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RESEARCH ARTICLE

ANTIOXIDANT PROPERTIES AND HEPATOPROTECTIVE EFFECT OF ANDROGRAPHIS PANICULATA ON PCM INDUCED HEPATOTOXICITY IN ALBINO RATS.

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Abstract

Methanolic extract of *Andrographis paniculata* was evaluated for hepatoprotective and antioxidant activities in albino rats. The plant extract (250 and 500 mg/kg, bw.) exhibited a remarkable hepatoprotective and antioxidant activity against paracetamol induced hepatotoxicity as judged from the serum marker enzymes and antioxidant levels in liver tissues. Paracetamol induced a significant rise in aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), lipid peroxidase (LPO) and bilirubin with a reduction of protein, superoxide dismutase (SOD). Treatment of rats with different doses of plant extract (250 and 500 mg/kg) significantly ($P < 0.001$) altered serum marker enzymes and antioxidant levels to near normal against paracetamol induced albino rats. The activity of the extract at dose of 250 mg/kg was comparable to more effective than dose of 500, mg/kg bw.). Histopathological changes of liver sample were compared with respective control. Results indicate the hepatoprotective and antioxidant properties of *Andrographis paniculata* against paracetamol -induced hepatotoxicity in albino rats.

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Introduction:-

Herbal medicines have recently attracted much attention as alternative medicines useful for treating or preventing life style related disorders and relatively very little knowledge is available about their mode of action. There has been a growing interest in the analysis of plant products which has stimulated intense research on their potential health benefits. 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. Several hundred plants have been examined for use in a wide variety of liver disorders. Antioxidants play an important role in inhibiting and scavenging free radicals and thus providing protection against infections and degenerative diseases. *Andrographis paniculata* is a herbaceous plant commonly known as "King of bitter" in the family Acanthaceae. Mostly the leaves and roots have been traditionally used over the centuries for different purposes. *Andrographis* has demonstrated a number of different pharmacological actions in in-vitro and/or animal studies. Anticancer, immunomodulatory, anti-inflammatory, antipyretic, hepatoprotective-hypotensive, hypoglycemic, antiplatelet and antithrombotic activity have all been reported. Andrographolide is considered one of the most active and important constituents in *andrographis* and is most concentrated within the leaf. The present study is aimed to evaluate the hepatoprotective and antioxidant activity of methanol extract of *Andrographis paniculata* against paracetamol -induced hepatotoxicity in albino rats.

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Materials and Methods:-

ANIMALS:-

The present study was conducted on Albino rats after approval from the Institutional Ethics Committee. Albino rats of Charles Foster strain of either sex weighing between 180-250g were procured from the Central Animal House, Institute of Medical Sciences, Banaras Hindu University, Varanasi.

All the animals were kept in colony cages at an ambient temperature of $25\pm 2^{\circ}\text{C}$ with 45-55% relative humidity and 10 : 12 h. light and dark cycle, Animals were kept on standard rodent diet and water ad libitum. All the experimental animals were acclimatized in the department for 3 days. Principles of laboratory animal care (NIH Publication No. 86-23 revised 1955) guidelines were followed.

PLANTS:-

The whole plant of *Andrographis paniculata* were collected from the local market after proper identification by expert of Botany and Dravyagunadepartmentof BHU.

EXTRACTION:-

The dried whole plant of *Andrographis paniculata* was powdered and extracted methyl alcohol for forty hours. The methanolic extract after filtration was concentrated and remove any trace of solvent.

Chemicals and Analysing Kits:-

Chemicals and analysing kits of different parameters of liver like SGOT, SGPT, ALP, Bilirubin, Protein, were procured from Avicondiagnostics, Varanasi. Chemicals for MDA and SOD were purchased from Gupta Enterprises, Varanasi.

Assay procedure:-

The serum samples were subjected to assay for hepatic marker enzymes such as Aspartate transaminase (AST) Alanine transaminase (ALT) and Alkaline phosphatase (ALP). Activities of AST and ALT were assayed according to the 2-4 DNP method. Values are expressed as IU/dl ALP activity was measured using the method of Kind and King (1954) and results are expressed as K.A. units/L, bilirubin (Mallay and Evelyn, 1937) and protein (Lowry et al., 1951). The LPO in the liver and serum were determined by the method of Ohkawa. The SOD level in serum and liver tissue were measured by using modified kakkar method.

Experimental Design:-

Paracetamol (PCM) induced hepatic damage (Chattopadhyay, 2003)- Animals were divided into eleven (V) groups of six animals in each group.-

Group I:- Normal control.

Group II:- Alcoholic group.

Group III:- Paracetamol (PCM) treated group- Animal were given paracetamol at 2g /kg body wt on 11 days.

Group IV:- Paracetamol with *A. paniculata*- In this group Animal received methanolic extract of *A. paniculata* 250 mg/kg body weight for 12 days – and 11th day paracetamol.

