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Therapeutic Efficacy of combined plant extract of *Andrographis panicultata* Wall. ex Nees and *Achyranthes aspera* L. against Paracetamol induced Hepato and Renal Toxicity in *Wistar albino* rats

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Abstract

The current study was designed to evaluate the therapeutic efficacy of *Andrographis paniculata* (*Ap*) and *Achyranthes aspera* (*Aa*) on Paracetamol (PCM) induced hepato and nephrotoxicity in *Wistar albino* rats. The activities of serum marker enzymes of the liver like alanine aminotransferase (ALT), asparate aminotransferase (AST), alanine phosphates (ALP) and bilirubin were increased by the administration of Paracetamol (500 mg/kg body weight). These activities were found to be reduced after the administration of plant (*Andrographis panicultata* Wall. ex Nees and *Achyranthes aspera* (L.) extracts in the toxicity induced groups. Lipid peroxidation level, which increased after administration of paracetamol, was significantly reduced in the liver. Levels of antioxidants defense enzymes, such as, superoxide dismutase (SOD), Catalase (CAT), glutathione peroxidase (GPx), and glutathione-S-transferase (GST) were lowered by paracetamol. They were elevated by the plant extracts (separate and combined). The kidney function markers like urea, uric acid and creatinine were relatively increased in Paracetamol treated animals. However, their levels were found to be decreased while treating with the plant extract either separately [*Ap* or *Aa*] or combined [*Ap* and *Aa*]). This study shows that the extracts of *Ap* and *Aa* extracts could play a significant role to protect and cure the liver and kidney cells against paracetamol induced toxicity. They also increase the immunity in hepatic and nephritic cells of *wistar albino* rats.

Keywords: *Andrographis paniculata, Acheranthes aspera*, Liver marker enzymes, Paracetamol, Hepatoprotective, Nephroprotective.

Introduction

The human body is complex, like a highly technical and sophisticated machine. It operates as a single entity, but it is made up of number of operational parts that work independently. Among them, the liver is the largest organ and it plays a vital role in metabolism and excretion. Many synthetic chemical substances/drugs are metabolized by liver. During this process, free radicals which are generated could cause hepatotoxicity by damaging the cell membrane and cell constituents (Wiseman *et al.,* 1996). Paracetamol is the most commonly used analgesic; it effectively reduces fever and pain and is considered to be safe at therapeutic doses. Overdose of Paracetamol has been reported to cause acute centrilobular hepatic necrosis (Zhang *et al.,* 2000) in liver and acute proximal tubular necrosis (Newton *et al.,* 1982) in kidney of rats (Newton *et al.,* 1983b) it binds covalently to renal protein (Nelson *et al.,* 2003) by an NADPH-dependent, cytochrome-P-450-mediated process (Newton *et al.,* 1983b). Alternatively, it is enzymatically de-acetylated to *para*-amino phenol. It causes proximal tubular necrosis in rats.

Human beings possess inbuilt natural endogenous antioxidant defense system against the oxidative stress by scavenging the free radicals generated. Many drugs are useful against diseases, but they are also responsible for severe side effects. Many of the antitumor drugs have been known to produce hepatotoxicity, cardiotoxicity and nephrotoxicity, which are mainly caused by the free radicals (Malik *et al.,* 2008). Liver is the main detoxifying organ of xenobiotics. Hence, chemicals and their byproducts are responsible for the damage caused to detoxifying and excretory organs. Most diseased conditions have also been linked to the generation of Reactive Oxygen Species (ROS) (Valko *et al.*,2007) and antioxidants have been reported to prevent the formation of ROS and scavenging of free radicals

(Valko *et al.,* 2006). Many of the naturally available antioxidants are plant by products. They are oils, alkaloids, saponins, carotenoids, phytosterols, phenolics-coumarines, flavonoids, tannins, vitamins A, C, and E, they protect against cardiovascular and malignant diseases (Haendeler *et al.,* 1996). Andrographolide is a diterpene lactone, which is found in *Andrographis panicultata*. It is used to treat a wide range of conditions such as hepatitis, diabetes and cardio toxicity (Khare *et al.,* 2004) scutellaria radix (Hwang *et al.,* 2005). It is also used as an anti-cancer (Sheeja and Kuttan, 2007) and cardio protective (Yoopan *et al.,* 2007) medicine. More recently, it has been adopted by western herbal practitioners as a modulator with hepatoprotective (Trivedi *et al.,* 2007) and tonic effects.

