Effect of *Semecarpus anacardium* nuts on lipid peroxidation

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Alcoholic extract of pericarp showed significant protection against FeSO$_4$ induced lipid peroxidation, as compared with whole native nut and seeds. Mechanism of action may be through metal chelation or activation of endogenous antioxidant enzymes, because the extract did not show hydroxyl and super oxide anion scavenging property. Further *in vitro* experiments against FeSO$_4$, it did not maintain the level of reduced glutathione.

Nuts of *Semecarpus anacardium*, Linn (Anacardiaceae), commonly known as marking nut (Bhilava in Hindi). It is a tree, growing in the tropical outer Himalayas and the hot temperate-zone in India. Many experimental evidences have been published earlier to support its therapeutic value, as described in the Ayurvedic literature.$^{1,2}$ It is used for management of rheumatism, wound healing, diabetes, urinary diseases, gout and cancer. It is also used as aphrodisiac, nerve-tonic and as anabolic medicine (*rasayana* drug) in the Ayurvedic system of therapy.$^{3,4}$ Several long chain alkenyl phenols, phenolic compounds (biflavone derivatives) have been isolated from the oil and pericarp of this nut. Some of the important phytochemicals are galuflavonane, jeedflavanone, seme arpuflavonane.$^{5,6}$ These compounds are polyhydroxy biflavones and have been isolated from the de-fatted nutshell. Pillay and Siddiqui$^7$ have also isolated several monophenolic compounds, known as semecarpal and bhilawanol. Further it is recommended that the selected nuts should be purified, before it is used for preparing the medicine.

For purification of nuts, there are 2 standard methods as per ayurvedic literature. One is to treat the nuts with the small brick powder and the other is to bury the small pieces of nuts beneath the soil in a cloth-envelop. It appears that the basic idea behind this purification process is to remove the oil content of the nut, which is considered to be toxic in high doses$^1$. Contrary to this concept, the use of oil as a medicine is also recommended in the ayurvedic system$^9$. Another possible hypothesis could be the removal of pericarp and oil both. In this case only the cotyledons should contain the active ingredient.

In the present study, we have tried to investigate the antioxidant property of three parts of the nut i.e. alcoholic extract of total nut (without sodhan) alcoholic extract of chemically defatted pericarp and alcoholic extract of the seeds only. All the fraction have been tested on the ferrous sulphate induced lipid peroxidation and other related anti-oxidan parameters.

Phosphotungstic acid, thiobarbituric acid trichloroacetic acid, acetic acid, sodium salicylate EDTA, were purchased from Central Drug House (Pvt.) Ltd. Reduced glutathione, and DTNB (5,5-Dithio-bis (2-nitrobenzoic acid) were from Sigma Chemical Co. St. Louis, MO, USA. Ascorbate, FeCl$_3$, sodium tungstate, sodium nitrite, and other reagents were of analytical grade, Albino Swiss Mice strain A (body weight 25-30g) from the colony were maintained with mice pellets (Hidustan Lever Ltd. Bombay) and given tap water *ad libitum*.

Preparation of alcoholic extract—Nuts of *S. anacardium* were purchased from the Ayurvedic Pharmacy of our Institute and its authenticity was checked on standard pharmacognostic parameter and by direct comparison with the specimen, preserved in the Department. Nuts, which were drowned in the water were selected and directly extracted with ethanol. Remaining nuts were mechanically separated out into pericarp and seeds. Pericarp was full of oil, so it was kept in the petroleum ether for 30 hr. Next day, it was filtered and washed twice with fresh petroleum ether. Washed pericarp (100g) was extracted
with ethanol in soxhlet extraction apparatus. Similarly the cotyledons (20g) were also defatted and extracted with ethanol. The three extracts were distilled under reduced pressure in Buchi type rotary evaporator. The concentrated extract was transferred to vacuum desicator and dried till constant weight was attained. Yield of these extracts were 19.8% (from nut) (b) 25% (from pericarp) (c) 14.37% (from seed).