Group V:- Paracetamol with *A. paniculata* 500 mg/kg body weight.-Animal received *A. paniculata* in the dose of 500mg/kg.b.w for 12 days on 11th day paracetamol- After 48 hours paracetamol administration, we sacrifice the rat, collect the serum for LFT (SGOT, SGPT, Alkaline Phosphatase, Bilirubin, Protein, SOD, and LPO). At the end of the experiment (48h after paracetamol administration), all the animals were sacrificed by cervical decapitation. Blood samples were collected, allowed to clot. Serum was separated by centrifuging at 2500 rpm for 15 min and analyzed for various biochemical parameters.

The liver was immediately isolated and washed with normal saline, blotted with filter paper and weighed. Liver tissue homogenate (LTH) was prepared in normal saline and used for estimation of endogenous marker enzymes and biological antioxidants superoxide dismutase (SOD) activities.

Histopathological studies:-

Liver slices fixed for 12 hrs in Bouin's solution were processed for paraffin embedding following standard micro techniques (Galigher and Kozloff, 1971). 5µm sections of liver stained with alum haematoxylin and eosin, were observed microscopically for histopathological changes.

Statistical Analysis:-

The result were expressed as mean \pm S.D of four animal from each group .The statistical analysis of variance were carried out by one way analysis of variance(ANOVA) P values<0.05 were consider significant.

Results:-

The effect of Andrographispaniculata on serum marker enzymes are presented in table 1. The levels of serum AST, ALT,ALP, bilirubin, were markedly elevated and that of protein decreased in paracetamol treated animals, indicating liver damage. Administration of Andrographispaniculata extract at the doses of 250 and 500 mg/kg remarkably prevented paracetamol induced hepatotoxicity in a dose dependent manner. Analysis of LPO levels by thiobarbituric acid reaction showed a significant (P<0.001) increase in the paracetamol treated rats. Treatment with Andrographispaniculata (250 mg/kg and 500 mg/kg) significantly (P<0.001) prevented the increase in LPO level which was brought to near normal. Paracetamol treatment caused a significant (P<0.001) decrease in the level of SOD, when compared with control group (table 2) The treatment of Andrographispaniculata at the doses of 250 and 500 mg/kg resulted in a significant increase of SOD, when compared to paracetamol treated rats. The high dose of combined extract of Picrorhizakurroa and Andrographispaniculata (500 mg/kg, b.w) was less effective in reducing PCM induced hepatotoxicity. All these results indicate a hepatoprotective potential of the extract.

Morphological observations showed an increased size and enlargement of the liver in acetaminophen treated groups. These changes were reversed by treatment with Andrographispaniculata at the doses tested (fig1). Histopathological studies, showed acetaminophen to produced extensive vascular degenerative changes and centrilobular necrosis in hepatocytes. Treatment with different doses of Andrographispaniculata 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.

Table 1:- Effect of Andrographispaniculata on biochemical parameters in acetaminophen induced hepatotoxicity in rats.

G r o u p s	D o s e mg/kg.b.w.	B I L I . mg/ml	AST IU/ml	ALT IU/ml	ALP KA.U/ml	PROT mg/ml
Normal		0.82 \pm .01	36.08 \pm 1.4	29.58 \pm 1.7	28.64 \pm 1.4	8.20 \pm .18
Alcoholic	4 % alcohol	0.87 \pm .01	43.38 \pm 1.8	33.99 \pm 1.4	36.11 \pm 1.3	7.35 \pm .12
Paracetamol	2 gm/kg b.w.	1.97 \pm .01	103.56 \pm 2.9	85.4 \pm 1.4	78.11 \pm 2.2	4.62 \pm .25
PCM+A.paniculata	2gm+250mg/kg b.w.	0.85 \pm .01	77.23 \pm 1.6	45.96 \pm 1.5	39.17 \pm 1.4	6.87 \pm .17
PCM+A.paniculata	2gm+500mg/kg b.w.	1.1 \pm .02	81.99 \pm 1.6	60.35 \pm 2.4	51.38 \pm 2.0	5.65 \pm .23

N=6; Values are expressed as mean \pm SEM; aP< 0.001; bP< 0.01; dP< 0.05 Vs Control; cP< 0.001 Vs Acetaminophen

Data were analyzed by using one way ANOVA followed by Tukey multiple comparison test.

Table 2:- Effect of Andrographispaniculata on antioxidants level in acetaminophen induced hepatotoxicity in rats.

G r o u p s	D o s e mg/kg.b.w.	liver weight/100gm	L P O nmol/ml	S O D U/mg
Normal		3.40 \pm .01	14.83 \pm 1.3	2.22 \pm .03
Alcoholic	4 % alcohol	3.45 \pm .01	18.00 \pm 1.0	1.25 \pm .02
Paracetamol	2 gm / kg b . w .	5.90 \pm .02	43.06 \pm 2.3	0.71 \pm .02
PCM+A.paniculata	2gm+250mg/kg b.w.	3.78 \pm .01	20.92 \pm 1.1	1.44 \pm .37
PCM+A.paniculata	2gm+500mg/kg b.w.	4.84 \pm .02	27.82 \pm 1.1	1.01 \pm .06

N=6; Values are expressed as mean \pm SEM; aP< 0.001; bP< 0.01; dP< 0.05 Vs Control; cP< 0.001 Vs