Every part of *Acheranthes aspera* has been utilized by ayurveda, siddha and yunani, practitioners to treat asthma, arthritis, cardiac dropsy, diabetes, wound, insect and snake bite, kidney stone, leprosy, and renal disorders (Kayani *et al.,* 2007) cough, dermatological disorders, dysentery, fever, hysteria, pyorrhea, rabies, toothache etc (Aziz *et al.,* 2005), first observed the hepatoprotective activity of *Acheranthes aspera*. Methanol extract of *Acheranthes aspera* was found to prevent the lead induced nephrotoxicity in albino rats.

These plants are popular folk remedy in the traditional system of medicine throughout the tropical Asian and African countries. Hence, we have selected these two plants to find their efficacy of hepatotoxicity caused by PCM individually and combined.

In this study, we have used Liv 52 (Himalayan drug) as a standard drug, against PCM-induced acute hepatic damage in rats. It has been undertaken to determine the hepatoprotective activities of the methanol extract of *Ap* and *Aa* (separate and combined) in animal model. In addition to finding the levels of liver marker enzymes, histopathological studies were done to prove the efficacy of their preventive and curative role against Paracetamol toxicity in vivo. The urinary system is one of the excretory systems of the body. It consists of kidneys which produce urine. Urine contains metabolic nitrogenous waste products, like urea, ureic acid, excess ions and metabolites of drugs.

Materials and Methods

Plant material

The whole plant of *Andrographis panicultata* Wall. ex Nees (Acanthaceae), *Achyranthes aspera* (Amaranthaceae) were collected in and around Vellore district, Tamil Nadu, and authenticated at the Department of Botany, C. Abdul Hakeem College, Melvisharam, Vellore District, Tamil Nadu. The plant materials were cleaned with double distilled water and shade and dried at room temperature.

Plants extract preparation

The shade dried plant materials were powdered separately in an electrical blender. The powdered material of the whole plant was extracted in a Soxhlet apparatus using Methanol (500ml for 100gms) as solvent for 3hrs. The extract was filtered and concentrated under reduced pressure on rotary evaporator to obtain (10%) the extract. The powder obtained was then subjected to phytochemical analysis to determine the chemical constituents present in the extract and the remaining was stored at 5^{o}C for further use.

Animals

Thirty six *Wistar albino* rats were used in this study. *Wistar albino* rats weighing 175-200g used in the present studies were procured from the animal house of C. Abdul Hakeem college, Melvisharam, Tamil Nadu, India. The animals were housed in a clean and well ventilated experimental unit of the animal house in a poly propylene cages with sterile inert husk materials as bedding. All the animals were kept under standard environmental condition at 25±2°C (12hrs light / 12hrs dark cycle) and maintained on commercial pellet diet, which was supplied by "HINDUSTAN LEVER" Limited Mumbai. Water was provided *ad libitum*. Experimental protocol and procedures employed in this study were approved by the Animal Ethics Committee of C. Abdul Hakeem College, Melvisharam, Tamil Nadu, India. The rats were kept in animal house for ten days before starting the experiments.

Experimental design

Paracetamol (PCM) was obtained from Indian pharmaceutical company (IPCA), Mumbai. The animals were divided into six groups consisting of six animals each for different experiments.
Group I rats served as normal control. They received only feed and water.

