NEPHROPROTECTIVE ACTIVITY OF VITEX NEGUNDO LINN BARK AGAINST CHEMICAL INDUCED TOXICITY IN EXPERIMENTAL RATS

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ABSTRACT:

The present study shows the nephroprotective activity of methanolic extracts of Vitex negundo linn bark (VBE) against chemical induced kidney damage. Kidney damage was induced by single oral administration of 750 mg/kg of paracetamol in male wistar rats (150-200) on the 14th day. Kidney damage was studied by assessing the biochemical parameters such as serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALT), Bilirubin, total protein and enzymatic antioxidants which includes SOD, CAT, GSSH, GPx, Px and non-enzymatic antioxidants, such as GSH. The methanolic extracts of Vitex negundo linn bark (200 mg/kg, 400 mg/kg) were orally administered to the animals once daily for 14th days. The methanolic extracts of Vitex negundo linn bark (200 mg/kg, 400 mg/kg) results in a significant reduction on biochemical parameters as well as enzymatic and non-enzymatic antioxidants when compared to the paracetamol induced kidney toxicity. The result concludes that the methanolic extract of vitex negundo linn bark (200, 400 mg/kg) has protected the kidney from paracetamol induced toxicity.

KEYWORDS: Paracetamol, nephroprotective, anti-oxidant, silymarin and vitex negundo linn bark.

INTRODUCTION

Vitex negundo linn is a large aromatic shrub with quadrangular, densely whitish tomentose branchlets, up to 4.5m, in height, or sometimes a small slender tree, found thought the greater part of India. Bark thin, grey; leaves 3-5 foliate; leaflets lanceolate, entire or rarely leaflets smaller flower bluish purple small in penduncled cymes, forming large terminate, often compound, pyramidal panicles, drupes globose, black when ripe, 5-6mm, in diameter, invested at the base with enlarged calyx. The shrub is very common in many parts of the country and often occurs gregariously and it is usually not browsed by cattle. The shrub can be reproduced readily from cuttings and it produces the root-suckers it is useful for planting against soil-erosion. The rate of growth of the shrub is moderate; with seven rings per 2.5cm of radius giving a mean annul girth-increment of 2.3cm. It is acrid, bitter, heating, astringent, stomachic, cephalic, anthelmintic and useful in treatment of inflammations, eye diseases, spleen enlargement, bronchitis, asthma, biliousness, painful teething of children etc. It has germicidal properties. It is easily digestible and can cure morbid vata and kapha and used in arthritis, cephalgia, otalgia, inflammatory,
glandular and rheumatic swellings, intestinal worms, fever, ulcers, skin diseases, nervous disorders and leprosy (Vishal R Tandon et al.). But still no scientific and methodical investigation on bark of *Vitex negundo linn* reported in literature regarding its action on kidney. Therefore, the present investigation has been designed to study the possible mechanism of methanolic extracts of bark of *Vitex negundo linn* on biochemical, enzymatic and non-enzymatic parameters against paracetamol induced kidney damage.

Kidney is the vital organ of excretion. About 20,000 deaths are found every year due to kidney disorder. Paracetamol is a widely used antipyretic and analgesic, produces acute kidney damage if overdoses are consumed. Paracetamol is metabolized primarily in the liver where 60-90% is converted to inactive compounds by conjugation with sulfate and glucronide and then excreted by the kidney.

**MATERIALS AND METHODS**

**Animals**

*Male wistar rats* weighing between 150 and 220 gm were used for this study. The animals will be obtained from animal house, IRT, Perundurai medical College, Erode, TamilNadu, India. On arrival, the animals were randomly grouped in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2°C and relative humidity of 30-70%. A 12:12 light: day cycle was followed. All the animals were allowed to free access to water and fed with standard commercials pelleted rat chaw (M/s.Hindustan Lever Ltd., Mumbi). All the experimental procedures and protocols used in this study were reviewed by the Institutional animal ethics committee (688/2/C-CPCSEA) of NCP and were accordance with the guidelines of the IAEC. Approval was obtained from the IAEC, NCP, erode (Proposal No: NCP/IAEC/PG/2010-01).

**Plant material**

The plant material consists of dried powdered bark of *Vitex negundo Linn*. Belonging to the family Verbenaceae.

**Preparation of plant extract**

Fresh bark of *Vitex negundo linn* was collected from koorapalayam, Erode, TamilNadu, India. The bark was dried for one month and latter powered. This powder was then macerated with methanol for 72 h with occasional shaking. It was then filtered and the solvent was evaporated under heating mandle. The yield of methanolic extract of bark of *Vitex negundo linn*, (VN) was 34% (w/w).

