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Hepatoprotective activity of *Andrographis paniculata* on lipid peroxidase, antioxidant enzymes and liver enzymes in Thioacetamide intoxicated rats

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ABSTRACT

The efficacy of *Andrographis paniculata* (AP) extract was studied on thioacetamide induced hepatic damage in rats. Treatment with aqueous extract of *A. Paniculata* (20mg/100gm body weight) was found to protect the rat from hepato-toxic action of thiacetamide as evidenced by significant reduction in the level of lipid peroxidation and increased the anti-oxidant defense system activity in the thioacetamide intoxicated rats. Histopathological studies show marked reduction in fatty degeneration and patchy focal necrosis in animals receiving *A. paniculata* along with thioacetamide as compared to the control group.

Keywords: *Andrographis paniculata*; Hepatoprotective; Thioacetamide.

Abbreviations: H&E - Heamatoxylin eosin; LPO - Lipid peroxidases; GPX - Glutathione peroxidase; SOD - Superoxide dismutase; CAT – Catalases; GSH - Reduced glutathione; GST - Glutathione-s-transferase; LDH - Lactate dehydrogenase; ALP - Alkaline phosphatase; ACP - Acid phosphates.

1. INTRODUCTION

Liver diseases cause a major case of mortality and give a serious of health problems. Thioacetamide is one of the acute hepatotoxic agent cause toxic effect primarily on liver secondly on kidney (Andesrson et al., 1974). Primary effect on liver lead to centrilobular necrosis, since liver necrosis by thiocetamide is the result of microsomal metabolism of thioacetamide to a toxic metabolite which binds to various molecules in liver directly or indirectly (Gabriel et al., 1994). The metabolite is S-oxide known as thioacetamide sulfine or thioacetamide sulfoxide which exhibit very intense necrogensis and carcinogensis effect (Ammon et al., 1967). Thioacetamide

sulfoxide is converted by mixed function oxides system to thioacetamide sulfone derivative of thiocarbonly compound are highly reactive species accounts for high hepatotoxicity of thioactamide (Hunter et al., 1977).

In the absence of reliable hepatotoxic drugs in modern medicine, herbs and plants play an important role in the management of several diseases (Maiti et al., 2006). In Ayurveda the ancient system of Indian medicine identifies liver disease quite early and recommend no of herbal remedies. Herbal medicine is gaining popularity since cheap, easily available and have rare or no side effect. *Andrographis paniculata* is mentioned as popular remedies in the treatment of liver disorders (Maiti et al., 2009) belong to the family (Acanthaceae). The whole plant are used in Indian medicine in the therapy main part used are stem and leaves for liver disorder (Chopra et al., 2007). The "King of bitter" is annual herb popularly known in the indigenous system of medicine as 'kal megh' (Nadkarni, 1976) act as hepatoprotective, hepatostimulant agent, antioxidant effect (Tridevi et al., 2001), anti-platelet, antithrombotic drug (Borhanuddin et al., 1994), antipyretic effect (Kanniappan et al., 1991), immunostimulatory, anticancer activity (Kumar et al., 2004; Puri et al., 1993), and hepatoprotective against carbon tetrachloride (Kapil et al., 1993; Handa and Sharma, 1990). Therefore the present study has been aimed to investigate the protective effect of *Andrographis paniculata* on lipid peroxidase, antioxidant enzymes and liver enzymes in thioacetamide intoxicated in wistar albino rats.

2. MATERIALS AND METHODS

2.1. Plant material

The aqueous extract of *Andrographis paniculata* was purchased from local herbal store and kept at 4^0 C at Department of biochemistry, University of madras, Chennai, India.

2.2. Chemicals

Thioacetamide was obtained from sigma chemicals.

2.3. Animals and exposure condition

Healthy male wistar albino rats (120-150gm) were housed in a macrolon cages under uniform husbandry condition and given paleted diet (Gold mohur foods and feed Ltd) and water ad-libitum. The animals were housed at temperature of 25±°C with a 12-12 hrs light and dark cycle was followed.

2.4. Study design

The six male wistar albino rats were allotted in to four experimental groups, Group 1 (control) was treated with corn oil, Group II (Thioacetamide alone treated), Group III (*Andrographis paniculata* pretreated and intoxicated with thioacetamide), Group IV (*Andrographis paniculata* pretreated alone). The Group II received Thioacetamide (sigma) where 50mg/kg body weight was given as suspension in water by oral administration for a period of two days to induce acute liver injury, Group III pretreated with *Andrographis paniculata* 20mg/100gm body weight for a period of ten days induced liver injury, Group IV pretreated with *Andrographis paniculata* 20mg/100gm body weight alone.