Group II (intoxicated group) single dose of Paracetamol (500 mg/kg b.wt.) dissolved in water and received by orally gavage,
Group III rats were intoxicated with PCM followed by Liv 52 (standard) commercial drug (250 mg/kg b.wt.) for 30 days.
Group IV rats were intoxicated with PCM (500 mg/kg b.wt.) followed by the extract of *Andrographis paniculata* (250 mg/kg b.wt.).
Group V rats were intoxicated with PCM (500 mg/kg b.wt.) followed by the extract of *Acheranthes aspera* (250 mg/kg b.wt.).
Group VI rats were intoxicated with PCM (500 mg/kg b.wt.) followed by the combined extract of *Andrographis paniculata* and *Acheranthes aspera* (125+125 mg/kg b.wt).

At the end of the experiment, the animals were sacrificed. The blood samples were collected by cardiac puncture using ether anesthesia without any anticoagulant and were allowed to clot for 10 minutes at room temperature. The blood was centrifuged at 3000 rpm for 15 minutes at 30˚C. The serum samples were stored at - 80°C before determination of the biochemical parameters. The liver and kidney were dissected into two parts, one portion for the preparation of homogenate and another portion for the histopathological examination. The part of the liver and kidney proposed for histopathological examination was washed in normal saline and fixed in 10% formalin for 2 days. These tissues were processed in alcohol-xylene series and stained with haemotoxylin and eosin. The 5µ thickness microtome sections were then made. The slides were studied under a light microscope (Strate et al., 2005) for any histological changes.

Preparation of hepatic homogenate

The liver was quickly removed, part of the liver perfused immediately with ice-cold saline (0.9% NaCl). The portion of the liver was then homogenized in chilled sodium phosphate buffer (0.1M, pH 7.4) using a Potter Elvehjehm Teflon homogenizer (Yamato L.S. G. L.H-21, Japan). The homogenate thus obtained was centrifuged in a cooling centrifuge at 12,000 rpm for 30 min at 4˚C to obtain a post-mitochondrial supernatant (PMS) which was used for evaluation of liver endogenous antioxidant enzymes at 4˚C.

Estimation of Biochemical parameters

The biochemical parameters like serum enzymes were analyzed. They include, ALT and AST (Reitman *et al.,* 1957), ALP (King *et al.,* 1934), serum bilirubin (Mally *et al.,* 1937), super oxide dismutase (Kakkar *et al.,* 1984), catalase (Sinha, 1972), glutathione peroxidase (Rotruck *et al.,* 1973), and Glutathione -S- tranferase (Habig *et al.,* 1974).

Statistical Analysis

The data of biochemical estimations were reported as mean ± SD. The statistical significance was determined by using one way analysis of variance (ANOVA) followed by Dunnet's multiple comparison tests. $P< 0.05$ was used to determine statistical significance.

Results

Hepatic damage is usually associated with elevated serum ALT, AST, ALP and bilirubin concentration. The results of the experiment show that the levels of marker enzymes were high in paracetamol induced group (ALT-110.37%, AST-81.22%, ALP-113.68% and bilirubin-338.04%) when compared to the normal. The separate plant extracts of *Ap* and *Aa* show a better reduction of liver marker enzymes (*Ap*: 18.41%, 26.34%, 31.39% and 29.28% and *Aa*: 15.43%, 24.78%, 26.13% and 26.05%) when compared with PCM toxicated rats. In this study, methanol combined plant extract of *Ap* and *Aa* (1:1) significantly reduced the levels of serum ALT, AST, ALP and bilirubin (44.39%, 40.85%, 48.88% and 75.68%), when compared to the Paracetamol induced *albino* rats. The methanol combined plant extracts exhibit better results, comparable with Liv 52 (ALT-43.22%, AST-38.53%, ALP-47.49% and bilirubin-74.94%), when compared to separate plant extracts of *Ap* and *Aa*, as seen in (Table - 1).