**Drugs and chemicals**

All the drugs and chemicals used in the study were obtained commercially and were of analytical grade.

**Experimental protocol**

The animals were divided in to five groups of six animals in each group. Group I served as control and fed orally with normal saline 5ml/kg body weight daily for 14 days. Group II rats were treated similarly as group I. group III and Group IV animals were treated with the 200 mg/kg, 400 mg/kg daily for 14th days through oral routes. Group V were fed with 25 mg /kg silymarin as standard drug. On the 14th day paracetamol suspenstion was given by oral, in a dose of 750 mg/kg to all rats except group I. the blood samples were collected for biochemical parameters including SGOT, SGPT, ALP, total protein, Bilirubin, urea and creatinine. Histopathological studies were carried for kidney.

**Statistical analysis**

Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison tests. P values <0.05 were considered significant.
Results

The results of nephroprotective activity on paracetamol treated rats are shown in results parts. The biochemical parameters such as SGOT, SGPT, ALP, Total protein, urea, creatinine, and Bilirubin in serum were significantly (P<0.01) increased in paracetamol treated group when compared to control. The VBE treatment (200, 400 mg/kg) significantly (P<0.01) reversed the levels of kidney enzymes when compared to paracetamol treated animals. Enzymatic antioxidants such as SOD, CAT, GSSH, GPx, Fx and non-enzymatic antioxidants GSH levels were significantly (P<0.01) increased in paracetamol treated group when compared to control.

The VBE treatment (200, 400 mg/kg) significantly (P<0.01) reversed the levels of kidney enzymes when compared to paracetamol treated animals. Silymarin treated group also shown the significantly (P<0.01) inverted the levels of both biochemical parameters and antioxidant levels.

Discussion

The present thesis entitled “Nephroprotective activity on the bark of Vitex negundo linn bark against chemical induced toxicity in the experimental rats” deals with the exploration of pharmacological and phytochemical screening of the selected Indian medicinal plant Vitex negundo linn belonging to the family Verbenaceae, Vitex negundo is traditionally used for eye disease, spleen enlargement, asthma, inflammatory, fever, ulcers, skin disease.

Biological systems can produce free radicals. Free radicals can attack many cellular energy-producing systems. For instance, it deactivates all the enzymes (Hyslop et al., 1988). It can be formed in vivo by many oxidizing enzymes such as SOD, CAT, Px, GPx, GSSH, and GSH. It can cross membranes and many slowly oxidize a number of compounds. It is used in the respiratory burst of activated phagocytes. Free radicals itself is not very reactive; however it can sometimes be toxic to cell because it my give rise to free radicals in the cells. (Gulcin et al.2004).

The kidney disorders are world problem. Despite its frequent occurance, high morbidity and high mortality, its medical management is currently inadequate, no therapy has successfully prevented the progression of kidney diseases, even though newly developed drugs have been used to treat chronic kidney disorders these drugs have often side effects. Therefore, that is an essential research about suitable herbal drugs, that could replace the chemical ones (Bruck et al., 1996). Plants extracts have been used by traditional medical practitioners for the treatment of kidney disorders for centuries (Schuppan et al., 1999). Phenylpropnoids or polyphenolic compounds are a large group of herbal chemical compounds with well-known treat mental and protective effects.

Paracetamol is a widely used antipyretic and analgesic, produces acute kidney damage if overdoses are consumed. Paracetamol is metabolized primarily in the liver where 60-90% is converted to inactive compounds by conjugation with sulfate and glucronide and then excreted by the kidney.

In the present investigation it was observed that the animals treated with paracetamol resulted significant kidney damage as shown by the elevated levels of serum markers. These changes in the marker levels will reflect in nephrotic structural integrity. 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.

The normalization of serum markers by VBE suggests that they are able to condition the tubular brush borders so as to protect the membrane integrity against paracetamol induced linkage of marker enzymes into the circulation.
The above changes can be considered as an expression of the functional improvement of tubular borders, which may be caused by an accelerated regeneration of parenchyma cells. Increase in serum level of ALP and bilirubin is due to increased synthesis in presence of increasing biliary pressure.

In the present study, it was observed that treatment with paracetamol induced a significant elevation in the levels of serum urea, creatinine and total protein. However, daily pretreatment with VBE for 14 days conferred nephroprotection on paracetamol induced rats in a dose dependent fashion and 400 mg/kg dose offered maximum protection.

