EFFECTS OF FEW INDIAN MEDICINAL HERBS ON CARBON TETRACHLORIDE INDUCED HEPATIC INJURY IN ANIMALS

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ABSTRACT: The Phyllantus nirruri (PN), Andrographis paniculata (AP) and Picrorhiza kurroa (PK) have been widely used in number of hepatoprotective formulations based on their traditional claims. However, no published data showing the comparative study of these herbs is available. The aim of the present study was to evaluate the hepatoprotective effect of standardised herbal extracts of PN 142.5 mg/kg, p.o), AP (300 mg/kg, p.o) and PK (200 mg/kg, p.o) on carbon tetrachloride (CCI₄) induced acute and chronic hepatic damage in rats. In chronic hepatitis, CCI₄ (0.2 ml/kg, p.o) was administered twice weekly during eight weeks of extract treatment, whereas, in acute hepatitis, CCI₄ (0.5 ml/kg, p.o) was given on 10th day of administration of extract. Liver injury was analyzed by estimating the biochemical marker enzymes levels and antioxidant activities. Histological studies were also carried out. Statistically significant reversal of the elevated serum levels and depleted tissue levels of biochemical marker enzymes were found as the biochemical indices for hepatoprotection. The histological scores and liver weight was significantly reduced as well as SOD and catalase activities were significantly increased in treated groups compared to CCI₄ control. Further, PK and PN were found to be most and least effective respectively in chronic CCI₄ induced liver damage, whereas, AP exhibited least protection than PN and PK in acute CCI₄ hepatic injury. These results show that the standardised extracts PN, AP and PK possess hepatoprotective potential and prevented hepatic damage induced by CCI₄.

KEYWORDS: Andrographis paniculata; carbon tetrachloride; Hepatoprotective; Picrorhiza kurroa; Phyllanthus nirruri.

INTRODUCTION:
The use of herbal resources such as Ayurvedic, Siddha and Chinese medicine for the treatment of liver diseases, quite an old approach, is experiencing a great demand in recent years. These medicinal systems conceptualize a general imbalance of the dichotomous energies leads to the disease and they focus on medicine that balance these energies and maintain good health. The increased demand for herbal medicine is attributed to wide biological activities, higher margin of safety than the synthetic drugs and lesser costs. It is important to note that most of the formulations available as remedy from hepatic manifestation are polyherbal without elucidating the efficacy of individual herb of the formulation.

Phyllanthus nirruri (Fam. Euphorbiaceae) found in the rainforests of the Amazon and other tropical areas throughout the world including southern India and China. It has been claimed to be an excellent remedy for jaundice and infective hepatitis. It is well known for its diuretic, analgesic, stomachic, antispasmodic, febrifugal, and cell protective properties. Phyllanthus may help decrease the amount of hepatitis B virus found in the bloodstream.

Andrographis paniculata (Fam. Acanthaceae), commonly known as kalmegh, found throughout plain regions in India and other Asian countries. The various parts of this plant are reported to possess anti-asthmatic, anti-inflammatory, antiviral, cardioprotective, antimicrobial, antiprotozoal and hepatoprotective properties. It was found to normalize completely the CCl₄-induced increase in the pentobarbitone sleep time of mice.

Picrorhiza kurroa (Fam. Scrofulariaceae) commonly grows in the himalayas, from kahsmir to Sikkim regions of India at elevation of 2700-4500 meters. It is found to be choleretic hepatoprotective, neuroprotective and immunostimulant.

As shown above, Phyllanthus nirruri (PN), Andrographis paniculata (AP) and Picrorhiza kurroa (PK) have been
shown to offer hepatoprotection against number of hepatotoxic agents in experimental animals. While the hepatoprotective activity of PN, AP and PK are well documented, no comparative systematic pharmacological screening of these herbs has been reported so far. Among number of Indian herbs for hepatoprotection, we selected these three Indian herbs as they are inexpensive, easily available and have a well-established place in traditional Indian system of medicine. They have similar antioxidant, hepatoprotective, stress relieving and cellular protective potential. Therefore, present study was undertaken to evaluate the hepatoprotective effect of standardised herbal extracts of PN, AP and PK on carbon tetrachloride (CCl₄) induced acute and chronic hepatic damage in albino rats.

