Evaluation of Hepatoprotective Activity of Flowers of “Tagetes erecta linn”

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ABSTRACT
In the present study, the hepatoprotective activity of the ethanolic extract of flowers of Tagetes erecta was carried out. For the study, male wister rats weighing 200-250gm were used and divided into six group of four in each. Experimentally induced hepatotoxicity by using CCl₄ /olive oil (1:1, 3ml/kg, i.p for seven days), silymarin suspended in 0.6% c.m.c. (100mg/kg, orally. For seven days) as standard reference and 100mg, 200mg, and 400mg/kg of ethanolic extract of flowers of plant Tagetes erecta for seven consecutive days orally followed by CCl₄ injection on day seven. Pre-treatment with ethanolic extract of Tagetes erecta reduced the biochemical markers of hepatic injury like SGPT, SGOT, ALP and bilirubin levels and histopathological examination showned 200 and 400mg/kg showed hepatoprotective property. This results indicate the flower of tagetes erecta possess hepatoprotective property may be attributed to the quercetin related flavonoids present in the flower of Tagetes erecta.

INTRODUCTION
Liver has a pivotal role in regulation of physiological processes. Liver diseases are among the most serious ailment. They may be classified as acute or chronic hepatitis (inflammatory liver diseases), hepatitis (non inflammatory diseases) and cirrhosis (degenerative disorder resulting in fibrosis of the liver). There are numerous plants and polyherbal formulations claimed to have hepatoprotective activities¹. Most of the studies on hepatoprotective plants were carried out using chemical-induced liver damage in rodents as models. Tagetes erecta is a flower, belonging to the family Compositae. Commonly cultivated in Indian gardens for their bright yellow to orange colored flowers². Extensive literature survey indicated the presence of sterols, glycosides, tannins, saponins, flavonoids from the flowers of the plant Tagetes erecta³. From the literature survey, it was learnt that no detailed pharmacological investigation done so far on the flowers of Tagetes erecta. Hence present study will be undertaken to investigate its hepatoprotective activity.

MATERIALS AND METHODS
For the study, Silymarin: Received as a gift sample from micro labs limited Hosur, tamilnadu. Male wistar rats weighing 200-250g were used. They had been given standard pellet diet supplied by Hindustan Lever Co. Mumbai and water adlibitum throughout the course of the study, flowers of Tagetes erecta were obtained from local market, and it was authenticated by Dr. Noeline.J.Pinto. H.O.D. Dept of Botany, St Agnes College, Mangalore-2. Thus obtained flowers were dried under the shade for 15 days and were pulverized in an electric grinder. The powdered material was soaked in 90% ethanol for four days. Stirring of the mixture was done twice daily. After the fourth day, the mixture was filtered and the marc was pressed. This process was repeated 3 times. All the alcoholic fractions were combined and the ethanol was subjected for evaporation. The syrupy consistency material obtained was heated on the water bath until dry extract was obtained. Thus obtained ethanolic extract of flowers of Tagetes erecta were labeled and stored in the desiccator for further usage⁴. The preliminary pharmacological studies were conducted to assess the acute pharmacological effects and LD₅₀ of the ethanolic drug extracts. The acute toxicity study was carried out in adult female albino rats by “up and down” method (OECD guidelines 425)⁵. The animals were fasted overnight.
and next day extracts of the flowers of the plant Tagetes erecta (suspected in 0.6 % w/v sodium CMC) were administered orally at different dose level (100 mg/kg-2000 mg/kg). Then the animals were observed continuously for three hour for general behavioral, neurological, autonomic profiles and then every 30 min for next three hour and finally death after 24 hour. The LD_{50} value will be determined by the graphic method. This experiment was carried out according to described by Akram Jamshidzadeh et al. Rats were divided into six groups of four animals in each group.

Group I received saline (10ml/kg, i.p.) as normal control for seven days.

Group II received CCl_{4} /olive oil (1:1, 3ml/kg, i.p.) as treated control group on day seven.

Group III received silymarin suspended in 0.6% c.m.c. (100mg/kg,) orally as standard reference for seven days followed by CCl_{4} injection on day seven.

Group IV, V, VI received 100 mg, 200 mg, and 400 mg/kg of ethanolic extract of Tagetes erecta for seven consecutive days orally, followed by CCl_{4} injection on day seven. The rats of all groups were sacrificed 48 hour after CCl_{4} injection. They were anaesthetized by diethyl ether and blood was collected from retro-orbital route, and serum was separated for assessment of different enzyme level (SGPT, SGOT & ALP) and bilirubin. The liver will be preserved in 10% formalin solution for histopathological Investigation. Histological damage will be scored as (0: absent; +: mild; ++: moderate; and +++: severe). The results were seen in table no 1 and figure no 1,2,3 and 4. The results will statistically analyzed by using one way analysis of variance (ANOVA), followed by Dunnet’s ‘t’ test.

