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# HEPATOPROTECTIVE AND ANTIOXIDANT PROPERTIES OF COMBINED PLANT EXTRACT OF ANDROGRAPHIS PANICULATA AND PICRORHIZA KURROA AGAINST $CCl_4$ INDUCED OXIDATIVE DAMAGE IN RATS

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**ABSTRACT**

The hepatoprotective effect of aqueous combined extract of *Andrographis paniculata* (Ap) and *Picrorhiza kurroa* (Pk) against $CCl_4$ induced oxidative damage was investigated in adult male Wistar *albino* rats. The hepatotoxicity was induced by the administration of 30% of $CCl_4$ in olive oil (1ml/kg body wt.,) to the animal in three successive doses for every 72 hours). The plant extracts of Ap and Pk (250 mg/kg body wt.,) administrated to toxicity induced rats (Separately and Combined) for 30days. The toxicity induced groups, the levels biochemical marker enzymes such as ALT, AST, ALP and bilirubin were increased significantly ($P<0.001$) which was reduced after administration of plant extracts. $CCl_4$treatment markedly decreased the level of GPx, SOD, CAT and GST in the liver, which were significantly enhanced by Ap and Pk plants extract treatment (Separate and Combined) in the liver. Also, lipid peroxidation level, which was increased after $CCl_4$ administration, was significantly reduced in the liver by plant extract treatment (Separate and combined).

**KEYWORDS:** *Andrographis paniculata* (Ap), *Picrorhiza kurroa* (Pk), Biochemical marker enzymes, $CCl_4$ (Carbon tetrachloride), Lipid peroxidation.

## INTRODUCTION

Liver is the first organ to metabolise all foreign compounds and hence it is susceptible to almost as many different diseases. Some are rare but there are a few, including hepatitis, cirrhosis, alcohol related disorders and liver cancer. A major cause of these disorders is due to exposure to different environmental pollutants and xenobiotics e.g., paracetamol, carbon tetrachloride, thioacetamide, alcohol, etc. These toxicants mainly damage liver by producing reactive oxygen species (ROS).Reactive oxygen free radicals have been known to produce tissue injury through covalent binding and lipid peroxidation and have been shown to augment fibrosis as seen from increased collagen synthesis [1]. Various xenobiotics are known to cause hepatotoxicity one among them is carbon tetrachloride ($CCl_4$) [2]. Reductive dehalogenation of $CCl_4$ by the P450 enzyme system to the highly reactive trichloromethyl radical initiates the process of lipid peroxidation which is considered to be the most important mechanism in the pathogenesis of liver damage induced by $CCl_4$[3]. Trichloromethyl radical can even react with sulfhydryl groups of glutathione (GSH) and protein thiols. Scavenging of free radicals by antioxidants could reduce the fibrosis process in the tissues [4]. Free radicals may also be a contributory factor in a progressive decline in the function of immune system [5]. Cooperative defense systems that protect the body from free radical damage include the antioxidant nutrients and enzymes. The antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). Their role as protective enzymes is well-known and has been investigated extensively with in vivo models.

Steroids, vaccines, and antiviral drugs, have been used as therapies for liver pathologies, have potential adverse side-effects, especially if administered chronically or sub-chronically. Therefore, herbal products and traditional medicines with better effectiveness and safe profiles are needed as a substitute for chemical therapeutics. As oxidative stress plays a central role in liver pathologies and their progression, the use of antioxidants have been proposed as therapeutic agents, as well as drug co-adjuvants, to counteract liver damage. A number of studies have shown that the plant extracts having antioxidant activity protect against $CCl_4$ hepatotoxicity by inhibiting lipid peroxidation and enhancing antioxidant enzyme activity [6, 7].

*Andrographis paniculata* (Family: *Acanthaceae*) is an important medicinal plant, occurring wild in India. The dried herb is a remedy for a number of ailments related to digestion, hepatoprotection, vermicidal, antiacne, analgesic, anti- inflammatory, antibacterial, antityphoid, antibiotic activities, hypoglycemic, besides immune enhancement [8]. This plant is well documented and several *in vitro* and *in vivo* studies describe its anti-cold, anti-hepatotoxic, anti-urothelial and anti-hepatotoxic properties [9].