Antioxidant defense enzymes

Antioxidant defense enzymes viz., superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) play a vital role in the protection of the body from toxic substances. The activity of liver antioxidant defense enzymes viz., SOD, CAT, GPx and GST were significantly decreased by 51.18%, 74.10%, 48.89% and 63.51%, respectively, in the liver tissue of paracetamol induced group, when compared to normal group. The methanol combined plant extract (*Ap* and *Aa* in the ratio of 1:1) was administered to the paracetamol treated groups. Hence, the levels of antioxidant enzymes were significantly increased by 87.37%, 234.92%, 74.73% and 146.93%, respectively. The decreased levels of enzymatic antioxidants such as SOD, CAT, GPx and GST

were increased in Liv 52 (75.72%, 224.295, 79.48%, and 122.15%) treated group, when compared to PCM control rats. The hike of the antioxidant defense enzyme levels was found to be more in combined plant extract (*Ap* and *Aa* in the ratio of 1:1) than separate (*Ap* or *Aa*) plant extract treated animals, as seen in (Table - 2).

Table - 1: Activity levels of serum Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline Phosphate (ALP), and bilirubin in normal, PCM intoxicated and plant extract treated rats

Groups	Parameters in the serum			
	ALT (IU/l/min/mg protein)	AST (IU/l/min/mg protein)	ALP (IU/l/min/mg protein)	Bilirubin (mg/dl)
Group-I (NaCl)	48.75±0.51	67.52±0.58	71.31±1.96	0.92±.0.061
Group-II PCM Control	102.56±0.004[a]	122.36±3.16[a]	152.38±0.44[a]	4.03±0.001[a]
% of change (Normal vs PCM)	+110.37	+81.22	+113.68	+338.04
Group-III PCM+Liv 52	58.23±1.15	75.21±0.04	80.01±0.15	1.01±0.048
% of change (PCM vs Liv 52)	-43.22	-38.53	-47.49	-74.94
Group –IV PCM +AP	83.67±0.065[b]	90.12±0.05[b]	104.54±1.24[b]	2.85±0.18[b]
% of Changes PCM vs Ap	-18.41	-26.34	-31.39	-29.28
Group –V PCM+Aa	86.73±0.09[b]	92.03±0.07[b]	122.5 ±1.80[b]	2.98±0.15[b]
% of Changes PCM vs Aa	-15.43	-24.78	-26.13	-26.05
Group VI PCM +Ap+Aa	57.03±0.17[b]	72.37±0.03[b]	77.89±0.02[b]	0.98±0.023[b]
% of changes PCM vs Ap+Aa	-44.39	-40.85	-48.88	-75.68

Values are mean ± S.D., n = 6. a $p < 0.05$ compared with normal control. b $p < 0.05$ compared with PCM intoxicated.

Renal markers

Renal function markers like urea, uric acid and creatinine were significantly higher in Group II animals than normal [(percentage of change Normal vs PCM) 171.69%, 65.36%, and 297.76%]. This hike in the renal function markers indicates renal damage. These increased levels of markers were decreased significantly (percentage of change Normal vs PCM) 51.24%, 33.44% and 63.97%) by the administration of methanol combined plant extract (*Ap* and *Aa* in the ratio of 1:1) than the separate plant extract administered groups (Table: 3).

Histopathological findings show Fig. 1. A: normal hepatic cell architecture in control group. 1B: abnormal cell architecture and centrilobular necrosis. 1C: Liv 52 treated liver shows nearly normal cell architecture. 1D: *Andrographis paniculata* extract restored normal cell architecture 1E: *Acheranthes aspera* shows nearly normal cell architecture. 1F *Ap* and *Aa* extract liver shows nearly similar cell architecture to

normal. Fig. 2. A: shows normal architecture of cell. 2B: PCM in intoxicated: shows damaged cell architecture. 2C: Liv 52 retain the normal cell structure. 2D: Ap restored nearly normal structure. 2E: Aa brought similar to normal structure. 2F: combined plant extract (Ap and Aa) shows the normal cell architecture, it proves the efficacy of the combined plant extract (Fig: 1, Fig: 2).