The biochemical alterations were corroborated by the histological findings of glomerular and tubule-interstitial necrosis. It can be concluded that the methanolic extract of *Vitex negundo linn* showed significant nephroprotective activity. The significant nephro activity of the extract may be due to the presence of isolated flavonoids. The isolation of other bioactive components in the extract could certainly help to ascertain the individual potency of the compounds. Exploitation of its antioxidiant, nephro activity could be further explored using *in vivo* assay systems, to increase the overall activity and to protect against various ailments that are induced by oxidative stress.

Further investigation on the isolation and identification of active components in the bark may lead to chemical entities with potential for clinical use in the prevention and treatment of nephrotoxicity.

### RESULTS

Table 1. Effect of *Vitex negundo linn* bark on kidney protein, MDA and LH in the experimental groups.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>DOSE</th>
<th>PROTEIN</th>
<th>MDA</th>
<th>LH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>5 ml/kg</td>
<td>1362.5±20.9</td>
<td>120±1.74</td>
<td>12±1.08</td>
</tr>
<tr>
<td>Positive control</td>
<td>25 mg/kg</td>
<td>1143.6±16.5c</td>
<td>132±2.55c</td>
<td>15±1.51c</td>
</tr>
<tr>
<td>Negative control</td>
<td>750 mg/kg</td>
<td>738±9a</td>
<td>160±1.53b</td>
<td>19±1.43b</td>
</tr>
<tr>
<td>VBE I</td>
<td>200 mg/kg</td>
<td>1144.8±10.9d</td>
<td>150±2.06d</td>
<td>17±1.72d</td>
</tr>
<tr>
<td>VBE II</td>
<td>400 mg/kg</td>
<td>1276.5±11.3c</td>
<td>146±1.38c</td>
<td>16±1.84d</td>
</tr>
</tbody>
</table>

Values are mean± SEM; n=6.

*P<0.05, **P<0.01 when compared to control

*P<0.01, **P>0.05 when compared to paracetamol control (one way ANOVA followed by Dunnett’s test)

Protein=mmoles/min/mg protein, MDA = nmoles/min/mg protein, LH= nmoles/min/mg protein.
There was a significant (P<0.01) decrease in the level of total protein and an increase in the level of malondialdehyde and lipid hydroperoxides in paracetamol-induced nephrotoxicity when compared to normal control. Treatment with VBE (200 mg/kg, 400 mg/kg) and silymarin simultaneously caused a significant (P<0.01) decrease in the malondialdehyde and lipid hydroperoxides and an increase in total protein content.

Table 2. Effect of Vitex negundo linn bark on kidney enzymatic and non enzymatic antioxidants in control and experimental groups.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>DOSE</th>
<th>SOD</th>
<th>CAT</th>
<th>GSSH</th>
<th>Px</th>
<th>GPx</th>
<th>GSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>5ml/kg</td>
<td>4.32±0.14</td>
<td>52±1.28</td>
<td>32±2.42</td>
<td>9±0.89</td>
<td>12±0.98</td>
<td>14±1.36</td>
</tr>
<tr>
<td></td>
<td>Normal saline</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive control</td>
<td>25 mg/kg</td>
<td>3.69±0.77b</td>
<td>50±3.28b</td>
<td>29±3.27b</td>
<td>8.6±0.57b</td>
<td>11±1.68b</td>
<td>13±0.62b</td>
</tr>
<tr>
<td>Negative control</td>
<td>750 mg/kg</td>
<td>2.36±0.01a</td>
<td>42±3.62a</td>
<td>19±2.62a</td>
<td>7.4±0.86a</td>
<td>8±0.72a</td>
<td>8±0.88a</td>
</tr>
<tr>
<td>VBE I</td>
<td>200 mg/kg</td>
<td>3.24±0.12b</td>
<td>43±5.23b</td>
<td>20±1.28b</td>
<td>7.9±0.19b</td>
<td>9.1±0.25b</td>
<td>10±0.96c</td>
</tr>
<tr>
<td>VBE II</td>
<td>400 mg/kg</td>
<td>3.62±0.79b</td>
<td>46±2.0b</td>
<td>26±2.15b</td>
<td>8.4±0.26b</td>
<td>10.6±0.36b</td>
<td>12±1.23b</td>
</tr>
</tbody>
</table>

Values are mean± SEM; n=6 in each

*P<0.01 when compared to control

bP<0.01, P>0.05 when compared to paracetamol control (one way ANOVA followed by Dunnett’s test)

CAT=µmoles/min/mg protein, GPx= nmoles/min/mg protein, GSH= nmoles/min/mg protein,
SOD= nmoles/min/mg protein, GSSH= nmoles/min/mg protein, and peroxidase= nmoles/min/mg protein

Effect of Vitex negundo linn bark on kidney enzymatic and non enzymatic antioxidants in control and experimental groups.