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*Picrorhiza* demonstrated antioxidant activity similar to that of superoxide dismutase and xanthine oxidase inhibitors [10]. Bioactivity studies on *Picrorhiza kurroa* established its anti-inflammatory [11], Immunomodulatory [12] and Hydrocholeretic effects in rats and dogs [13] and antiviral activity on vaccinia virus [14]. In our present study we evaluated the protective effect of combined aqueous extract of *Andrographis paniculata* and *picrorhiza kurroa* against $CCl_4$ induced hepatotoxicity in rats.

## MATERIALS AND METHODS

### Plant Material

The leaves of *Andrographis paniculata* (Family: *Acanthaceae*) were collected during the month of Oct - Dec, 2009 from in and around Vellore District, Tamilnadu, India. The rhizomes of *Picrorhiza kurroa* (Family: *Scrophulariaceae*) were collected from Sikkim State, India.The plant materials were cleaned with distilled water and shade dried at room temperature. The plant materials were authenticated by the Department of Botany, C. Abdul Hakeem College, Melvisharam, Vellore District, Tamilnadu, India and voucher specimens were kept at the Department of Botany, C.AbdulHakeemCollege, Melvisharam, Vellore Dt., Tamil nadu, India. The shade dried plant materials were powdered by using electric blender.

### Preparation of plant extract

The shade dried plant materials were powered separately in an electrical blender and stored at 5°C until further use. 100gms of plant leaf power was taken, mixed with 500 ml of distilled water and stirred magnetically in separate containers overnight at room temperature. The residue was removed by filtration and the aqueous leaf extracts were concentrated under vacuum to get 20% solid yield.The plant extracts were tested for hepatoprotective effect in the albino rats at the selected optimum dosage of 250 mg/kg body weight and administered orally in aqueous solution.

### Animals

Adult male *albino* rats of Wistar strain weighing around 180 to 200gms were procured from Tamilnadu Veterinary and Animal Sciences University, Chennai, India. The animals were kept in polypropylene cages (four in each cage) at an ambient temperature of 25±2°C and 55-65% relative humidity. A 12±1hr light and dark schedule was maintained in the animal house till the animals were acclimatized to the laboratory conditions. They were fed with commercially available rat chow (Hindustan Lever Ltd., Bangalore. India) and had free access to water. The experiments were designed and conducted with the approval of the institutional animal ethics committee (No: 1011/c/06/CPCSEA).

**Experimental induction of hepatotoxicity**

The rats were treated with three successive doses of 30% $CCl_4$ in olive oil (1ml/kg body wt., ip) every 72 hours for hepatotoxicity studies.

**Experimental protocol**

Group I: Normal rats.
Group II: Rats were treated with Olive oil (1ml/kg body wt., ip) every 72 hrs with3 successive doses.
Group III: Rats were treated with 30% $CCl_4$ in olive oil (1ml/kg body wt., ip) every 72 hrs with 3 successive doses.
Group IV: The $CCl_4$ injected rats were administrated aqueous extract of *Andrographispaniculata* (250mg/kg body wt.,) orally by using intra gastric tubes for 30 days.
Group IV: The $CCl_4$ injected rats were administrated aqueous extract of *Picrorrhizakurroa* (250mg/kg body wt.,) orally by using intra gastric tubes for 30 days.
Group V: The $CCl_4$ injected rats were administrated combined (1:1) aqueous extract of *Andrographis paniculata* and *Picrorrhiza kurroa* (250mg/kg body wt.,) orally by using intra gastric tubes for 30 days.
Group VI: The $CCl_4$ injected rats were administrated with standard dose of silymarin (25 mg/kg body wt.,) orally for 30 days.

**Preparation of tissue homogenate**

Known amount of hepatic tissue was homogenized in suitable buffer and centrifuged at 12,000 rpm for 20 min at 4°C. The supernatant was collected and stored at -80°C for assay of the marker enzymes and antioxidant studies.

