Evaluation of Antioxidant Potential of *Kaempferia rotunda* Linn

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Mohanty, et al.: Antioxidant Potential of *Kaempferia rotunda* Linn

The plant *Kaempferia rotunda* Linn. has been explored for its antioxidant potential in the present study. The antioxidant property was assessed by lipid peroxidation markers such as malonaldehyde (MDA) and 4-hydroxyl-2-nonenal (4-HNE). The lipid peroxidation byproducts are highly toxic and responsible for various diseases like myocardial infarction, diabetes mellitus, hepatic injury, atherosclerosis, rheumatoid arthritis and cancer. The chemical constituents of the plant were critically and qualitatively analyzed to confirm the presence of flavonoids and phenolic derivatives. Hence our objective has been designed to evaluate the antioxidant effect of *Kaempferia rotunda* linn. and its contribution to control the lipid peroxidation.

Key words: Lipid peroxidation, antioxidants, malonaldehyde, 4-hydroxyl-2-nonenal, *Kaempferia rotunda* Linn

The free radicals such as hydroxyl radicals (OH-), superoxide radicals (O2-), singlet oxygen (O) and hydrogen peroxide radicals (H2O2) play a significant role in age dependent diseases such as atherosclerosis, myocardial infarction, diabetes mellitus, rheumatoid arthritis and cancer1,2. The free radicals deliberately activate the lipid peroxidation3. Hence various toxic metabolites are generated i.e. MDA and 4-HNE. The malonaldehyde can attack NH2 group of protein molecules to form both intramolecular and intermolecular cross links between different proteins causing severe damage to membrane proteins. These metabolites have been associated with damaging effects of oxidative stress, oxygen toxicity and liver injury4.

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The plant *Kaempferia rotunda* linn. belongs to the family Zingiberaceae also named *bhuichampaka* (Sanskrit), *bhuchampa* (Hindi) and blackhorm (English). It is a fragrant aromatic herb with a tuberous rhizome distributed throughout India5. In some districts of Maharashtra the powder root is popular in mumps and also said to be used in the form of poultice, promotes suppuration. The main constituent crotepoxide is useful for the inhibition of tumors6.

Phytochemically the plant has been attributed to contain flavonoids, crotepoxide, chalcones, quercetin, flavonols, β-sitosterol, stigmasterol, syringic acid, protocatechuic acid and some hydrocarbons have been previously reported7. The recent literature review revealed that abundant presence of flavonoids in the plant of interest plays prime role in antioxidant mechanisms8-10.

The tuberous rhizomes of *Kaempferia rotunda* Linn were collected during July-August from the various areas of Sikkim Himalayan region and authenticated by Botanical Survey of India, Gangtok, Sikkim. The dried, powdered rhizomes were subjected to Soxhlet extraction successively using methanol. The extract was filtered, concentrated in vacuum under reduced pressure. The yield value was found to be 8.5%. The extract was subjected to qualitative chemical investigation for phytochemical constituents like flavonoids, steroids, triterpenoids and crotepoxide.

The liver was chosen to estimate the markers of lipid peroxidation because the metabolism of toxic metabolites and free radicals occur mainly in liver. The metabolites from liver may diffuse into various extra hepatic tissues causing lipid peroxidation and cellular damage11.

The fresh goat liver was obtained from local market, stored in phosphate buffer, homogenized (1g/ml) and
filtered to get clear homogenate. The lipid peroxidation indicator i.e. MDA was estimated using thiobarbituric acid reacting substances (TBARS) by the method of Ohkawa et al. The lipid peroxidation was induced in goat liver homogenate by ferrous sulphate. The generated MDA reacts with thiobarbituric acid at pH 3.5, produces a pink coloured complex, which has λmax at 530 nm. The concentration of MDA was calculated by calibration of standard graph through regression method. The significance of the results was analyzed by statistical method.

The liver supernatant was mixed with 2,4-dinitrophenylhydrazine (DNPH) and incubated for 1 h at room temp. The formed adduct of 4-HNE and DNPH was measured at 350 nm in UV/Vis spectrophotometer. The quantity of 4-HNE was calculated by calibration of standard graph through regression method. The concentration of MDA was calculated by one way analysis of variance (ANOVA) followed by Bonferroni multiple range test. The results are statically significant at P <0.05 levels.