MATERIALS AND METHODS:

Chemicals & reagents
All chemicals used were of analytical grade and purchased from standard companies. Nitroblue tetrazolium (NBT) was procured from Loba Chemicals. AST, ALT, ALP and Bilirubin kits were purchased from Crest Biosystems (Goa, India).

Plant extracts
Silymarin was procured from Micro labs, Hosur, Tamil Nadu (India). The standardized extracts of Phyllanthus nirruri, Andrographis paniculata and Picrorhiza kurroa were gifted by Phytotech extracts Bangalore (India), Natural Remedies Pvt Ltd, Bangalore (India) and All Seasons Herbs, Bangalore (India) respectively.

Experimental animals
Laboratory bred female Sprague-Dawley (SD) rats weighing 150-200 g were housed at 25° ± 5°C in a well-ventilated animal house under 12:12 h light dark cycle. Institutional Animal Ethics Committee approved the experimental protocol. The animals were maintained under standard conditions in an animal house as per the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). The rats had free access to standard rat chow (Amrut Laboratory Animal feed, Maharashtra, India) containing (% w/w) protein 22.10, oil 4.13, fibre 3.15, ash 5.15, sand (silica) 1.12, and water ad libitum.

Experimental protocol
The animals were divided into six groups consisting of six animals each. Group I and II served as normal and CCl₄ control (vehicle treated). The group III was given silymarin, 100 mg/kg, p.o. The group IV, V and VI were administered orally standardized Phyllanthus nirruri extract (PN), 142.5 mg/kg, Andrographis paniculata extract (AP), 200 mg/kg and standardized Picrorhiza kurroa extract (PK), 200 mg/kg respectively.

Carbon tetrachloride induced chronic hepatitis model
The animals were treated in their respective groups for eight weeks. During this treatment, animals in groups II to VI received CCl₄ (0.2 ml/kg, p.o) diluted with liquid paraffin (1:1) twice weekly. Eight weeks after CCl₄ administration started, and 24 hours after the last treatment, the rats were sacrificed. Blood samples were collected by the retro orbital puncture method. Serum was separated and biochemical markers - alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin (total and direct) were estimated. Liver tissue homogenate (LTH) was prepared in sucrose solution (0.25 M) and used for estimation of endogenous biochemical markers and biological antioxidants viz., superoxide dismutase (SOD) and catalase activities. The liver was then subjected to histopathological examination.

Carbon tetrachloride induced acute hepatitis model
At the end of 10 days treatment in their respective groups, group II to VI received a single dose of CCl₄ (0.5ml/kg, p.o) diluted with liquid paraffin (1:1). After 24 hours of CCl₄ administration the animals were sacrificed and blood was collected by retro orbital puncture. Serum was separated and biochemical markers [ALT, AST, ALP and bilirubin (total and direct)] were estimated. Liver tissue homogenate (LTH) was prepared in sucrose solution (0.25M) and subjected to estimation of biochemical markers and antioxidants. The liver was also subjected to histopathological examination.

Statistical analysis
Results are expressed as mean ± SEM. Statistical significance was assessed using One-way Analysis of variance (ANOVA) followed by Tukey multiple comparison tests. P<0.05 was considered significant.

Histopathological studies
The liver tissue was dissected out and fixed in 10% formalin, dehydrated in gradual ethanol (50–100%), cleared in xylene, and embedded in paraffin. Sections were prepared and then stained with hematoxylin and eosin (H–E) dye for photomicroscopic observations. The hepatic damage was determined by giving scores depending on the intensity as follows: normal hepatic architecture and hepatocytes – score 00; focal degeneration of hepatocytes (1/3rd or less) – score 01; diffuse degeneration of hepatocytes (1/3rd - 2/3rd) – score 02; diffuse degeneration of hepatocytes (2/3rd or more) – score 03; diffuse degeneration of hepatocytes + Fatty vacuoles / vesicular formations – score 04.