**Biochemical assay**

The biochemical marker enzymes like ALT and AST were measured by the method of Reitman and frankel, 1957 [15], ALP was measured by themethod of King and Amstrong, 1934 [16] and Serum Bilurubin was measured by the method of Mally and Evelyn 1937 [17]. The liver antioxidant enzymes namely super oxide dismutase (SOD), catalase (CAT), glutathione-S-tranferase (GST), glutathione peroxide (GPx) profiles were estimated as per standard protocol. SOD levels were measured by the method developed by Kakkar *et al.,* 1984 [18], CAT activity was measured by the method of Sinha, 1972 [19], GPx was assayed according to the method of Rotruck *et al.,* 1973 [20]. The activity of GST was estimated by the method of Habig *et al.,* 1974 [21].

**Chemicals**

Ethanol was purchased from Hayman Ltd., (Witham, Essex) CM8 3YE, England.Bovine serum albumin (BSA), 1-chloro-2,4-dinitrobenzene (CDNB), 2,4-dinitrophenylhydrazine (DNPH), 5,5-dithiobis (2-nitrobenzoic acid) (DTNB), ethylene diamine tetra acetic acid (EDTA), reduced nicotinamide adenine dinucleotide (NADH), nitro blue tetrazolium (NBT), phenazine methosulfate (PMT), potassium dihydrogen phosphate, reduced glutathione (GSH), sodium azide, sodium pyrophosphate, trichloro acetic acid (TCA), thiobarbituric acid (TBA), 5-thio- 2-nitrobenzoic acid (TNB), hydrogen peroxide ($H_2O_2$) and ascorbic acid were purchased from Sisco Research Laboratory, Mumbai, India. All chemicals and reagents used were of analytical grade.

**Histopathological studies**

The liver was removed and stored immediately in 10% formalin initially for 48 hours; thereafter the materials were transferred to 70% alcohol and stored. After fixation, the tissue was subsequently put in paraffin. Thin (5µm) sections were drawn using a microtome and then stained with hematoxylin and eosin and mounted in neutral di-styrene-dibutyl propylene (DPX) medium and examined using photomicroscopy [22].

**Statistical analysis**

Data were statistically calculated by utilizing one way ANOVA and expressed as Mean±SEM followed by Fisher's LSD post-hoc test using SPSS 10.0 software (SPSS, Inc, Chicago). The values were considered significant when $P<0.05$.

**RESULTS**

The serum activities of Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP) and bilirubin were used as biochemical markers for the assessment of early acute hepatic damage.

In group II when olive oil was injected to animals intraperitoneally, the levels of marker enzymes like ALT, AST and bilirubin were insignificantly elevated by 4.70%, 2.04%, and 2.5% respectively (Table1), but ALP was significantly elevated by 9.21% when compared in the level of normal group. $CCl_4$ was given to group III animals. The levels of biochemical marker enzymes were found to have been elevated by 107.36%, 40.13%, 96.49% and 50% respectively which was considered as significant (Table 1) when compared to the levels in normal animals.

**Table 1.** Effect of combined plant extract of *Andrographis paniculata* and *Picrorhiza kurroa* on $CCl_4$ induced hepatotoxicity: Activity of ALT, AST, ALP and Bilirubin.

| Experiment | ALT (IU/l/min/mg Protein) | AST (IU/l/min/mg Protein) | ALP (IU/l/min/mg Protein) | Bilirubin Mg/dl |
|---|---|---|---|---|
| Group-I Normal (N) | 56.5±1.87 | 147±4.8 | 228±5.7 | 0.40±0.01 |
| Group-II Olive Oil | 59.16±1.47 NS | 150±2.48 NS | 249±3.5* | 0.41±0.01 NS |
| Group-III $CCl_4$ (C) | 117±2.63** | 206±6.02** | 448±5.44** | 0.60±0.07** |
| Group-IV $CCl_4$+AP | 76.16±2.85** | 170.33±2.58** | 286±2.63** | 0.49±0.03** |
| Group-V $CCl_4$+PK | 62.6±2.50** | 155.8±2.85** | 260±6.9** | 0.48±0.01** |
| Group-VI $CCl_4$+(AP+PK) Combined (1:1) | 60.5±3.61** | 153±3.40** | 252.8±4.87** | 0.40±0.05** |
| Group-VII $CCl_4$+Silymarin | 60.23±2.59** | 152±2.78** | 250±4.68** | 0.49±0.05** |

Values are mean of six individual observations in each group ± SEM.
P Denotes statistical significance *P<0.05. **P<0.001. NS - Non significance.