2.5. Biochemical analysis

After the experimental period the animals were scarified by cervical decapitation the liver was excised and washed in ice cold saline the homogenized in poter elvejhens homogenizer with Tris HCl buffer (0.1 M) pH 7.4 at 4^0 C. The homogenate was centrifuged and supernatant was used for various assays a portion of the liver tissue stored in 10 % formal saline for carrying out histopathological studies (using 5&10 micrometer thick section stained with heamatoxylin eosin (H&E).

Lipid peroxidases (LPO) was determined by the method of Ohkawa by spectrophotometric method and the color produced during the reaction to TBA with MDA for this purpose to the 2.0 ml of tissue homogenate, 2.0ml of 20% TCA were added the content mixed and centrifuged at 4000 rpm for 20 min at4^0 C ,2.0 ml of the supernatant mixed with 2.0ml of TBA reagent . Blank and standard also treated similarly the content heated in boiling water bath for 20 min and cooled in tap water and its absorbance was measured using Shimadu UV spectrophotometer at 523 nm. The content of MDA was calculated by the absorbance coefficient of MDA-TBA complex and expressed as nano moles of MDA /gm tissue protein.

Glutathione peroxidase (GPX) was determined by the method of Paglia and Valentine (1967), 0.1ml of enzyme preparation was added to 0.4ml of phosphate buffer of pH7.4 ,0.1ml of glutathione reductase ,0.1ml of reduced glutathione ,0.1 ml of sodium azide the reaction mixture incubated at 37^0 C for 10 min ,0.1 ml of NADPH ,0.1 ml of Hydrogen peroxide to the test cuvette and change in

the absorbance monitored using Shimadu UV spectrophotometer at 340 nm for 5min at 30 sec interval against the reference cuvette containing mixture expect NADPH & Hydrogen peroxide the activity as nano mole of GSH oxidized / min / mg tissue protein.

Superoxide dismutase (SOD) was determined the method of Misra and Fridovich based on the inhibition of epinerphrine autooxidation by the enzyme ,0.5ml of enzyme preparation was diluted with 0.5ml of water, added 0.25ml of ethanol,0.15 ml of chloroform centrifuged at 4000 rpm for 20 min at 4^0 C , to the 0.5ml of the supernatant ,1.5 ml of carbonate bicarbonate buffer PH 10.2 added and the reaction initiated by the addition of 0.1ml of epinerphine added and change in the absorbance at 30 sec interval / min measured at 480nm using Shimadu UV spectrophotometer , the activity expressed as one unit of SOD(amount of protein required to give 50% inhibition of epinephrine auto oxidation)/min /mg tissue protein.

Catalases (CAT) was determined by the method of Bergmeyer, to the 2.0 ml of phosphate buffer pH 7.0, 30 micro liter of enzyme preparation was added and reaction started by the addition of 1.0ml of hydrogen peroxide solution the decrease in absorbance was measured at 240nmm at 30 sec interval for 3 min using Shimadu UV spectrophotometer, the activity expressed as nano mole of hydrogen peroxide decomposes /min / mg tissue protein.

Reduced glutathione (GSH) was determined by the method of Moron 0.5ml of tissue homogenate was mixed with 1.0 ml of 5% TCA precipitation removed by centrifugation 2000 rpm 20 min at 4^0 C then to 1.0 ml of supernatant,2.0ml of DTNB (5 5'- dithio bis-2- nitro benzoic acid) reagent were added and final volume adjusted to

4.0ml with phosphate buffer pH 8.0, the blank and standard solution of glutathione was prepared treated in the similar manner the absorbance measured at 412nm using Shimadu UV spectrophotometer

Glutathione-s-transferase (GST) was determined by the method of Habig 0.1ml of enzyme homogenate was added to reaction mixture containing 1.0ml of phosphate buffer 6.5ml, 0.1ml of CDND (1-chloro-2,4-dinitro benzene) made up to 2.5ml with water and the reaction mixture incubated at 37^0C for 5min, 0.1ml of GSH reduced glutathione was added and change in the absorbance was measured at 340 nm at 30sec using Shimadu UV spectrophotometer.

Lactate dehydrogenase (LDH) was determined by the method of king with slight modification, 1.0ml of the lithium lactate, 0.1ml of enzyme solution added incubated at 37^0C for 15 min, 0.2ml of NAD added and incubation continued for another 30 min, 1.0 ml of DNPH (2,4 dinitro phenyl hydrazine) added and incubated for another 15 min at 37^0C after than 7.0 ml of 0.4 N sodium hydroxide added and the absorbance measured at 520 nm using Shimadu UV spectrophotometer.