RESULTS:
Effect on carbon tetrachloride induced chronic hepatitis model
Administration of CCl₄ resulted in elevation of biochemical markers ALT, AST, ALP and bilirubin (Total and Direct) in serum and depletion in LTH significantly compared to vehicle control. In animals pretreated with PN, AP and silymarin, there was significant fall and rise of biochemical marker levels (p<0.001) in serum and LTH respectively compared to
CCL4 control. The liver weight was significantly (p<0.001) lowered in pre-treated groups compared to CCL4 control. Further, we found that upon pre-treatment of animals with PN, AP, PK and silymarin, the activities of SOD and catalase were reverted back to normal when compared to CCL4 control. Moreover, there was significant (p<0.05) decrease in histological scores of microscopic slides of hepatocytes in animals of all treated groups compared to CCL4 control. The observation of microscopic slides of CCL4 control group showed fatty changes with necrosis, ballooning degeneration, broad infiltration of lymphocytes and loss of cellular boundaries with formation of fibrosis and steatosis (Figure Ib). However, in treated groups, there was mild to moderate necrosis, ballooning, degeneration with least infiltration of lymphocytes and cellular boundaries (Figure Ic to Ie). Comparative assessment of different treated groups indicated that standardized extract of PK was most effective, while standardized extract of PN was least effective.

**Effect on carbon tetrachloride induced chronic hepatitis model**

Administration of single dose of CCL4 showed significant (p<0.001) rise in biochemical marker enzyme levels in serum and fall in LTH compared to vehicle control. Pretreatment of animals with PN, AP, PK and silymarin showed a significant (p<0.001) recovery in biomarker enzymes such as ALT, AST, ALP and bilirubin (Total and Direct) levels in serum as well as in LTH. Moreover, the liver weight was significantly (p<0.001) decreased in pretreated groups compared to CCL4 control. Similarly, we also found that in all prophylactically treated groups, there was significant (p<0.001) inclination in the SOD and catalase activities in LTH compared to CCL4 control. Moreover, biochemical estimations were confirmed by histological determination of hepatocyte sections prepared from treated animals. There was rise in histological scores in CCL4 control group (Figure IIa) showing massive infiltration of lymphocyte, ballooning degeneration, necrosis, loss of cellular boundaries with formation of fibrosis and steatosis. Prophylactic administration of silymarin, PN (Figure IIb), AP (Figure IIc) and PK (Figure IId) decreases the histological scores revealing mild to moderate infiltration of lymphocytes, less degeneration, and few losses of cellular boundaries with mild steatosis. Among the three herbs investigated in the study, AP was found be least effective than PN and PK was found to be most effective.