**Preparation of tissue homogenate**—Liver was perfused with phosphate buffer saline through hepatic portal vein and harvested. Its lobes were dried between blotting papers (to remove excess of blood) and were cut into small pieces with a heavy-duty blade. They were then homogenized in glass-Teflon homogenizing tube in phosphate buffer saline (pH 7.4) in cold condition. It was centrifuged at 2000 rpm for 10 min and supernatant was diluted with PBS upto final concentration of protein 0.8-1.5mg/0.1 mL. Protein concentration was measured by using the standard method of Lowery et al. 10.

**Experimental design**—Effect of different fractions of nut on lipid peroxidation and glutathione was estimated in the liver homogenate. Liver homogenate (5%; 3mL) was aliquoted to each 35mm glass petri-dishes. In the control plates, different volumes of drug vehicle (Tween 80: water, 1: 9) were added. To study the comparative response, the experiment was divided into 4 groups. The first group was treated as control where only buffer was added. In the second, third and fourth group, different concentrations of drug extract and vitamin E were added. All the concentrations were expressed as μg because the drug extract could not be expressed in mM.

**Estimation of thiobarbituric acid reactive substances (TBA-RS)**—lipid peroxidation was induced by adding 0.5mM FeSO₄. Liver homogenate (5%; 3mL) was preincubated with different concentrations of extract before induction of lipid peroxidation. After incubation for 30 min, 0.1mL incubation mixture was transferred to a tube containing 1.5mL of 10% TCA. After 10 min, tubes were centrifuged and TCA soluble fraction was kept safely to develop the colour reaction, OD was read at 535nm as described by Ohkawa et al. 11 with modification (Tripathi et al. 12).

**Estimation of glutathione (GSH)**—Liver homogenate (10%) was mixed with different concentrations of the extract. After every 10 min, 250 μL of the incubation mixture was taken out and processed for estimation of reduced GSH by the method of Ellman et al. 13 as described earlier 14.

**Estimation of superoxide anion scavenging potential**—It was assayed in a purely chemical reaction system, by estimating the degree of reduction of nitro blue tetrazolium (NBT) and measured in terms of blue formazan. Absorbance was taken at 560 nm at different time intervals (Flehe et al. 15).

**Estimation of hydroxyl radicals scavenging potential**—The concentration of hydroxyl radicals was also determined in a chemical reaction system according to Reddy et al. 16 with minor modifications as described earlier 14.

**Interaction of the extract with ferrous ions**—Effect of the extracts on un-chelated Fe²⁺ was estimated in terms of ferrous-2,2′ bipyridyl complex at 525nm 18.

**Statistical analysis**—ANOVA analysis was performed to verify the degree of significance.

<table>
<thead>
<tr>
<th>Conc. of drug in μg/ml</th>
<th>Nut (564.88 ± 49.96)</th>
<th>Pericarp (564.88 ± 49.96)</th>
<th>Seed (564.88 ± 49.96)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control with FeSO₄ (0.5mM)</td>
<td>452.69 ± 12.91(20%)*</td>
<td>445.58 ± 12.67(21%)*</td>
<td>483.51 ± 8.99(14%)</td>
</tr>
<tr>
<td>1</td>
<td>437.06 ± 11.34(23%)*</td>
<td>308.11 ± 8.75(45%)*</td>
<td>483.98 ± 10.7(14%)</td>
</tr>
<tr>
<td>2</td>
<td>293.89 ± 8.93(48%)**</td>
<td>98.67 ± 11.52(82.5%)**</td>
<td>395.34 ± 19.19(30%)*</td>
</tr>
<tr>
<td>4</td>
<td>199.08 ± 12.84(64%)**</td>
<td>66.34 ± 10.7(88%)**</td>
<td>322.61 ± 69.69(42%)*</td>
</tr>
<tr>
<td>6</td>
<td>069.67 ± 5.32(87%)**</td>
<td>069.67 ± 5.32(87%)**</td>
<td>069.67 ± 5.32(87%)**</td>
</tr>
</tbody>
</table>