Induction with paracetamol (750 mg/kg) produced a significant (P<0.01) decrease in the enzymatic antioxidants like catalase, superoxide dismutase, peroxidase, glutathione peroxidase and glutathione reductase and the non-enzymatic antioxidants reduced glutathione in the kidney homogenate when compared to normal control.

Treatment with VBE (200 mg/kg, 400mg/kg) significantly (P<0.01) restored the levels of both enzymatic and non-enzymatic antioxidant enzymes which is almost similar to the control group. The activity produced by the standard silymarin was found to be the highest among the groups tested.
Table 3. Biochemical parameters on paracetamol induced Hepato and nephrotoxicity studies on bark Vitex negundo linn.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>SGOT</th>
<th>SGPT</th>
<th>ALP</th>
<th>BILIRUBIN</th>
<th>TP</th>
<th>UREA</th>
<th>CREATININE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>41.38±2.63</td>
<td>40.32±5.72</td>
<td>12.10±1.45</td>
<td>0.78±0.09</td>
<td>7.76±0.81</td>
<td>15.98±0.68</td>
<td>0.63±0.07</td>
</tr>
<tr>
<td>Positive</td>
<td>43.65±3.72</td>
<td>38.90±3.21</td>
<td>11.6±0.96</td>
<td>0.92±0.06</td>
<td>8.56±0.91</td>
<td>14.25±1.78</td>
<td>1.08±0.09</td>
</tr>
<tr>
<td>Negative</td>
<td>86.65±10.26</td>
<td>112.65±14.8</td>
<td>42.35±5.32</td>
<td>5.38±0.14</td>
<td>17.25±1.67</td>
<td>36.10±3.97</td>
<td>1.25±0.3</td>
</tr>
<tr>
<td>VBE 200mg/kg</td>
<td>40.22±3.72</td>
<td>35.56±1.62</td>
<td>9.50±0.92</td>
<td>0.65±0.07</td>
<td>8.23±0.89</td>
<td>13.32±0.98</td>
<td>0.95±0.08</td>
</tr>
<tr>
<td>VBE 400mg/kg</td>
<td>42.16±5.77</td>
<td>40.65±2.80</td>
<td>13.12±1.36</td>
<td>1.05±0.26</td>
<td>7.35±0.92</td>
<td>14.07±1.26</td>
<td>1.05±0.02</td>
</tr>
</tbody>
</table>

Values are mean± SEM; n=6; P<0.01 when compared to control

bP<0.01, cP>0.05 when compared to paracetamol control (one way ANOVA followed by Dunnett’s test)

SGOT= U/L, SGPT= U/L, ALP=KA units/dl, Bilirubin= mg/dl, Total protein= g/dl, urea= mg/dl, and creatinine=mg

Biochemical parameters on paracetamol induced nephrotoxicity studies on Vitex negundo linn bark.

The result of the biochemical parameters revealed the elevation of enzymes levels in the paracetamol induced treated group, indicating that paracetamol induces damage to the liver. Treatment with the paracetamol significantly increasing the serum SGOT, SGPT, ALP, TP, UREA, Bilirubin and Creatinine. Treatment with the silymarin 25 mg/kg and VBE (200, 400mg/kg) prevented the elevation of serum marker enzymes. The study demonstrated that VBE (200, 400 mg/kg) significantly prevented paracetamol induced nephrotoxicity.

HISTOPATHOLOGICAL EVALUATION OF KIDNEY

(A) Normal section of kidney

Fig. A: Represents the kidney section of control group showing normal tubular brush borders, intact glomeruli and Bowman’s capsule
(B) Paracetamol treated kidney
Fig. B: Represents the kidney section of paracetamol treated group showing the severe tubular necrosis and degranulation.

(C) Extract VBE (200 mg/kg) + Paracetamol (750 mg/kg)
Fig. C: Represents the kidney section of methanolic extracts of *Vitex negundo* linn 200 mg/kg body weight showed normal tubular pattern with a mild degree of necrosis, swelling and degranulation.

(D) Extract VBE (400 mg/kg) + Paracetamol (750 mg/kg)
Fig. D: Represents the kidney section of methanolic extracts of *Vitex negundo* linn 400 mg/kg body weight showed the ameliorated and toxic manifestations in kidney.
Silymarin (25 mg/kg) + Paracetamol (750 mg/kg).

Fig. E: Represents the kidney section of silymarin treated group showing normal tubular brush borders, intact glomeruli and Bowman's capsule.

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