**DISCUSSION:**

The present research was carried out to compare the hepatoprotective efficacy of standardized herbal extracts of *Phyllanthus niruri*, *Andrographis paniculata* and *Picrorrhiza kurroa* using acute and chronic hepatotoxicity induced by CCL4 in rat model and find out the therapeutically better efficacious extract. The result of the study indicate that *Picrorrhiza kurroa* (PK) is most effective in both models of acute and chronic hepatic damage induced by CCL4, whereas, *Phyllanthus niruri* (PN) exhibited least and moderate protection in chronic and acute models respectively. Further, *Andrographis paniculata* (AP) provided moderate and least cover to hepatic damage in acute and chronic hepatic damages respectively in experimental animals. The mechanism by which CCL4 manifests its injurious effects on the liver is varied. Since the liver is an organ with diverse functional activity, the hepatoprotective activity of a drug should be based on its ability to reduce the injurious effects or preserve the architecture and physiological functions of the liver, disturbed by a hepatotoxin. The hepatotoxic effects of CCL4 are largely due to its active metabolite, trichloro methyl radical. The activated radicals bind covalently to the macromolecules and induce peroxidative degradation of membrane lipids of endoplasmic reticulum (ER) that is rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxides, which in turn give products like malondialdehyde that cause damage to the membrane. The lipid peroxidative degradation of biomembranes is one of the principal causes of hepatotoxicity of CCL4. Damage to the membrane leads to leakage of endogenous biomarkers into the circulation and thereby decreasing the intracellular enzyme levels. This is evidenced by an elevation in the serum marker enzymes such as AST, ALT, ALP and Direct and Total bilirubin in the circulation and depletion of these enzymes in the liver tissues. Prophylactic administration of all standardized extracts was found to revert back the normal condition to the hepatic musculature as shown by biochemical and histological findings. The stimulation of hepatic regeneration was considered as the possible hepatoprotective mechanism of standardized herbal extracts. Such stimulation was known to cause the liver to become more resistant to hepatotoxins induced liver injuries. Likewise, activation of the functions of the reticuloendothelial system or inhibition of protein biosynthesis were also considered as some possible hepatoprotective mechanisms, which could reduce the hepatotoxicity of CCL4. Picroside II of PK could be attributed for enhanced hepatoprotection than PN and AP. This active principle of PK was known to prevent hepatocytes from necrosis by the mechanism of up regulating the bcl-2 gene expression and antioxidation. A major defense mechanism involves the antioxidant enzymes, including SOD and catalase, which convert active oxygen molecules into non-toxic compounds. The lipid peroxidation is accelerated when free radicals are formed as the results of losing a hydrogen atom from the double bond in the structure of unsaturated fatty acids. Scavenging of free radicals is one of the major antioxidation mechanisms to inhibit the chain reaction of lipid peroxidation. The free radical scavenging activity of the crude drug extracts was evaluated by measuring SOD and catalase in liver tissue homogenate (LTH). The endogenous antioxidants were remarkably decreased at times of stress induced by xenobiotic hepatotoxin such as CCL4. Pretreatment of animals with standardized herbal
extracts shows the significant rise in SOD and catalase activities in liver tissue homogenate. Elevated activity of catalase in LTH is more important than rise in SOD activity alone as without a simultaneous rise in catalase activity, elevated SOD activity may lead to intracellular accumulation of H₂O₂ with detrimental effects 35. The maximum incline was seen in groups treated with PK. It is speculated that the augmented antioxidant activities could be due to the presence of more potent active constituents in PK than in PN and AP. The hepatotoxin such as CCl₄ induces a significant increase in liver weight by blocking of secretion of hepatic triglycerides into the plasma 36. Administration of PN, PK and AP prevents this blockage and thereby keeps normal weight of liver at times of stress.

Since saponins are thought to have a membrane-stabilizing action 37, the cytoprotective effect of PN may be related to this capability. This membrane-stabilizing role of PN was predominantly found during acute than chronic hepatic damage. The picrosides I, II, III could be responsible for highly protective role of PK at times of both acute and chronic damage to hepatocytes. Even though, the standardized extract of AP provided cover to hepatic damage which is inferior to PN and PK, nevertheless, they enhances the anti-oxidation due to presence of flavonoid.

Thus the results of the study shows that the standardized herbal extracts of PN, AP and PK were found to be effective in ameliorating the manifestations of acute and chronic hepatic damage induced by CCl₄. Among them, PK was found to be superior in preventing the damage to hepatic musculature than PN and AP. It is often speculated that the herbs individually might not be so efficacious than when they are combined together. Hence it is found that many formulations that are polyherbal are highly effective in reversing the manifestation of fatty changes, degenerations, necrosis, hepatitis and cirrhosis.

In conclusion, all three herbs were found to be effective in preventing hepatotoxicity, considering the profile of liver enzymes. The standardized extract of PK was found to be superior in preventing the damage to hepatocytes during acute or chronic injury, whereas, AP was inferior to PN in avoiding the acute damage caused by CCl₄. However, PN was less effective than AP in providing protection to the hepatic cell during chronic injury in experimental animals. Histologically, near normal morphology of liver was observed with PK, PN and AP suggesting their superior cellular protective potential even in the presence of toxins such as CCl₄.