The group IV hepatotoxicity induced animals, were fed with aqueous plant extract of *A. paniculata* orally. The elevated levels of marker enzymes were found to have significantly decreased by 34.90%, 17.31%, 36.16% and 18.33% respectively (Table1) when compared to the level in $CCl_4$ induced group III control animals. The hepatotoxicity induced group V animals were fed with *P.kurroa* rhizome extract, the increased marker enzymes were found to have significantly decreased by 46.49%, 24.36%, 41.96% and 20% respectively when compared to the levels in $CCl_4$ induced control groups (Table1).

The group VI hepatotoxicity induced animals were fed with combined plant extract (1:1) of *A. paniculata* and *P.kurroa.* The elevated levels of marker enzymes were found to have significantly decreased by 48.52%, 26.21%, 44.19% and 18.33% respectively when compared to the levels in control groups. The levels of decrement were higher in combined plant extract treated groups when compared to the levels in control as well as individual plant extract treated groups (Table 1).

The levels of lipid profile comprising total cholesterol, triglycerides, free fatty acids and phospholipids in serum were estimated in both normal, $CCl_4$ and plant extract fed animals. The cholesterol and triglycerides level were significantly elevated in olive oil treated group II animals by 13.40% and 6.66% respectively but the other lipid profile like free fatty acids and phospholipids were insignificantly elevated (Table2) when compared to levels in normal animals. The Group III $CCl_4$ treated animals, the levels of lipid profile enzyme were found to have been significantly elevated (Table2). After administration of plant extracts individual and combined the elevated levels were found to have reduced significantly (Table 2) when compared to levels in $CCl_4$ induced animals. The decrement was

higher in combined plant extract treated groups were recorded (Table 2).

The oxidative stress in the liver tissue was assessed by measuring the levels of thiobarbituric acid reactive substances (TBARS), lipid hydro peroxides and antioxidant defense enzymes viz., Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx) and Glutathione–S- transferase (GST) in $CCl_4$ administrated as well as plant extract treated groups.
The levels of tissue TBARS and lipid peroxides were not significantly elevated in Group-II olive oil treated animals, when compared to levels in normal animals (Table3). In group III animal, $CCl_4$ was given by intraperitoneal injection. In these animals the levels of TBARS and lipid peroxides were found to have been significantly increased when compared to levels in normal animals (Table3). The plant extract of *A.paniculata* and *P.kurroa* were administrated individually as well as combined to the respected hepatotoxicity induced groups. The combined plant extract treated groups, the elevated levels of TBARS and lipid hydro peroxides were decreased significantly by 47.55% and 31.48% respectively when compared to the level in control as well as individual plant extract treated groups (Table3).

The activity of liver antioxidant defense enzymes viz., SOD, CAT and GPx were decreased by 2.43%, 4.66% and 2.49% respectively in the liver tissue of olive oil induced Group-II animals. This was not considered to be significant. In these animals the levels of GST was found to have significantly decreased by 8.29% (Table3) when compared to levels in normal animals. In group III animals which were treated with $CCl_4$, the levels of SOD, CAT, GPx and GST were found to have significantly decreased by 51.62%, 49.20%, 45.98% and 55.55% respectively when compared to the level of normal group I animals. The plant extract was fed with hepatotoxic induced animals individually and combined to the respected groups. The combined extract treated groups the decreased levels of antioxidant defense enzymes were significantly increased by 100.67%, 85.16%, 71.28% and 105.59% respectively. The increment of antioxidant defense enzymes was higher in combined plant extract treated groups were recorded (Table3).

**Table 2.** Effect of combined plant extract of *Andrographis paniculata* and *Picrorhiza kurroa* on $CCl_4$ induced hepatotoxicity: Activity of TC, TG, FFA and Phospolipids

| Experiment | TC | TG | FFA | Phospolipid |
|---|---|---|---|---|
| Group-I Normal (N) | 37.3±1.75 | 114±3.74 | 61±3.7 | 79±1.41 |
| Group-II Olive Oil | 42.3±1.63* | 121.6±1.75* | 62.5±1.04 NS | 81.5±1.04 NS |
| Group-III $CCl_4$ (C) | 88.3±2.25** | 160.5±3.08** | 107.6±1.75** | 128.3±2.16** |
| Group-IV $CCl_4$+AP | 58.8±1.94** | 121.8±1.47** | 78.5±2.88** | 97.6±1.86** |
| Group-V $CCl_4$+PK | 50.51±1.87** | 123±1.41** | 68.83±1.47** | 96.6±1.78** |
| Group-VI $CCl_4$+(AP+PK) Combined (1:1) | 44.5±2.66** | 120±1.26** | 64.12±1.68** | 82.41±1.77** |
| Group-VII $CCl_4$+Silymarin | 41.7±2.89** | 119±1.87** | 64.12±1.68** | 82.41±1.77** |