Alkaline phosphatase (ALP) was determined by the method of king to 1.5ml of carbonate bicarbonate buffer pH 9.8, 0.1ml of magnesium chloride, 1.0ml of Disodium phenyl-phosphate, 0.4ml of water added 0.1ml of enzyme preparation incubated 15 min at 37^0C, 1.0ml of folin ciocalteau reagent added to all the tubes to the blank also after arresting the reaction centrifuged at 2000 rpm for 10 min, to 1.0 ml of the supernatant ,2.0 ml of 15 % sodium carbonate added and the absorbance measured after 10 min at 640 nm using Shimadu UV spectrophotometer. Acid phosphates (ACP) the method is similar to the above except the Citrate buffer pH 4.9 the activity expressed for ALP and ACP as nano mole of phenol liberated/min /mg tissue protein.

Estimation of protein determined by the method of Lowry et al. (1951) , 0.1ml of the tissue homogenate was made up to 1.0ml with water, 4.5ml of alkaline copper sulphate solution ,0.5ml of folin ciocalteau reagent added mixed well the absorbance measured at 640 nm after 20min Shimadu UV spectrophotometer.

Table 1 Protein level in liver of group I (control), group II (Thioacetamide Treated), group III (*Andrographis paniculata* pretreated and intoxicated with thioacetamide), group IV (Andrographis paniculata pretreated alone)

Groups	Proteins mg/gm
I	210 ±1.4
II	158 ± 2.8***
III	205±2.8 NS
IV	208±2.8 NS

Values are expressed as mean ± SD; n=6,
Statistical significance at p<*0.05*.
Group II compared with group 1.
Group II compared with group IV.

Table 2 Activities liver enzymes LDH, ALP, ACP in liver of group I (control), group II (Thioacetamide Treated), group III (*Andrographis paniculata* pretreated and intoxicated with thioacetamide), group IV (Andrographis *paniculata* pretreated alone)

TG	LDH	ALP	ACP
I	1.20±0.28	4.10±0.26	9.0+0.37
II	2.40±0.28**	6.90±0.28***	10.12+1.57 NS
III	1.10±0.50*	5.40±0.26*	8.17+0.20 ***
IV	1.15±0.41 NS	4.20±0.30 NS	9.2+ 0.26 NS

Values are expressed as mean ± SD; n=6, Statistical significance at p<*0.05*
Group II compared with group 1. Group II compared with group IV.

2.6. Statistical analysis

The data were expressed as mean ± SD and analyzed by repeated measure of variance. t test was used to test for difference among mean significant (P<0.05).Statistically significant variation were compared with respective control, Group II, Group III ,Group IV vs Group I. ***P< 0.001,*P<0.01,*P<0.05, NS - not significant.

3. RESULTS

The result for the hepatoprotective effect of *Andrographis paniculata* on lipid peroxide, antioxidant enzymes and liver enzymes are shown in Tables 1-4. The intoxication of thioacetamide resulted in a significant elevation of lipid peroxidation, liver enzymes and decreased antioxidant enzyme level. The hepatotoxic effect of thioacetamide was significantly controlled in animals pretreated with aqueous extract of *Andrographis paniculat* the histopathological studies confirms the hepatoprotective activity.

3.1. Statistical analysis

All values are expressed as mean ± SD, p<0.05 statistically significant variation was compared.

3.2. Histopathological Examination

Histopathological observation basically supported the results obtained from the liver enzymes assays. The liver section of control group showed normal architecture with distinct hepatic cells, sinusoidal spaces (Fig.1). Group II animals of thioacetamide treated showed perivascular infiltration of mononuclear cells, sinusoids also shows mononuclear infiltrate and the patchy focal necrosis (Fig.2). Group III animals pretreated with Andrographis panniculata showed the congested results with sinusoidal dilatation more or less normal histological architecture of the liver having reserved to a large extent (Fig.3). The hepatic injury produced by the toxin almost comparable to the normal control and Andrographis Panniculata alone treated groups (Fig.4).

Table 3 Levels of Lipid peroxides (Lpo) in liver of group I (control), group II (Thioacetamide Treated), group III (*Andrographis paniculata* pretreated and intoxicated with thioacetamide), group IV (Andrographis *paniculata* pretreated alone)

TG	Lpo
I	0.80 ± 0.02
II	2.50 ± 0.24***
III	1.12 ± 0.23 *
IV	0.96 ± 0.02 NS

Values are expressed as mean ± SD; n=6,
Statistical significance at p<*0.05*.
Group II compared with group 1. Group II compared with group IV.