### Table I: Effect on Serum AST, ALT, ALP, Direct (D-BIL) and Total bilirubin (T-BIL) levels and liver weight in CCl₄ induced chronic hepatitis in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AST(U/L)</th>
<th>ALT(U/L)</th>
<th>ALP(U/L)</th>
<th>Serum Bilirubin (mg/dl)</th>
<th>Liver Weight (Grams/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>118±6.6</td>
<td>115±10.3</td>
<td>343±23</td>
<td>1.3±0.10</td>
<td>2.4±0.10</td>
</tr>
<tr>
<td>HP control (CCl₄)</td>
<td>732±25.5***</td>
<td>636±22.5***</td>
<td>1200±28.5***</td>
<td>3.1±0.10***</td>
<td>5.7±0.13***</td>
</tr>
<tr>
<td>Silymarin + CCl₄</td>
<td>176±8.13***</td>
<td>186.5±27.5***</td>
<td>480±15.2***</td>
<td>1.65±0.05***</td>
<td>3.23±0.07***</td>
</tr>
<tr>
<td>PN + CCl₄</td>
<td>403±14.47***</td>
<td>342±35***</td>
<td>773±12.01***</td>
<td>2.1±0.10***</td>
<td>4.46±0.26*</td>
</tr>
<tr>
<td>AP + CCl₄</td>
<td>275±4.59***</td>
<td>306±25***</td>
<td>554±21***</td>
<td>1.85±0.05***</td>
<td>3.95±0.05***</td>
</tr>
<tr>
<td>PK + CCl₄</td>
<td>209±22.9***</td>
<td>254±32***</td>
<td>476±26***</td>
<td>1.65±0.05***</td>
<td>3.69±0.01***</td>
</tr>
</tbody>
</table>

Values are mean ±S.E.M, n=6 Symbols represent statistical significance.
***P<0.001; ** P<0.01 & *P<0.05, comparison between control and Hepatotoxic (HP) control,
**a***P<0.001; **aP<0.01 & *aP<0.05 Vs hepatotoxic control. HP: Hepatotoxic; Silymarin:100 mg/kg, p.o; PN: Phyllanthus nirruri 142.5 mg/kg, p.o; AP: Andrographis paniculata 200 mg/kg , p.o; PK: Picrorrhiza kurroa 200 mg/kg , p.o.
Table II: Effect on AST, ALT, ALP, Direct (D-BIL) & Total bilirubin (T-BIL) levels, SOD, Catalase (CAT) activities in liver homogenate and histological scores in CCl4 induced chronic hepatitis in rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AST(U/L)</th>
<th>ALT(U/L)</th>
<th>ALP(U/L)</th>
<th>Serum Bilirubin (mg/dL)</th>
<th>SOD</th>
<th>CAT</th>
<th>Histologica l scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>473±12</td>
<td>606±8.8</td>
<td>290±5.7</td>
<td>3.06±0.06</td>
<td>4.43±0.23</td>
<td>4.36±0.18</td>
<td>12.2±0.25</td>
</tr>
<tr>
<td>HP control (CCl4)</td>
<td>113±18.5***</td>
<td>193±8.8***</td>
<td>80±5.7***</td>
<td>0.9±0.05***</td>
<td>1.4±0.10***</td>
<td>1.16±0.08***</td>
<td>4.5±0.5***</td>
</tr>
<tr>
<td>Silymarin + CCl4</td>
<td>423±17.6***</td>
<td>543±12***</td>
<td>266±6.6***</td>
<td>2.83±0.03***</td>
<td>3.86±0.08***</td>
<td>4.0±0.15***</td>
<td>11.7±0.25***</td>
</tr>
<tr>
<td>PN + CCl4</td>
<td>250±17.3***</td>
<td>353±20***</td>
<td>153±3.3***</td>
<td>1.9±0.05***</td>
<td>2.56±0.12***</td>
<td>2.5±0.05***</td>
<td>8.5±0.5***</td>
</tr>
<tr>
<td>AP + CCl4</td>
<td>343±12***</td>
<td>473±12***</td>
<td>230±11.5***</td>
<td>2.3±0.08***</td>
<td>3.23±0.14***</td>
<td>3.46±0.08***</td>
<td>9.75±0.25***</td>
</tr>
<tr>
<td>PK + CCl4</td>
<td>416±20.2***</td>
<td>530±30.5***</td>
<td>266±14.5***</td>
<td>2.76±0.21***</td>
<td>3.9±0.15***</td>
<td>4.4±0.10***</td>
<td>12.18±0.18</td>
</tr>
</tbody>
</table>