Values are mean of six individual observations in each group ± SEM.
P Denotes statistical significance *P<0.05. **P<0.001. NS - Non significance.

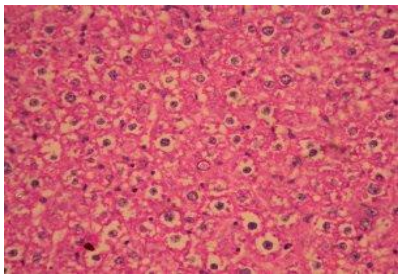

**Figure 1.** Normal rat liver

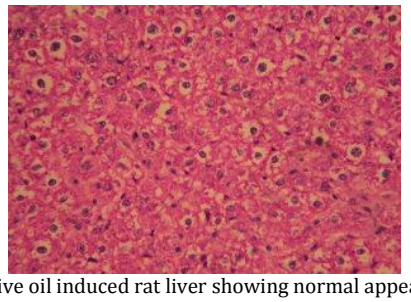

**Figure 2.** Olive oil induced rat liver showing normal appearance

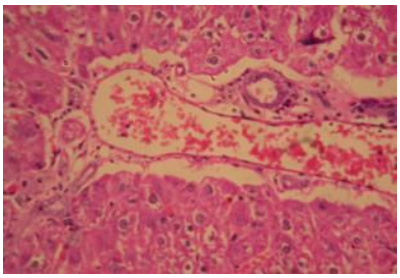

**Figure 3.** $CCl_4$ induced liver showing necrosis, fatty changes, vacuolization, ballooning degeneration and loss of cellular boundaries.

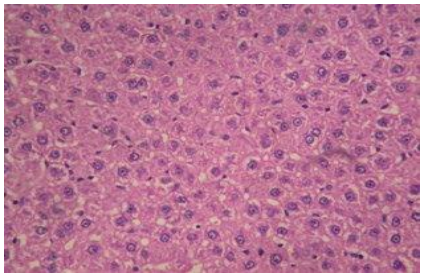

**Figure 4.** $CCl_4$ + plant extract (Ap) fed liver showing near normal appearance

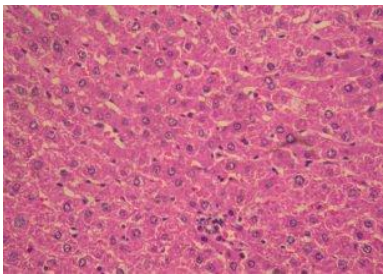

**Figure 5.** $CCl_4$ + Plant extract (Pk) fed liver showing near normal appearance

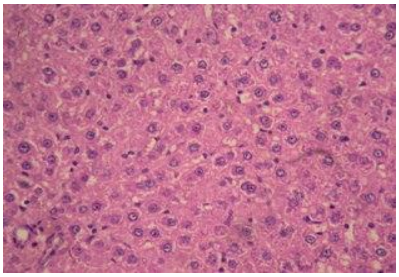

**Figure 6.** $CCl_4$ + combined plant extract fed liver showing near normal appearance

Histopathological studies also provide supportive evidence for biochemical analysis. Histopathological examination of liver sections of normal groups showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein (Figure 1). In group – II olive oil induced liver damaged animals also no pathological changes were detected in hepatocytes (Figure 2). Disarrangement of normal hepatic cells with necrosis, massive fatty changes, vacuolization, ballooning degeneration, broad infiltration of the lymphocytes and

kuffer cells around the central vein and the loss of cellular boundaries are observed in $CCl_4$ intoxicated liver (Figure 3).