Table 2: Levels of SOD, CAT, GPX and GST in the liver homogenate of the normal, PCM intoxicated and plant extract treated rats

Groups	Parameters in the liver			
	SOD (mg/dl)	CAT (mg/dl)	GPX (mg/dl)	GST (mg/dl)
Group-I (NaCl)	2.11±0.16	87.12±0.064	9.06±0.061	4.33±0.05
Group-II PCM Control	1.03±0.05[a]	22.56±0.71[a]	4.63±0.05[a]	1.58±0.03[a]
% of change (PCM Vs Normal)	-51.18	-74.10	-48.89	-63.51
Group-III PCM+Liv 52	1.81±0.03[b]	73.16±0.35[b]	8.31±0.34[b]	3.51±0.24[b]
% of change (Liv52 vs PCM)	+75.72	+224.29	+79.48	122.15
Group –IV PCM +AP	1.79±0.04[b]	67.72±0.08[b]	7.33±0.02[b]	3.23±0.07[b]
% of Changes PCM vs Ap	73.78	200.17	58.31	104.43
Group –V PCM+Aa	1.48±0.03[b]	33.16±0.02[b]	6.94±0.41[b]	3.02±0.16[b]
% of Changes PCM vs Aa	43.68	179.96	49.89	91.13
Group VI PCM +Ap+Aa	1.93±0.13[b]	75.56±0.06[b]	8.09±0.16[b]	3.87±0.03[b]
% of changes PCM vs Ap+Aa	87.37	234.92	74.73	146.93

Values are mean ± S.D., n = 6. a $p < 0.05$ compared with normal control. b $p < 0.05$ compared with PCM. 'P' denotes statistical significance. "a"- '+' and '-'indicates % of changes over the normal and "b"-'+' and '-'indicates % of changes over the PCM control.

Discussion

The present work explored the potential effect of the combined methanol extract of *Andrographis paniculata* and *Achyranthes aspera* (1:1) and separate (*Ap* or *Aa*) to offer protection against hepatotoxicity induced by the over dose of paracetamol. Liver is an important metabolic organ affected by various hepatotoxins. This has been recognized as a major toxicological problem for years (Azer *et al.,* 1997). In the absence of suitable hepato protective drugs in modern medical practices, herbal medicines play an important role in the management of various liver disorders. Many of the plants show hepatoprotective activity (Hwang *et al.,* 2009; Hamza, 2010; Olaleye *et al.,* 2010). Our traditional healers use *A. paniculata* extensively in the Indian traditional system of medicine as a hepatoprotective and hepatostimulative agent. The BHC induced hepato toxicity was inhibited by the aqueous extract of *A. paniculata* in Swiss male mice (Trivedi *et al.,* 2007; Sutha *et al.,* 2010) and ethanol induced hepatotoxicity in *wistar albino* rats (Vetriselvan *et al.,* 2011). Andrographolide protects the hepatic cells against galactosamine or paracetamol induced toxicity (Handa and Sharma, 1990).

Table - 3: Levels of Urea, Uric acid and Creatinine in serum of normal, PCM intoxicated plant extract treated rats

Groups	Parameters in the serum		
	Urea (mM/L)	Uric Acid (µM/L)	Creatinine (µM/L)
Group-I: (NaCl)	15.37±0.57	1.79±0.04	1.34±0.06
Group-III: PCM Control	41.76±0.39[a]	2.96±0.11[a]	5.33±0.10[a]
% of change (Normal vs PCM)	171.69	65.36	297.76
Group –IV: PCM +AP	24.79±0.27[b]	2.22±0.10[b]	2.21±0.11[b]
% of Changes PCM vs Ap	-40.63	-25	-58.53
Group –V: PCM+Aa	26.55±0.27[b]	2.34±0.14[b]	2.43±0.21[b]
% of Changes PCM vs Aa	-36.42	-20.94	-54.40
Group VI: PCM +Ap+Aa	20.36±0.39[b]	1.97±0.08[b]	1.92±0.10[b]
% of changes PCM vs Ap+Aa	-51.24	-33.44	-63.97

Values are mean ± S.D., n = 6. a $p < 0.05$ compared with normal control. b $p < 0.05$ compared with PCM. 'P' denotes statistical significance. "a"- '+' and '-'indicates % of changes over the normal and "b"-'+' and '-'indicates % of changes over the PCM control.