Values are mean ±S.E.M, n=6 Symbols represent statistical significance.
***P < 0.001; ** P < 0.01 & * P < 0.05, comparison between control and Hepatotoxic (HP) control,
***P < 0.001; ** P < 0.01 & * P < 0.05, comparison between HP and hepatotoxic control. HP: Hepatotoxic; Silymarin: 100 mg/kg, p.o.
PN: Phyllanthus niruri 142.5 mg/kg, p.o; AP: Andrographis paniculata 200 mg/kg, p.o.
PK: Picrorrhiza kurroa 200 mg/kg, p.o.

Table III: Effect on Serum AST, ALT, ALP, Direct (D-BIL) and Total bilirubin (T-BIL) levels and liver weight in CCl4 induced acute hepatitis in rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AST(U/L)</th>
<th>ALT(U/L)</th>
<th>ALP(U/L)</th>
<th>Serum Bilirubin (mg/dL)</th>
<th>Liver Weight (Grams/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Direct</td>
<td>Total</td>
<td>Direct</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>107±12.47</td>
<td>74.3±3.4</td>
<td>533±21.8</td>
<td>1.75±0.09</td>
<td>3.95±0.15</td>
</tr>
<tr>
<td>HP control (CCl4)</td>
<td>1285±155***</td>
<td>996±27***</td>
<td>1216±20.2***</td>
<td>3.52±0.13***</td>
<td>5.55±0.05***</td>
</tr>
<tr>
<td>Silymarin + CCl4</td>
<td>533±8.89***</td>
<td>230±15.2***</td>
<td>596±12***</td>
<td>2.29±0.21***</td>
<td>4.25±0.05***</td>
</tr>
<tr>
<td>PN + CCl4</td>
<td>624±24.39***</td>
<td>240±25***</td>
<td>593±21***</td>
<td>2.44±0.21***</td>
<td>4.03±0.03***</td>
</tr>
<tr>
<td>AP + CCl4</td>
<td>716±37.5***</td>
<td>433±27.2***</td>
<td>706±29***</td>
<td>2.39±0.22***</td>
<td>4.4±0.10***</td>
</tr>
<tr>
<td>PK + CCl4</td>
<td>628±22***</td>
<td>226±12***</td>
<td>583±14.5***</td>
<td>2.48±0.26***</td>
<td>4.09±0.01***</td>
</tr>
</tbody>
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<th>ALP(U/L)</th>
<th>Serum Bilirubin (mg/dl)</th>
<th>SOD</th>
<th>CAT</th>
<th>Histological scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>443±23</td>
<td>633±8.8</td>
<td>237.5±12.5</td>
<td>3.55±0.05</td>
<td>6.35±.212</td>
<td>2.75±.35</td>
<td>11.24±.024</td>
</tr>
<tr>
<td>HP control (CCl₄)</td>
<td>67±11.8***</td>
<td>133±12***</td>
<td>57.5±7.5***</td>
<td>1.25±0.05***</td>
<td>3.4±0.4***</td>
<td>0.45±0.07***</td>
<td>3.25±0.25***</td>
</tr>
<tr>
<td>Silymarin + CCl₄</td>
<td>310±15.2aaa</td>
<td>540±20aaa</td>
<td>162±8aaa</td>
<td>3.1±0.1aaa</td>
<td>6.31±0.06aaa</td>
<td>2.15±0.21aaa</td>
<td>9.7±0.510aaa</td>
</tr>
<tr>
<td>PN + CCl₄</td>
<td>320±17.3aaa</td>
<td>513±17.6aaa</td>
<td>160.5±4.5aaa</td>
<td>2.95±0.05aaa</td>
<td>5.95±0.05aaa</td>
<td>2.6±0.11aaa</td>
<td>9.5±0.510aaa</td>
</tr>
<tr>
<td>AP + CCl₄</td>
<td>196±12aa</td>
<td>283±20.2aaa</td>
<td>115±5.0a</td>
<td>2.9±0.1aaa</td>
<td>5.55±0.05aa</td>
<td>1.65±0.15a</td>
<td>7.25±0.25**aa</td>
</tr>
<tr>
<td>PK + CCl₄</td>
<td>306±21aaa</td>
<td>510±15.2aaa</td>
<td>175±5.0aaa</td>
<td>3.25±0.25aaa</td>
<td>6.39±0.11aaa</td>
<td>2.54±0.26aaa</td>
<td>9.65±0.1510aaa</td>
</tr>
</tbody>
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**Figure 1a: Normal control** - Section stained with hematoxylin and eosin (H&E- 100X); Liver section of normal rats showing normal hepatic cells with well-preserved cytoplasm, well brought out central vein prominent nucleus and nucleolus.