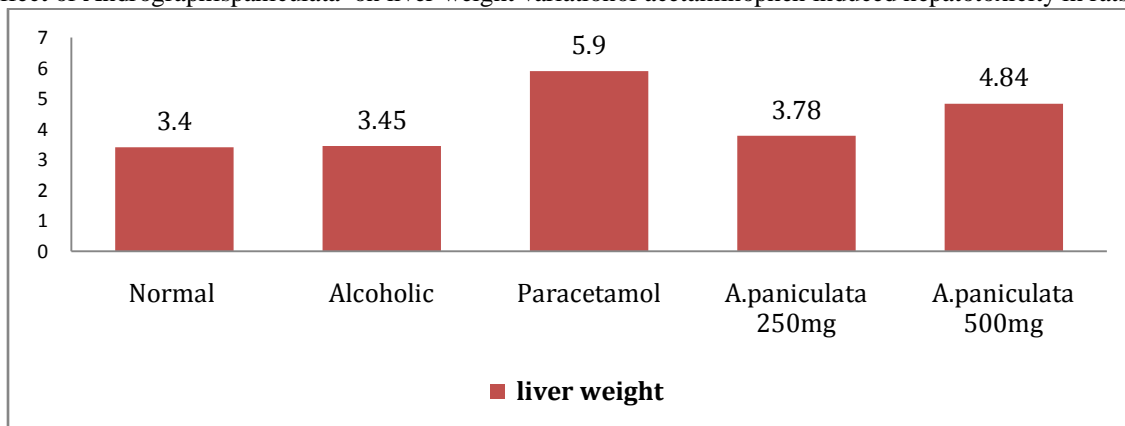
Acetaminophen

Data were analyzed by using one way ANOVA followed by Tukey multiple comparison test.

LPO = μ moles of MDA/ min/mg protein

SOD = Units/min/mg protein

Fig. 1: Effect of *Andrographispaniculata* on liver weight variation of acetaminophen induced hepatotoxicity in rats.



Discussion:-

Paracetamol (Acetaminophen) a widely used antipyretic analgesic drug produces acute hepatic damage on accidental over dosage (Hazai et al. 2002). It is established that, a fraction of acetaminophen is converted via the cytochrome P450 pathway to a highly toxic metabolite; N-acetyl-p-benzoquinamine (NAPQI) (Dahlinet al., 1984) which is normally conjugated with glutathione and excreted in urine. Overdose of acetaminophen depletes glutathione stores, leading to accumulation of NAPQI, mitochondrial dysfunction (Parmar, and Kandakar, 1995) and the development of acute hepatic necrosis. Several P450 enzymes are known to play an important role in APAP bioactivation to NAPQI. P450 2E1 have been suggested to be primary enzymes for acetaminophen bioactivation in liver microsomes (Raucy et al., 1989). Studies demonstrated that acetaminophen induced hepatotoxicity can be modulated by substances that influence P450 activity. In the assessment of liver damage by paracetamole the determination of enzyme levels such as AST, ALT is largely used. Necrosis or membrane damage releases the enzyme into circulation and hence it can be measured in the serum. High levels of AST indicates liver damage, such as that caused by viral hepatitis as well as cardiac infection 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 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 and Garcipiana, 1992). Administration of paracetamole caused a significant (P<0.001) elevation of enzyme levels such as AST, ALT, ALP, bilirubin and decrease in protein when compared to control. There was a significant (P<0.001) restoration of these enzyme levels on administration of the extract in a dose dependent manner. The reversal of increased serum enzymes in paracetamole induced liver damage by the extract may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity. This is in 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 secretory mechanism of the hepatic cells. The efficacy of any hepatoprotective drug is dependent on its capacity of either reducing the harmful effect or restoring the normal hepatic physiology that has been distributed by a hepatotoxin. The plant extract decreased paracetamole induced elevated enzyme levels in tested groups, indicating the protection of structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells. The increase in LPO level in liver induced by paracetamole suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive free radicals. Treatment with *Andrographispaniculata* significantly reverses these changes. Hence it is likely that the mechanism of hepatoprotection of *Andrographispaniculata* is due to its antioxidant effect. 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. 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 *Andrographis paniculata* causes a significant increase in hepatic SOD activity and thus reduces reactive free radical induced oxidative damage to liver, number of deleterious effects due to the assimilation of superoxide radical and hydrogen peroxide.

Extensive vascular degenerative changes and centrilobular necrosis in hepatocytes was produced by paracetamol. Treatment with different doses of water extract of *Andrographis paniculata* 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 *Andrographis paniculata* on paracetamol 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.

Conclusion:-

Based on results of our study, it can be concluded that paracetamol might disturb the balance between reactive oxygen species production and antioxidant protection in liver in paracetamol hepatotoxicity. The low dose of *Andrographis paniculata* (250 mg/kg bw.) possess good hepatoprotective activity against PCM induced liver damage, whereas high dose (500 mg/kg, bw.) of *Andrographis paniculata* failed to produce similar effect. The protective effect of the extract may be due to its antioxidant activity.

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