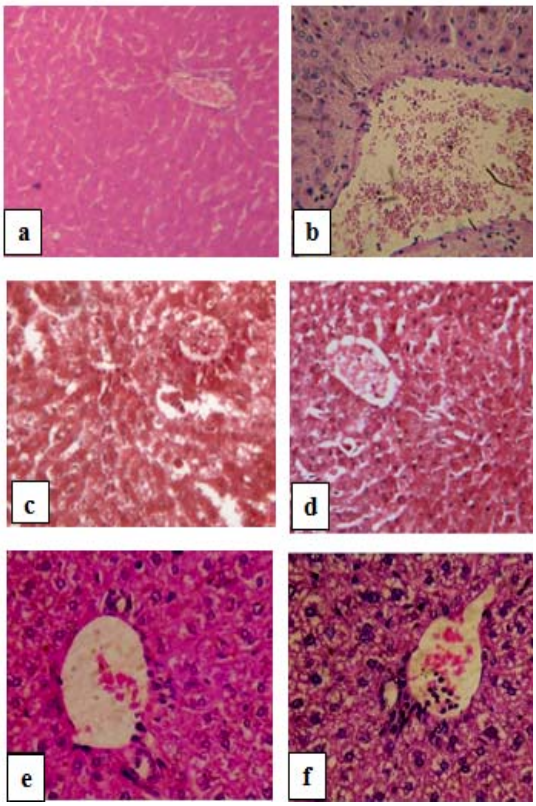

Fig.1: Shows that histopathology of liver.
(a)- Normal, (b)- PCM intoxicated, (c)- PCM+Liv 52 treated, (d) PCM+*Ap* treated, (e)-PCM+*Aa* treated, and (f) PCM+*Ap*+*Aa* treated.

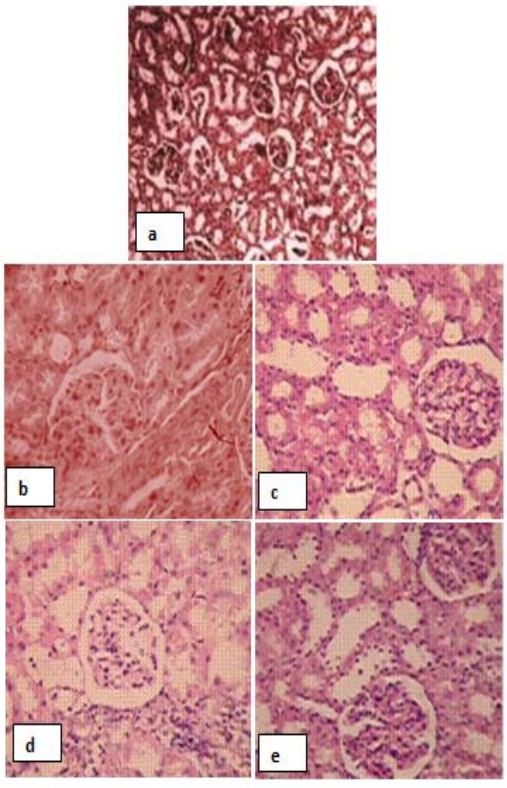

Fig.2: shows that histopathology of kidney. (a) - Normal, (b) - PCM intoxicated, (c) - PCM+*Ap* treated, (d) - PCM+*Aa* treated, and (e) - PCM+*Ap*+*Aa* treated.