**Figure 1b: Hepatotoxic(CCl₄) control in chronic model** - Section stained with hematoxylin and eosin (H&E- 100X); liver section showing fatty changes necrosis, ballooning degeneration, broad infiltration of lymphocytes and loss of cellular boundaries with formation of fibrosis and steatosis.

**Figure 1c: CCl₄+Phyllanthus nirruri in chronic model** - Section stained with hematoxylin and eosin (H&E- 100X); liver section shows fatty changes necrosis, ballooning degeneration, few infiltration of lymphocytes and less loss of cellular boundaries with mild fibrosis and steatosis.
Figure Id: CCl₄ + *Andrographis paniculata* in chronic model - Section stained with hematoxylin and eosin (H&E- 100X); liver section showing less fatty changes, mild necrosis, degeneration, little infiltration of lymphocytes with moderate fibrosis and steatosis.

Figure 1e: CCl₄ + *Picroriza kurroa* in chronic model - Section stained with hematoxylin and eosin (H&E- 100X); liver section showing moderate fatty changes, necrosis, ballooning degeneration, no infiltration of lymphocytes and mild loss of cellular boundaries with formation of fibrosis and mild steatosis.

Figure IIa: Hepatotoxic (CCl₄) control in acute model - Section stained with hematoxylin and eosin (H&E- 100X); liver section shows fatty changes with necrosis, ballooning degeneration and massive infiltration of lymphocytes and loss of cellular boundaries with formation of fibrosis and steatosis.

Figure IIb: CCl₄ + *Phyllanthus nirruri* in acute model - Section stained with hematoxylin and eosin (H&E- 100X); liver section shows mild fatty changes necrosis, mild ballooning with degeneration, mild infiltration of lymphocytes and lesser degree of loss of cellular boundaries and mild steatosis.

Figure IIc: CCl₄ + *Andrographis paniculata* in acute model - Section stained with hematoxylin and eosin (H&E- 100X); liver section shows moderate fatty changes, necrosis, moderate ballooning degeneration, few infiltration of lymphocytes and less loss of cellular boundaries with moderate steatosis.
Figure IIId: CCl₄+ Picroriza kurroa in acute model - Section stained with hematoxylin and eosin (H&E- 100X); liver section shows mild fatty changes, necrosis, mild ballooning degeneration, mild infiltration of lymphocytes and less loss of cellular boundaries with mild steatosis.

REFERENCES:

19. Rana A.C. and Avadhoot Y., Hepatoprotective effects of Andrographis paniculata against carbon tetrachloride-


