Original Article

Phytochemical Screening and Evaluation of in vivo Anti inflammatory activity of Fruit of Vitis vinifera

M Kavitha, R Kavitha *, N Sisindri, P Swathi
Department of Pharmacology, Teja College of Pharmacy, Kodad, Nalgonda-508206, Telangana State, India.

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The main objective of the present research work was to determine the various bioactive molecules and to evaluate the in vivo anti inflammatory activity of different fruit extracts of Vitis vinifera by Carrageenan induced Paw Edema Method in Rats. The activity was studied at a 200 mg/kg b.w. p.o. and their responses were measured at 30, 60, 120 and 180 min. The present experimental data displayed that all the extracts exhibited mild to good anti-inflammatory activity. All the extracts exhibited highest activity at 120 min. In vivo experimental data had shown that the various extracts ELN; MLN; ACT and CLF of fruit of Vitis vinifera had the potential ability to reduce inflammation which was induced by carrageenan in albino rat and had percentage protection of extract CLF 68.9%, ELN 68.5%, MLN 60.5%, and ACT 58.54%.

Key words: Bioactive molecules, Anti inflammatory, Carrageenan, Paw Edema

Correspondence Address:
R Kavitha, E mail: bhaumik.asish@gmail.com

1. INTRODUCTION

Vitis vinifera (common grape vine) is a species of Vitis, native to the Mediterranean region, central Europe, and southwestern Asia, from Morocco and Portugal north to southern Germany and east to northern Iran 1. There are currently between 5000 and 10,000 varieties of Vitis vinifera grapes though only a few are of commercial significance for wine and table grape production 2. It is a liana growing to 35 yards (32 m) tall, with flaky bark. The leaves are alternate, palmately lobed, 5–20 cm (2.0–7.9 in) long and broad. The fruit is a berry, known as a grape; in the wild species it is 6 mm (0.24 in) diameter and ripens dark purple to blackish with a pale wax bloom; in cultivated plants it is usually much larger, up to 3 cm (1.2 in) long, and can be green, red, or purple (black). The species typically occurs in humid forests and streamsides.

The wild grape is often classified as V. vinifera subsp. sylvestris (in some classifications considered Vitis sylvestris), with V. vinifera subsp. vinifera restricted to cultivated forms. Domesticated vines have hermaphrodite flowers, but subsp. sylvestris is dioecious (male and female flowers on separate plants) and pollination is required for fruit to develop. The main differences in grapes based on skin color, such as between black grapes or green grapes, are in the context of their antioxidant properties. Darker colored grapes are found to display higher anti-oxidant activity as compared to lighter varieties. As most of the health benefits in grapes derive from flavonoids and phytoneutrients that lend them their color, black grapes are thought to be even more nutritious as compared to most other varieties. Black grapes also derive some of their special health benefits from the presence of nocyanosis, a substance that lends it its distinctive color and tonic properties as well.

Grapes are a type of fruit that grow in clusters of 15 to 300, and can be crimson, black, dark blue, yellow, green, orange and pink. "White" grapes are actually green in color, and are evolutionarily derived from the purple grape. Mutations in two regulatory genes of
white grapes turn off production of anthocyanins, which are responsible for the color of purple grapes. Anthocyanins and other pigment chemicals of the larger family of polyphenols in purple grapes are responsible for the varying shades of purple in red wines. Grapes are typically an ellipsoid shape resembling a prolate spheroid.

**PHARMACOLOGICAL ACTIVITY OF BLACK GRAPES**

*Controlling blood Sugar:* Several studies have shown that consumption of black grapes helps to cure diabetes. Resveratrol, present in these grapes are responsible for increasing insulin secretion and insulin sensitivity, thereby improving and maintaining blood sugar balance and insulin levels in the body. The blood sugar benefits rendered by black grapes are clearly associated with their low Glycemic Index (GI) Value. These grapes also help in controlling blood pressure by increasing the blood flow.

*Improving brain function:* Regular consumption of black grapes helps to improve concentration, memory and also verbal and spatial recall. The polyphenol present in them also aid in curing migraine, dementia and preventing Alzheimer’s.

*Improving Cardiac Health:* the phytochemicals present in them help in reducing damage of the heart muscles and also aid in reducing and regulating cholesterol levels in the body thereby preventing heart attacks and other cardiovascular diseases.