**Table 3.** Effect of combined plant extract of *Andrographis paniculata* and *Picrorhiza kurroa* on $CCl_4$ induced hepatotoxicity: Activity of TBARS, Lipid hydro peroxide, SOD, CAT, CPx and GST

| Experiment | TBARS (nm/100g tissue) | Lipid hydro peroxide (nm/100g tissue) | SOD (U1/mg Protein) | CAT (U2/mg Protein) | GPx (U3/mg Protein) | GST (U4/mg Protein) |
|---|---|---|---|---|---|---|
| Group-I Normal (N) | 0.65±0.01 | 68.16±1.72 | 6.16±0.10 | 168±1.54 | 10.83±0.21 | 6.03±0.10 |
| Group-II Olive Oil | 0.67±0.01 NS | 71.33±1.21 NS | 6.01±0.02 NS | 160.16±1.3* | 10.56±0.17 NS | 5.53±0.12 NS |
| Group-III $CCl_4$ (C) | 1.43±0.04** | 111.16±2.71** | 2.98±0.07** | 85.33±2.80** | 5.85±0.18** | 2.68±0.09** |
| Group-IV $CCl_4$+AP | 0.93 ±0.22** | 85.5±2.94** | 4.98±0.14** | 146.6±2.80** | 9.18±0.14** | 4.98±0.14** |
| Group-V $CCl_4$+PK | 0.84±0.36 ** | 82.12±1.32** | 5.23±0.18** | 148±2.1** | 9.11±0.14** | 5.08±0.11** |
| Group-VI $CCl_4$+(AP+PK) Combined (1:1) | 0.75±0.02 ** | 76.16±1.94** | 5.93±0.12** | 155.3±3.55** | 9.95±0.24** | 5.50±0.17** |
| Group-VII $CCl_4$+Silymarin | 0.70±0.85** | 70.23±1.72** | 5.98±0.13** | 158±3.67** | 10.02±0.38** | 5.51±0.18** |

Values are mean of six individual observations in each group ± SEM.
P Denotes statistical significance *P<0.05. **P<0.001. NS - Non significance.
SOD – $U_1$- One unit of activity was taken as the enzymes reaction which gives 50% inhibition of NBT reduction in one minute.
CAT – $U_2$- µmoles of hydrogen peroxide consumed per minute.
GPx – $U_3$- µg of glutathione consumed per minute.
GST – $U_4$- µmoles of CDNB – GSH conjugate formed per minute

*Andrographis paniculata* (Ap) leaf extract was given $CCl_4$ injected groups. In the liver sections of these groups a few micro and macro vesicular type of fatty droplets were observed. No portal inflammation, vacuolization, ballooning degeneration, and necrosis were observed in this group (Figure 4).

The leaf extract of *Picrorhiza kurroa* (Pk) treated liver cells revealed restoration of the hepatic tissue architecture. In the liver sections of animals which were given $CCl_4$ and treated with *Picrorhiza kurroa* (Pk) restoration of the hepatic cells was seen. A few micro and macro-vesicular type of fatty droplets, less vacuole formation, absence of necrosis and less visible changes overall were observed in the plant extract treated hepatocytes (Figure 5).

The combined plant extract treated rats, the liver cells showed complete reversal of toxic effects. No necrosis, vacuolization, ballooning degeneration or broad infiltrations of the lymphocytes were observed in this group. The central vein and portal triads appears normal. Some of the hepatocytes showed binucleation suggesting regenerative activity with feathery degeneration of hepatocytes. Micro and macro vesicular type of fatty droplets were also not seen (Figure 6).

**DISSCUSSION**

The present study reports the potential hepato-protective activity of *A. paniculata* and *P. kurroa* against hepatic injury produced by carbon tetrachloride in rats. $CCl_4$ is a well-known hepatotoxic agent and the preventive action of liver damage by $CCl_4$ has been widely used as an indicator of liver protective activity of drugs in general. The changes associated with $CCl_4$ induced liver damage are similar to that of acute viral hepatitis. Leakage of cellular enzymes into plasma is a hall mark sign of hepatic injury or damage. In addition, the extent and type of liver injury or damage can be assessed based on the presence or absence of specific marker enzymes in the bloodstreams. Generally, the measurement of ALT and AST are commonly used as marker enzymes of hepatotoxicty [23,24]. When liver call plasma is damaged a variety of enzymes located normally in cytosol is released into the blood, thereby causing increased enzyme level in the serum. The estimation of enzymes in the serum is a useful quantitative marker of the extent and types of hepatocellular damage [25].