Table 4 Antioxidant enzymes level in liver of group I (control), group II (Thioacetamide Treated), group III (*Andrographis paniculata* pretreated and intoxicated with thioacetamide), group IV (Andrographis *paniculata* pretreated alone)

TG	GPX	SOD	CAT	GST	GSH
I	7.28±0.17	36.93± 1.8	115.29+1.6	0.34±0.02	8.74 ± 0.21
II	5.30±0.26***	21.01±2.5***	106.98+1.9***	0.20±0.03 ***	4.64±0.21 **
III	8.0±0.70*	38.08± 2.6^{NS}	110.0+2.8**	0.38 ± 0.02*	8.82 ± 0.24 ^{NS}
IV	7.4± 0.28^{NS}	36.83 ± 2.5 ^{NS}	117.29 + 1.5 ^{NS}	0.32 ± 0.02 ^{NS}	8.92 ± 0.21 ^{NS}

Values are expressed as mean ± SD; n=6

Statistical significance at p<*0.05*. Group II compared with group 1. Group II compared with group IV.

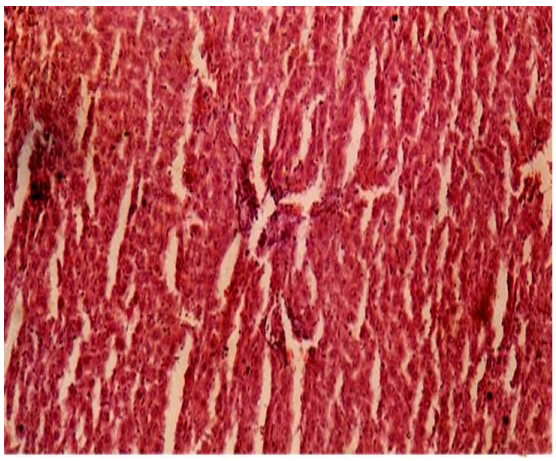

Figure 1
Control rat liver section revealing normal hepatic parenchyma cells

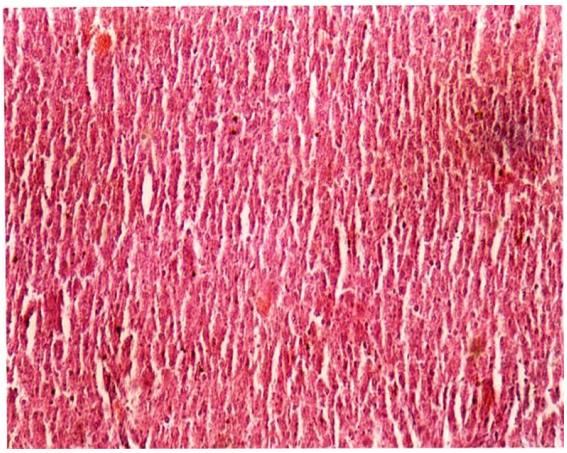

Figure 4
Architecture of the liver in rats given Andrographis paniculata alone, the parenchymal cells are normal with minimal sinusoidal dilatation

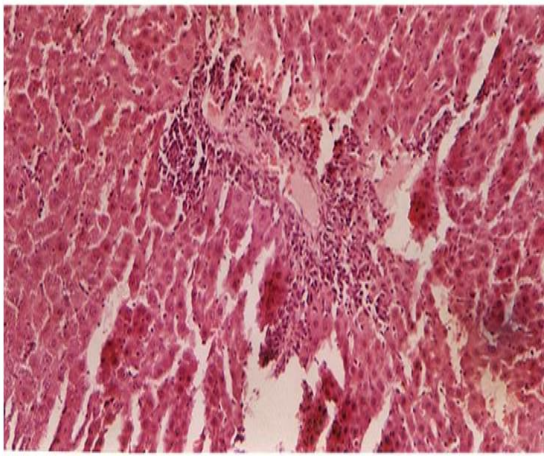

Figure 2
Architecture of the liver in rats induced with thioacetamide showing perivasular infiltration of mononuclear cells. Sinusoids also shows mononuclear infiltrate, patchy focal necrosis is also seen