*Prevention of Cancer:* Consumption of these grapes can be highly beneficial in preventing and treating skin, gastrointestinal, prostate, breast, lung and pancreatic cancer.

*Triggering weight Loss:* Antioxidant properties of black grapes help in releasing the unwanted toxins accumulated in the body which results in loss of weight.

*Protecting against infections and inflammations:* Resveratrol, in black grapes, is an excellent bactericide and fungicide and therefore helps to prevent pathogenic infections and inflammations. It has antiviral properties which are effective against diseases like polio and herpes. It also helps to cure asthma by increasing the level of moisture present in the lungs.

*Relieving constipation, indigestion and treating kidney disorders:* These grapes are rich in sugar, organic acid and polyose and hence act as a mild laxative to help cure constipation. They are effective in curing indigestion and irritation of stomach, and preventing dyspepsia. They also help in reducing acidity caused by uric acid thereby minimizing pressure on the kidneys.

*Improving Vision:* Black grapes are well known for their effect on the eyesight. These grapes contain Lutein and Zeaxanthin which help in maintain a good eyesight and proper vision.

*Maintaining a Healthy and youthful Skin:* Antioxidants present in black grapes render anti ageing benefits like reduction of wrinkles, increase in elasticity of skin, proper blood circulation which lead to a healthy, youthful and glowing skin. Vitamin E present in these grapes secures the moisture of the skin and hence they are used as natural moisturizers. The Vitamin C content of these grapes ensures rejuvenation of skin cells. Due to the presence of antioxidants, black grapes extracts can be used as natural sunscreen to protect against the damage caused by harmful ultra violet rays from the sun and thereby reduce the damage on the skin cells.

*Maintaining long and healthy tresses:* Consumption of black grapes is an ideal way to battle the general hair related problems faced by everyone these days, be it dandruff, hair fall, split ends or early greying. The high antioxidant and Vitamin E content of these grapes, whether consumed or used in form of grape seed oil,
helps to increase the blood circulation in the scalp, strengthens blood vessels and resultantly leads to healthy hair growth.

2. MATERIALS AND METHOD

2.1 Drug and chemicals

The standard drug diclofenac sodium purchased from Local Retail Pharmacy Shop and solvents and other chemicals were used for the extraction from Institutional Store and were of AR grade. In experimental animal inflammation was induced by Carrageenan.

2.2 Experimental animals

White male albino rats weighing about 200-250 g were used. They were obtained from the animal house of C.L. Baid Metha College of Pharmacy, Chennai. They were kept under observation for about 7 days before the onset of the experiment to exclude any intercurrent infection, had free access to normal diet and water. The animals were housed in plastic well aerated cages at normal atmospheric temperature (25±5 °C) and normal 12-hour light/dark cycle under hygienic conditions.

2.3 Methodology for extraction

Weigh 20 g of black grapes paste (ripen can be mashed to prepare a paste) into a 250 ml round-bottomed flask. Add 50 ml of ethanol and 60 ml of dichloromethane. Heat the mixture under reflux for 5 min on stem-bath with frequent shaking. Filter the mixture under suction and transfer the filtrate to a separatory funnel. Wash this mixture containing bioactive compounds with three portions of 150 ml each with sodium chloride solution. Dry the organic layer over anhydrous magnesium sulfate. Filter and evaporate most of the solvent in vacuum without heating. Same procedure is followed for the extraction of MLN; ACT and CLF extracts.

2.4 Preliminary Phytochemical screening

Preliminary Phytochemical screening of various extracts (ELN; MLN; ACT and CLF) of black grapes (Vitis vinifera) had shown the presence of various bioactive compounds such as carbohydrates aminoacids and peptides phytosterols carotenoids and polyphenols (higher concentration).

2.5 Evaluation of Acute Oral Toxicity

In the present study acute oral toxicity of the various extracts were performed by the acute toxic class method. No sign of toxicity and mortality were observed at 200 mg/kg b.w to the group of animals, and the LD50 value of the title extracts (ELN; MLN; ACT and CLF) expected to exceed at 200 mg/kg b.w and represented as class 5 (200 mg/kg < LD50 < 2500 mg/kg). From the toxicity studies the data revealed that all the extracts proved to be non toxic at tested dose levels and well tolerated by the experimental animals as their LD50 cut of values > 2500 mg/kg b.w.