In the present investigation, the dose of $CCl_4$ used, caused liver injury in rats. The rats treated with an overdose of $CCl_4$ developed significant hepatic damage, which was observed through a substantial increase in the concentration of serum parameters. Pretreatment of the rats with *A. paniculata* and *P. kurroa* (separate and combined)extract at 250mg/kg body wt., for 30 days before $CCl_4$ administration resulted in a significant protection of $CCl_4$ induced by the elevation of serum marker enzymes. This suggests that the plant extracts could repair the hepatic injury and or restore the cellular permeability, thus reducing the toxic effect of $CCl_4$ induced liver toxicity and preventing enzymes leakage into the blood circulation. The results are in agreement with the commonly accepted view that serum level of transaminase returns to normal with healing of hepatic parenchyma and the regeneration of hepatocytes. Other investigators have reported similar observations [26,27].

The activity of serum alkaline phosphatase (ALP) was also elevated during $CCl_4$ administration. Alkaline phosphatase is excreted normally via bile by the liver. In liver injury due to hepatotoxin, there is a defective excretion of bile by the liver which is reflected in their increased levels in serum. Hyperbilirubinaemia is a very sensitive test to substantiate the functional integrity of the liver and severity of necrosis which increases the binding, conjugation and excretory capacity of hepatocytes that is proportional to the erythrocyte degeneration rate [28]. Depletion of elevated bilirubin level together with the suppression of activity of ALP in serum of rats treated with *A. paniculata* and *P. kurroa* (Separate and Combined) suggest to stabilize biliary dysfunction of rat liver during chronic injury with $CCl_4$.
Biological systems try to protect themselves against from several toxicants. These include free radical scavengers and chain reaction terminators; enzymes such as SOD, CAT, and GPx system. These enzymes constitute a mutually supportive team of defense against ROS [29]. The balance between ROS production and these antioxidant defenses may be lost, and 'oxidative stress' results, which through a series of events deregulates the cellular functions leading to hepatic necrosis. Under the oxidative stress, some endogenous protective factors such as GPx and catalase are activated in the defense against oxidative injury.

Reactive oxygen species (ROS), such as superoxide anions and $H_2O_2$, are produced throughout cells during normal aerobic metabolism. The intracellular concentration of ROS is a consequence of both their production and their removal by these antioxidants. These enzymes work in concert to detoxify superoxide anion and $H_2O_2$ in cells. Our results indicated that pretreatment of *A. paniculata* and *P. kurroa* (Separate and Combined) caused an increase in the activity of antioxidant enzymes. Recent studies on the antioxidant properties of flavonoids from various plant extracts reveal their stimulatory action on antioxidative enzymes [30].

Some flavonoids exert a stimulatory action on transcription and gene expression of certain antioxidant enzymes [31]. The antioxidant enzyme system plays an important role in the defense of cells against oxidative insults. The study examined the ameliorating effect of the extracts from *A. paniculata* and *P. kurroa* (Separate and Combined) on

oxidative stress induced by $CCl_4$. The levels of TBARS approached the normal control in all *A. paniculata* and *P. kurroa* (Separate and Combined) treated animals exposed to $CCl_4$. Restoration of TBARS and to nearly normal levels by these extracts may be due to an enhancement of antioxidant enzymes, such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione-S-transferase. In the present study, $CCl_4$ treatment significantly decreased the activity of antioxidant enzymes levels, indicating that the plant extract could reduce the generation of free radicals and increase free radicals scavenging mechanism.

Most of the hepatotoxic chemicals including $CCl_4$ damaged liver mainly by inducing lipid peroxidation directly or indirectly. In higher animals peroxy radicals are important agents that mediate lipid peroxidation thereby damaging cell membrane, leading to liver injury, artherosclerosis and kidney damage [32]. In the present study, *A.paniculata* and *P.kurroa* (Separate and Combined) extract was effective in reducing the production of TBARS. They reported the strongest inhibition of malondialdehyde (MDA), MDA is a major reactive aldehyde that appears during the peroxidation of biological membrane polyunsaturated fatty acid [33]. Therefore, the hepatic content of MDA is used as an indicator of liver tissue damage involving a series of chain reactions [34-37]. This significant report confirms that pretreatment with *A.paniculata* and *P.kurroa* (Separate and Combined) extracts could effectively protect against the hepatic lipid peroxidation induced by $CCl_4$.