The experimental study was based on the estimation of MDA and 4-HNE and their suppression by *Kaempferia rotunda* Linn. and presented in Table 1. From the experimental results, it proved that the methanol extract of the plant has significant antioxidant property. The quantification of MDA and 4-HNE can be directly correlated with the lipid peroxidation inhibition capacity of the extract. The antioxidant property was studied for dose dependency. From the fig. 1 it was concluded that the antioxidant property has inverse relationship with dose i.e. high at low dose and vice versa. The extract 100 µg/ml and 200 µg/ml have significant and moderate antioxidant property; respectively but 500 and 1000 µg/ml has insignificant property. The antioxidant property has inverse relationship; it may be due to the presence of crotepoxide, which increases the peroxidation.

**TABLE 1: IN VITRO EFFECT OF KAEMPFERIA ROTUNDA ON LIPID PEROXIDATION**

<table>
<thead>
<tr>
<th>Concentration of extract (µg/ml)</th>
<th>MDA (µM)</th>
<th>4-HNE (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>18.87±0.15</td>
<td>97.59±0.18</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>13.57±0.22</td>
<td>75.82±0.16</td>
</tr>
<tr>
<td>100</td>
<td>14.44±0.21</td>
<td>82.90±0.23</td>
</tr>
<tr>
<td>200</td>
<td>16.86±0.15</td>
<td>88.98±0.14</td>
</tr>
<tr>
<td>500</td>
<td>17.29±0.10</td>
<td>93.58±0.28</td>
</tr>
<tr>
<td>1000</td>
<td>17.95±0.08</td>
<td>95.09±0.13</td>
</tr>
</tbody>
</table>

Each value is the mean concentration ± standard error of the mean. All values are significant at P<0.05

At low dose the level of crotepoxide is low hence peroxidation is less and the flavanoids can scavange all the radicals. As a result the antioxidant property is more but in higher dose the total phenomena is reversed. The antioxidant property of extract was compared with standard antioxidant (ascorbic acid). The scavenging of the radicals by the methanol extract of *Kaempferia rotunda* in the above study has thus been correlated with the antioxidant potential of the plant and this information can be used to control the age dependent diseases mentioned above.

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Ficus carica leaves were identified by comparing them with Herbarium and by carrying out their microscopical and physical evaluation study. The leaves of Ficus carica were collected from Nanded district, Maharashtra. The leaves were dried in the shade, powdered and used for extraction.

The petroleum ether (60-80°) extract of the above crude drug was prepared by Soxhlet extraction. The extract was tested for hepatoprotective activity in rats. Four groups (1-IV) of six rats each, of either sex weighing between 150-200 g, were selected. Group-I was normal control, fed with vehicle for 60 days. The animals in groups II and IV were orally administered with rifampicin 50 mg/kg for 60 days. Animals in group III were administered orally the extract of Ficus carica leaf (200 mg/100 g) for 10 days. To animals in the group-IV petroleum ether extract of Ficus carica was given for after 61 days for 10 days. On the 71st day, pentobarbitione sleeping time was determined after which animals were weighed and sacrified. The liver was examined morphologically, dissected out, weighed and stored in 10% formalin. Blood was collected by cardiac puncture, serum separated.


Hepatoprotective Activity of Ficus carica Leaf Extract on Rifampicin-Induced Hepatic Damage in Rats

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Gond, et al.: Hepatoprotective Activity of Ficus carica Leaf Extract

Shade dried leaves of Ficus carica were extracted using petroleum ether (60-80°) and tested for antihepatotoxic activity on rats treated with 50 mg/kg of rifampicin orally. The parameters assessed were serum levels of glutamic oxaloacetate transaminase, glutamic pyruvic transaminase, bilirubin and histological changes in liver. Liver weights and pentobarbitione sleeping time as a functional parameter were also monitored. There was significant reversal of biochemical, histological and functional changes induced by rifampicin treatment in rats by petroleum ether extract treatment, indicating promising hepatoprotective activity.

Key words: Ficus carica, hepatoprotectice activity, rifampicin-induced hepatic damage

Liver diseases remain one of the serious health problems. In the absence of reliable liver protective drugs in allopathic medical practices. Herbs play important role in the management of various liver disorders. However, in ayurveda many indigenous plants have been used as hepatoprotective agents. A number of reviews are published stating the importance of plant drugs in the diseases of liver. Indigenous plant Ficus carica was selected for investigating hepatoprotective activity.

Ficus carica Linn. (Moraceae), commonly known as Anjir. The plant is considered to be a native of carica in Asia Minor and is grown in nearly all tropical and sub-tropical countries. In India its commercial production is limited to a few centers near Pune. During casual conversation with tribal people of Maharashtra, it was found that they chewed the leaves of Ficus carica to treat Jaundice. However, no scientifi work has been carried out on the leaves of Ficus carica to prove the hepatoprotective activity.