*p value: <0.01; **p value: <0.001
Table 2 — Effect of alcoholic extract of S. anacardium on different antioxidant parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Nut</th>
<th>Pericarp</th>
<th>Seed</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH in nmol/mg protein (after 20 min)</td>
<td>11.13 ± 0.26</td>
<td>15.52 ± 0.31</td>
<td>10.12 ± 0.21</td>
<td>12.62 ± 0.36</td>
</tr>
<tr>
<td>Hydroxylation of salicylate (in nmole of 2,3-dihydroxybenzoate)</td>
<td>199.66 ± 5.60</td>
<td>189.84 ± 5.02</td>
<td>192.62 ± 4.28</td>
<td>194.10 ± 7.52</td>
</tr>
<tr>
<td>Super oxide anion radical generation expressed in terms of formazon (OD 540 nm)</td>
<td>0.239 ± 6.34</td>
<td>0.229 ± 6.01</td>
<td>0.232 ± 4.77</td>
<td>0.234 ± 6.22</td>
</tr>
</tbody>
</table>

No significant change was noted as compared to control.

Table 3 — Effect of alcoholic extract of S. anacardium on Fe²⁺ metal chelation.

<table>
<thead>
<tr>
<th>Iron:</th>
<th>Control</th>
<th>Nut</th>
<th>Pericarp</th>
<th>Seed</th>
</tr>
</thead>
<tbody>
<tr>
<td>0:0</td>
<td>.227±0.044</td>
<td>.27±0.044</td>
<td>.227±0.044</td>
<td>.27±0.044</td>
</tr>
<tr>
<td>1:0.5</td>
<td>.223±0.034 (1.7%)</td>
<td>.231±0.021</td>
<td>.240±0.0024</td>
<td>.27±0.044</td>
</tr>
<tr>
<td>1:1</td>
<td>.194±0.028 (14.5%)</td>
<td>.223±0.023</td>
<td>.225±0.031</td>
<td>.225±0.031</td>
</tr>
<tr>
<td>1:2.5</td>
<td>.143±0.0031* (37%)</td>
<td>.218±0.0026</td>
<td>.222±0.040</td>
<td>.216±0.0041</td>
</tr>
<tr>
<td>1:5</td>
<td>.134±0.0028* (40%)</td>
<td>.216±0.0041</td>
<td>.227±0.0032</td>
<td></td>
</tr>
</tbody>
</table>

Fe²⁺ was estimated by spectrophotometric measurement of Fe²⁺ bipyridyl complex at 525 nm. *P value <0.01

Effect of different parts of nuts of S. anacardium on ferrous sulphate (FeSO₄) induced lipid peroxidation in mice liver homogenate showed significant inhibition (Table 1). Best response was found with alcoholic extract of pericarp, which contained maximum oil content. The seed had less response (42%) as compared to pericarp (88%) and total nut (64%). This data indicated that active principle of this herbal medicine might be due to fatty portion of this nut. It was observed that native nut extract showed slight scavenging potential for super oxide anion (21%) and metal chelation but other fractions had no effect at all. On glutathione level, all the three fractions did not show any protective response. Regarding its mechanism of action there could be several reasons as lipid peroxidation is a chain reaction. Possible mechanism could be the (a) removal of iron from the reaction system; (b) maintenance of reduced glutathione level; (c) acting as electron acceptor or electron donor, which neutralizes the free radical; (d) super oxide scavenger; or (e) hydroxyl radical scavenger. Interestingly, all the samples of nut did not show any protective response on glutathione oxidation, hydroxyl radical scavenging property and super oxide radical scavenging property. Contrary to these findings, the total nut fraction showed the metal chelation property (40%), but this was not directly proportional to its anti-lipid peroxidative property, because this fraction did not show maximum response on lipid peroxidation. It was less as compared to pericarp fraction (88%), which showed no metal chelation property.

The observations given above indicate that S. anacardium, unlike other medicinal plants, had different mechanism of action. At this moment it is difficult to predict any mechanism of action, but it could be due to metal chelation as well as the inhibition of cyclooxygenase pathway, which is also involved in the process of iron induced lipid peroxidation.

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References
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