Hyperlipidemia is an important associated complication of $CCl_4$ induced liver injury. The imbalances in the lipid metabolism play a role in aggravating the lipid peroxidation [38]. Lipids when react with free radicals undergo a highly damaging chain reaction of lipid per oxidation (LP) leading to both direct and indirect effects. HMG CoA reductase catalyses rate limiting cholesterol biosynthesis and its activity directly correlates with the extent of tissue cholesterol synthesis, which is significantly activated during $CCl_4$ ingestion. Plasma lecithin cholesterol acyl transferase (LCAT) is the enzyme involved in the esterification of cholesterol in the plasma [39]. The enzyme lipoprotein lipase is involved in the uptake of triglycerides rich lipoprotein by extra hepatic tissue. Phospholipids are vital components of bio-membranes. Their primary target is lipid peroxidation [40].

The hyper-lipidemic condition seen in the serum of $CCl_4$ administered animals was restored to normal after the supplementation of the plant extract. The decreased serum cholesterol in the plant extract administrated rat might be due to increased activity of enzyme LCAT involved in esterification of cholesterol in the plasma. The significant decrease in the triglycerides (TG) in serum in the *A. paniculata* and *P. kurroa* (Separate and Combined) plant extract administrated animals might be due to decreased accumulation of lipoprotein. This might be due to increased activity of lipoprotein lipase, which is involved in the uptake of TG rich lipoprotein by extra hepatic tissue. The significant decrease in the free fatty acid accumulation in serum of plant extract administrated animals, might be due to decreased lipid breakdown, which corroborates with results obtained where in a decreased lipid peroxidation and increased activity levels of antioxidant defense enzymes were recorded. The significant decrease of the phospholipids in the serum of plant extract administrated animals might be due to decreased peroxidation in the biomembrane of hepatocytes.

Histological changes such as steatosis (Fatty changes in heapatocytes), perivenular fibrosis and significant pathomorhological alteration were observed in $CCl_4$ induced liver damaged groups. These changes can alter the properties of a cell. Morphologic features that include vacuolization, ballooning degeneration, inflammatory cell infiltrate and mixed macro and micro vesicular steatosis were observed in the liver of $CCl_4$ induced liver damaged groups. Fat accumulation in the liver induced by $CCl_4$ administration also seems to be associated with depletion of hepatic *S*-adenosylmethionine (SAM) leading to serious biochemical disturbances including inhibition of essential methylation reaction. Inhibition of these methylation reactions lead to steatosis, apoptosis and accumulation of damaged proteins with isoasparty residues. Hepatic damage observed in the present study may be partially attributed to cytochrome P-450 generated metabolic cytochrome-P-450 dependent enzyme activities in the liver that tend to be present at their greatest concentration near the central vein and at their lowest near the peripheral site [41]. This could be due to the formation of highly reactive free radicals because of oxidative threat caused by $CCl_4$. The free radical generation would lead to auto-oxidation of the fatty acid present in the cytoplasmic membrane phospholipids and cause functional and morphological changes in the cell membrane [42]. The accumulated hydroperoxides can cause cytotoxicity, which is associated with the peroxidation of membrane phospholipids by lipid peroxides [43].

All the changes observed were very much reduced in the animals after the oral administration of aqueous extracts of *Andrographis paniculata* and *Picrorhiza kurroa* (Separate and Combined). Histopathological observations suggested the ability of these extracts to condition the hepatic cells to a state of accelerated regeneration thus decreasing the leakage of ALT, AST and ALP into the circulation and increasing the antioxidant defense enzymes. In combinedplant extract administered animals the hepatic cell architecture appeared to be almost normal.

Based on the above results, it could be concluded that the combined plant extract of *Andrographis paniculata* and *Picrorhiza kurroa* is a more potent hepato-stimulant exerting a significant hepatoprotective action against $CCl_4$ induced liver toxicity.

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