2.6 Evaluation Anti inflammatory activity by Carrageenan Induced Paw Edema Method in Rats

Anti-inflammatory activity was performed by carrageenan induced paw oedema method in rats. Diclofenac sodium (10 mg/kg i.p) was administered as standard drug for comparison. The various extracts (ELN; MLN; ACT and CLF) of black grapes (Vitis vinifera) were administered at one dose level (200 mg/kg) by orally then 30 minutes prior to the administration of 0.1ml/kg body weight of carrageenan used in saline (1%w/v) into the lateral malleolus of sub-planter region of the rats left their hind paw. The paw volumes were measured using the mercury displacement technique with the help of a plethysmonograph immediately before and 30 minutes, 1, 2 and 3 hour after carrageenan injection. The percentage inhibition of paw oedema was calculated by using the following formula: Percentage protection = [(control-test)/control] ×100.

3. RESULTS AND DISCUSSION

3.1 Phytochemical screening
Preliminary Phytochemical screening of various extracts (ELN; MLN; ACT and CLF) of black grapes (Vitis vinifera) had shown the presence of various bioactive compounds such as carbohydrates aminoacids and peptides phytosterols carotenoids and polyphenols (higher concentration).

Fig 1: Percentage protection of the various extracts of fruit extracts of Vitis vinifera with reference to standard drug diclofenac sodium.

3.2 Pharmacological screening

In vivo anti-inflammatory activity of the various extracts ELN; MLN; ACT and CLF of fruit of Vitis vinifera was evaluated by carrageen an induced paw edema method. The activity was studied at a 200 mg/kg b.w. p.o. and their responses were measured at 30, 60, 120 and 180 min. The present experimental data shown in Table 1, displayed that all the extracts exhibited mild to good anti-inflammatory activity. Graded dose response was also observed. All the extracts exhibited highest activity at 120 min. When compared with standard drug diclofenac sodium (10 mg/kg i.p), it was found that the extract CLF (percentage of protection 68.9%), ELN (percentage of protection 68.5%), MLN (percentage of protection 60.5%), ACT (percentage of protection 58.54%), were exhibited good anti inflammatory activity.

4. CONCLUSION

In vivo experimental data had shown that the various extracts ELN; MLN; ACT and CLF of fruit of Vitis vinifera had the potential ability to reduce inflammation which was induced by carrageen in albino rat and had percentage protection of extract CLF 68.9%, ELN 68.5%, MLN 60.5%, and ACT 58.54%.

Table 1: For the evaluation of In vivo Anti inflammatory activity

<table>
<thead>
<tr>
<th>Extracts</th>
<th>30 min %</th>
<th>60 min %</th>
<th>120 min %</th>
<th>180 min %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.70±0.08</td>
<td>0.72±0.07</td>
<td>0.74±0.34</td>
<td>0.72±0.04</td>
</tr>
<tr>
<td>Diclofenac sod. (10mg/kg p.o.)</td>
<td>0.42±0.06</td>
<td>40.00±3.00</td>
<td>58.33±2.23</td>
<td>70.27±0.72</td>
</tr>
<tr>
<td>ELN (200mg/kg p.o.)</td>
<td>0.40±0.049</td>
<td>38.42±0.58</td>
<td>57.10±0.23</td>
<td>68.5±0.34</td>
</tr>
<tr>
<td>MLN (200mg/kg p.o.)</td>
<td>0.37±0.06</td>
<td>36.30±0.54</td>
<td>50.8±0.24</td>
<td>60.5±0.32</td>
</tr>
<tr>
<td>ACT (200mg/kg p.o.)</td>
<td>0.37±0.09</td>
<td>37.11±0.23</td>
<td>48.72±0.25</td>
<td>58.54±0.33</td>
</tr>
<tr>
<td>CLF (200mg/kg p.o.)</td>
<td>0.41±0.05</td>
<td>39.42±0.49</td>
<td>57.11±0.22</td>
<td>68.9±0.34</td>
</tr>
</tbody>
</table>

Significant differences with respect to control was evaluated by (ANOVA), Dunnet’s t test * P<0.05, **P<0.01, NS (Non significant) % (Percentage reduction of edema).

5. REFERENCES

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