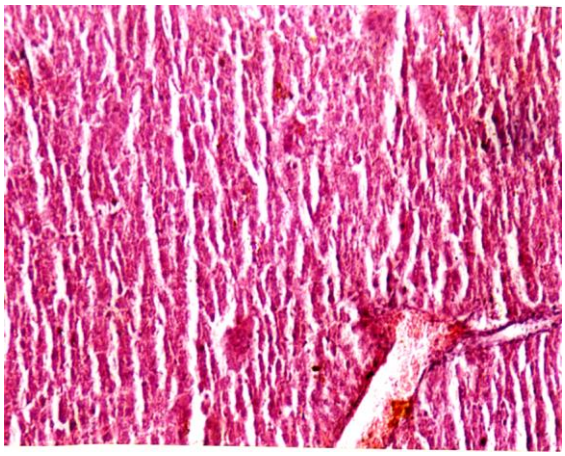

Figure 3
Architecture of the liver in rats given Andrographis paniculata and then treated with thioacetamide, shows congested with sinusoidal dilatation

4. DISCUSSION

Thioacetamide exert its toxic effect on DNA, RNA and protein synthesis at macro molecular level (Chakrabarty et al., 1978) the decrease level of protein in group II and pretreatment with *Andrographis paniculata* in group III increased the ribosomal activity and increase the protein level in liver. The raised level of liver marker enzyme is due to the disturbed/damaged integrity of liver

(Chandan et al., 1991) the increase in the ALP activity occur mainly in liver, billiary and bone disease it is highly probable that liver injury was responsible for the elevated level of ACP, ALP (Mazumdar et al., 1996), the group II shows the elevated level of LDH, ACP, ALP and decreased activates of these enzymes are represented in group III indicate the protective effect of *Andrographis paniculata*.

The initiation of lipid peroxidation is carried out by free radical such as superoxides, hydroxyl radical and hydrogen peroxide all of which cause cellular injury (Manemegalai et al., 1996), the reaction of hydroxide with polyunsaturated lipid leads to the formation of lipid radical and eventually short chain aldehyde and hydroxyl alkenes lipid peroxidation worsen the tissue injury (Shyamala and Devaki, 1996).The increase in lipid peroxidation is further adducting to more oxidative stress making the cell more vulnerable to damage. Lipid peroxidation reaction has been linked with altered membrane structure and enzyme inactivation, the lipid peroxide product malondialdehyde showed a significantly elevation in group II when compared to group I. Thioacetamide is known to induce membrane damage and cell injury increase in lipid peroxidation in thioacetamide hepatotoxicity has already been reported ,group, III prevented these alteration showed the decreased level which might be due to the trapping of hydroxyl free radical produced during toxicity.

Liver posses a wide range of anti-oxidant enzymes SOD, CAT, GST, GPX as well non enzymic antioxidants such as glutathione. *Andrographis paniculata* prevents induced increase in the activities of enzymes gamma-glutamyl transpeptidase, glutathione –s-tranferase and lipid peroxidation and increased antioxidant enzymes like SOD, CAT, glutamine peroxidase glutathione reductase and decrease level of glutathione. Antioxidant constitute the foremost defense system that limits the toxicity associated with free radical in normal cellular process the process of lipid peroxidation in cell termed as biological antioxidant, when lipid peroxidation take place the activities of these antioxidants are reduced, group II showed the significantly decrease in the level of these enzymes the level of these enzyme were significantly increased and reached to near normal level in group III. The protective activity might be due to the interaction of *Andrographis paniculata* with reactive oxygen species there inhibiting the process of lipid peroxidation, the increased level of these enzymes protect the membrane lipid against free radical mediated peroxidative damage induced by thioacetamide. The recovery towards normalization of LPO, antioxidant and liver enzymes and histological architecture caused by *Andrographis panniculata* in the present study Andrographis panniculata is a known hepatoprotective compound.

5. CONCULSION

In conclusion that prior oral administration of the aqueous extract of *Andrographis paniculata* offered significant protection to the liver against hepatotoxicity induced by thioacetamide hence avenues are wide open for *Andrographis paniculata* to be used as a potent hepactoprotecant.

Summary of research

1. The therapeutic activity and potency of Andrographis paniculata (AP) extract was studied on thioacetamide induced hepatic damage in rats.
2. Histopathological studies were carried out and it shows marked reduction in fatty degeneration and patchy focal necrosis in animals receiving A. paniculata along with thioacetamide as compared to the control group

Future issues

1. What are all the active ingredients in the extract of Andrographis paniculata?
2. Among the active ingredients of Andrographis paniculata, which chemical entity acts as hepactoprotecant?
3. Is Andrographis paniculata produces maximum efficacy against hepatotoxicity, when correlated with other medicinal plant for liver hepatotoxicity?

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Conflict of Interest:

The authors declare that there are no conflicts of interests.

Data and materials availability:

All data associated with this study are present in the paper.

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