Andrographis paniculata*. Acetaminophen treated rat liver sections showed cloudy swelling and fatty degeneration of hepatocytes, necrosis of cells were also seen . The drug treatment (250mg/kg and 500mg/kg ethanol extract) almost normalized these effects in the histoarchitecture of liver.

The Acetaminophen induced liver injury was delaying the metabolism of barbiturates, thereby, slowing their excretion rate and leading to an increase in the duration of barbiturate induced hypnosis ((from 9.78 ± 0.12 min in normal control to 15.5 ±0.22 min in Acetaminophen induced hepatotoxicity group; P<0.05) (Table 3). With Acetaminophen treatment, the mean liver weight was increased to 11.8±0.5 g per cent of body weight from that of normal control (5.82±0.18%), which was found to be statistically significant. The low dose of 250mg/Kg with *Andrographis paniculata* extract also resulted in an slight increase in the mean liver weight as compared to normal, which was statistically significant in the *Andrographis paniculata* treated groups.

DISCUSSION

Paracetamol is a known antipyretic and an analgesic which produces hepatic necrosis in high doses (Boyd and Bereczky 1966). Paracetamol is normally eliminated mainly as sulfate and glucuronide. Higher doses of Paracetamol and N-acetyl-p-benzoquineimine can alkylate and oxidize intracellular GSH, which results in the depletion of liver GSH pool subsequently leads to increased lipid peroxidation and liver damage (Diadelis *et al.,* 1995). Induction of cytochrome or depletion of hepatic glutathione is a prerequisite for Paracetamol induced hepatotoxicity(Moron *et al.,* 1979).

Table 2. Effect of treatment of *Andrographis paniculata* (APEE) on Biochemical Parameters and liver weight of albinao rats in Paracetamol induced hepatatoxicity

Treatment	Dose (mg/kg)	Biochemical Parameters				Liver weight (g)
		SGOT (IU / L)	SGPT (IU / L)	ALP (IU / L)	Serum Bilurubin (IU / L)	
Group 1	-	23.9 ±0.61	32.5±7.5	153.7±12	0.60±0.14	5.82±0.18
Group 2	*250*	*199* ± 0.62*	*241*±0.46**	*368*±0.6*	*1.15*±0.6*	*11.8*±0.5*
Group 3 Acetaminophen + *APEE* (250 mg/kg)	*250*	43 ±0.14*	*46*±0.16*	*215*±0.14**	*0.75*±0.11**	*9.6*±0.43**
Group 4 Acetaminophen + *APEE*(500 mg/kg)	*500*	*23* ± 0.17*	*26*±0.11*	*111*±0.11*	*0.70*±0.12*	*6.1*±0.52*
Group 5 Acetaminophen + *100 mg/kg Silymarin*	*100*	*22* ±0.15*	*34*±0.7*	*160*±0.07*	*0.6 1*±0.15*	*6.0*±0.38*

The values are mean ± SEM (n=6 animals / group) * P< 0.05 is considered significant when compared to group 1 , ** P< 0.05 is considered significant when compared with group 2 by Dunnett's multiple comparison test.

Table 3. Effect of *Andrographis paniculata Extract* on GSH *TBARS, SOD and CAT* Levels in Paracetamol induced hepatatoxicity

Treatment	Dose (mg/kg)	Biochemical Parameters			
		GSH (IU / L)	*TBARS* Mmol/ml	*SOD* (IU / L)	*CAT* (IU / L)
Group l	-	*0.05 ±1.08*	9.*78 ± 0.12*	387± 2.87	7.51±1.8
Group 2	250	0.29±*1.19**	15.5 ± *0.22**	323± 4.45*	*5.04*±2.4*
Group 3 Acetaminophen + *APEE* (250 mg/kg)	250	*0.0810.01**	10.9 ± *1.07**	256 ± 2.4**	6.92±0.57*
Group 4 Acetaminophen + *APEE* (500 mg/kg)	500	*0.06±1.09* **	*9.01±1.61***	378 ± 4.50*	7.1±.11**
Group 5 Acetaminophen + *100 mg/kg Silymarin*	*100*	*0.05 ±1.08**	*9.02*±0.21	*377*± 1.2**	*7.4*±0.7*

The values are mean ± SEM (n=6 animals / group) * P< 0.05 is considered significant when compared to group 1 , ** P< 0.05 is considered significant when compared with group 2 by Dunnett's multiple comparison test.

The Present study reveal that *Andrographis paniculata* extract inhibit the Cytochrome enzyme activity and provides protection against hepatotoxicity induced by Acetaminophen and it is observed that the lipid peroxidation levels in the Paracetamol group is increased. This clearly indicates that there is a significant hepatic damage due to Paracetamol and this is further evident from the fact that there is elevation in the levels of various markers of hepati damage like SGOT, SGPT and ALP. Bilirubin which are enzymes originally present in higher concentration in cytoplasm. When there is hepatotoxicity, these enzymes leak into the blood stream in conformity with the extent of liver damage (Nkosi *et al.,* 2005). The elevated level of these hepatic marker enzymes in the group II(Paracetamol treated rats) in this present correspond to the extensive liver damage induced by toxin. The reduced concentrations of SGPT, SGOT and ALP among the plant extract administered groups, may be due to the presence of flavonoids.

The enzymic antioxidant defense system is the nature protector against lipid peroxidation. SOD and CAT enzymes are important scavengers of superoxide ion and hydrogen peroxide. These enzymes prevent generation of hydroxyl radical and protect the cellular constituents from oxidative damage Scott *et al.,*1991. In the present study, it was observed that the *Andrographis paniculata* extract significantly increased the hepatic SOD activity in paracetamol induced liver damage in rats. This show *Andrographis paniculata extract* can reduce reactive free radicals that might lessen oxidative damage to the tissues and improve the activities of the hepatic antioxidant enzyme. Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues and the highest activity is found in the red cells and in the liver. CAT decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals. Therefore the reduction in the activity of these enzymes may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide. Administration of *Andrographis paniculata extract* increased the activities of CAT in paracetamol-induced liver damage in rats to prevent the accumulation of excessive free radicals and protected the liver from paracetamol intoxication.

Liver protective herbal drugs contain a variety of chemical constituents like phenols, coumarins, lignin, essential oil, monoterpenes, carotinoids, glycosides, flavanoids, organic acids, lipids, alkaloids and xanthines (Gupta and Misra, 2006).Bilirubin is one of the most useful clinical markers to indicate to the severity of necrosis and its accumulation is a measure of binding, conjugation and excretory capacity of hypatocyte. *Andrographis paniculata* extract. has decreased the levels of lipid peroxidation and the maintenance of biochemical markers to the near normal levels. It may be concluded that the hepatoprotective effect of the extract is due to the prevention of the depletion in the tissue GSH levels and there by the liver enzyme parameters are not altered. Further the effect was highly dose dependent and preventive in inducing oxidative stress. Preliminary phytochemical studies revealed the presence of flavonoids and terpenoids. The terpenoids and flavonoids are well known for their hepatoprotective potential The observed hepatoprotective activity of *Andrographis paniculata* may be due to the presence of flavonoids and terpenoids. Therefore the study reveals that the extract of *Andrographis paniculata* is considered to be one of the herbal remedies for Liver ailment by the significant decrease in serum enzyme levels. It may also be concluded from the results of the study that the hepatoprotective activity of *Andrographis paniculata* extract

is comparable to that of the commercially available silymarin preparations. A further study is very much essential to characterize the active principles and to elucidate the exact role played the active ingredient.

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