In this study, paracetamol was used as a model system to induce acute hepatotoxicity in albino rat. Assessment of liver function was made by estimating the activities of serum ALT, AST, ALP and bilirubin which were present at higher concentration in cytoplasm. When there is hepatopathy, these molecules leak into the blood stream in compliance with the extent of liver damage (Nkosi *et al.,* 2005). Bilirubin is one of the most useful clinical clues to the severity of necrosis and its accumulation is a measure of binding, conjugation and excretory capacity of hepatocyte. When high doses are taken in, the major portion of paracetamol dose is available to undergo bioactivation by the cytochrome P450 system (CYP2E1, CYP3A4, CYP1A2) to create a highly reactive intermediate, N-acetyl-p-benzoquinoneimine (NAPQI) (Nelson 1995; Patten *et al.,* 1993). This intermediate can covalently bind to cellular macromolecules, causing damage and cell death (Rogers *et al.,* 1997). Accidental or incidental paracetamol overdose may be associated with toxic liver damage leading to potentially fatal, hepatic centrilobular necrosis and liver failure (James *et al.,* 2003; Bergman *et al.,* 1996). Toxicity begins with the change in endoplasmic reticulum, which results in the loss of metabolic enzymes located in the intracellular structures (Recknagel, 1983). The significant decrement of cellular antioxidant enzymes and molecule levels were observed in paracetamol intoxicated animals. The declined antioxidant enzyme activity was responsible for the increased lipid peroxidation measured as thiobarbituric acid reacting substance malondialdehyde (MDA), which causes loss of membrane fluidity, membrane integrity, and finally loss of cell functions of liver (Halliwell and Gutteridge, 1989). This peroxidative damage to membranes results in the leakage of enzymes, and metabolic byproducts into circulation. In the present study, it was observed that, the animals treated with paracetamol showed elevated levels of serum markers such as ALT, AST, ALP and bilirubin. Due to hepatocyte necrosis or abnormal membrane permeability, these enzymes are released from the cells, Hence the levels of the liver marker enzymes increase in the blood. GPT is a sensitive indicator of acute liver damage and elevation of this enzyme in non hepatic diseases is unusual. GPT is more selectively a liver paranchymal enzyme than GOT (Shah *et al.,* 2002). The rise in the SGOT is usually accompanied by an elevation in the levels of SGPT, which play a vital role in the conversion of amino acids to keto acids (Sallie *et al.,* 1999).

Oral administration of the combined methanol extract of *A. paniculata* and *Achyranthes aspera* (1:1) and separate at the dose of 250 mg/kg significantly reduced the elevated levels of the serum markers of hepatotoxicity and restored the tissue antioxidant levels in liver to normal. The results would suggest that the extracts protected the membrane integrity of the liver cells against paracetamol induced leakage of marker enzymes into the circulation. The elevated levels of alkaline phosphatase due to the administration of paracetamol reflected the pathological alteration in biliary flow (Ploa and Hewitt, 1989). In the present study also, it was found that the administration of paracetamol induced the elevation of ALP and serum bilirubin. Administration of the combined methanol extract of *A. paniculata* and *Achyranthes aspera* (1:1) and separate decreased the ALP activity, serum bilirubin levels, stabilized biliary dysfunction and normal functional status of the liver (Suky *et al.,* 2011).

Administration of the combined methanol extract of *A. paniculata* and *Achyranthes aspera* (1:1) and separately were effectively restored the decreased levels of SOD, CAT, GP_X and GST activities which may be due to the scavenging of radicals by the extract. Thus the present study indicates that the combined methanol extract of *A. paniculata* and *Achyranthes aspera* (1:1) and separately maintain the cellular integrity of hepatic tissues and helped in its regeneration. The protective ability of these plant extracts towards paracetamol induced hepatotoxicity might be attributed to the free radical scavenging activity of the extracts. The findings suggest the therapeutic use of these plants for liver ailments for ameliorating hepatotoxicity. In similar way these *Ap* and *Aa* shows the curative efficacy on damaged kidney cells, hence the affected kidney cells restored to nearly normal condition.

Conclusion

The present investigation shows changes in the levels of liver marker enzymes, renal markers in serum and antioxidant enzymes in the liver tissue of paracetamol induced rats. The changes appeared in the body of paracetamol intoxicated rats, due to an increase of oxidative stress. In addition, changes were seen in the architecture of hepatic cells. Elevated levels of serum liver

marker enzymes and reduced levels of enzymatic antioxidants were restored to near normal levels after the administration of methanol combined plant extract. Metabolites of paracetamol are excreted out through kidneys; hence some changes may occur in the architecture of the kidneys. Abnormal architecture and elevated levels of renal function markers were brought to near normal levels by the combined plant extract. These results show the protective effect of the above mentioned combined plant extract.

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