**RESULTS AND DISCUSSION**

The rats of all the three groups treated with alcoholic extract of Tagetes erecta at different dose levels showed significant reduction in SGOT, SGPT, ALP and total bilirubin, compared to the CCl_{4} treated group. The results obtained so are statistically significant and comparable to the silymarin treated group as shown in the table no 1 and fig no 1,2,3 and 4. SGOT in CCl_{4} treated group have significantly increased compared to control group (p<0.01). SGOT were decreased significantly in treatment group up to 353.72±1.59 (p<0.01), 221.91±1.63 (p<0.01), and 152.60±1.72 (p<0.01), at doses of 100, 200 and 400mg/kg body wt. respectively as compared to the only CCl_{4} treated group. Silymarin also have decreased SGOT level to 131.75±1.61 (p<0.01) compare to CCl_{4} treated group. Serum enzyme SGPT levels were increased significantly in CCl_{4} treated group as compared to the control rats. The values were increased up to 296.46±1.46 (p<0.01), compared to control group, which was 48.55±2.25. The serum SGPT were decreased significantly in treatment group up to 117.21±2.30 (p<0.01), 89.09±0.87 (p<0.01), 59.41±1.90 (p<0.01) at doses of 100,200 and 400 mg/kg body wt. respectively as compared to the only CCl_{4} treated group. Silymarin also have decreased the serum SGPT level to 57.77±2.10 (p<0.01). Serum ALP levels were increased significantly in CCl_{4} treated group as compared to the control rats. The values were increased up to 250.50±1.80 (p<0.01), compared to control group, which was 126.15±1.82. The serum ALP were decreased significantly in treatment group up to 181.28±1.90 (p<0.01) 122.98±1.72 (p<0.01), 81.84±1.90 (p<0.01) at doses of 100, 200 and 400 mg/kg body wt. respectively as compared to the only CCl_{4} treated group. Silymarin also have decreased the serum ALP level to 91.79±2.92 (p<0.01). Total S. Bilirubin levels in CCl_{4} treated group have significantly increased compared to control group. The values were increased up to 4.23±0.02 (p<0.01), compared to control group, which was 0.99±0.01. The serum total bilirubin values were reduced significantly in treatment group up to 2.89±0.06 (p<0.01), 2.08±0.01 (p<0.01), and 1.69±0.01 (p<0.01), at doses of 100, 200 and 400mg/kg body wt respectively as compared to the only CCl_{4} treated group. Silymarin also have reduced serum total bilirubin level to 1.24±0.02 (p<0.01).

**Table 1: Effect of Ethanolic extract of flowers of Tagetes erecta on biochemical parameters of ccl4 induced hepatic injury.**

<table>
<thead>
<tr>
<th>Group No</th>
<th>Treatments</th>
<th>SGOT</th>
<th>SGPT</th>
<th>ALP</th>
<th>Total BILIRUBIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (normal saline)</td>
<td>95.30±1.59</td>
<td>48.55±1.21</td>
<td>126.15±1.82</td>
<td>0.99±0.01</td>
</tr>
<tr>
<td>2</td>
<td>CCl_{4} treated(CCl_{4} in olive oil:1)</td>
<td>481.00±1.80*</td>
<td>296.46±1.46*</td>
<td>250.50±1.80</td>
<td>4.23±0.02*</td>
</tr>
<tr>
<td>3</td>
<td>CCl_{4} +Silymarin (P.O)</td>
<td>131.75±1.61*</td>
<td>57.77±2.10*</td>
<td>91.79±2.92*</td>
<td>1.24±0.02*</td>
</tr>
<tr>
<td>4</td>
<td>CCl_{4} + Tagetes erecta extract 100mg/kg</td>
<td>353.72±1.59*</td>
<td>117.21±2.30*</td>
<td>181.28±1.90*</td>
<td>2.89±0.02*</td>
</tr>
<tr>
<td>5</td>
<td>CCl_{4} + Tagetes erecta extract 200mg/kg</td>
<td>221.91±1.62*</td>
<td>89.09±2.30*</td>
<td>122.98±1.72*</td>
<td>2.08±0.05*</td>
</tr>
<tr>
<td>6</td>
<td>CCl_{4} + Tagetes erecta extract 400mg/kg</td>
<td>152.60±1.72*</td>
<td>59.41±1.90*</td>
<td>81.84±1.90*</td>
<td>1.69±0.01*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± sem, n=6 rats in one group.
Significant (p<0.01) compared to control,*significant (p<0.01) compared to CCl_{4}.
Figure No. 1: Effect of alcoholic extract of flowers of plant *Tagetes erecta* on SGOT in CCl₄ treated rats.

Figure No. 2: Effect of alcoholic extract of flowers of plant *Tagetes erecta* on SGPT in CCl₄ treated rats.

Figure No. 3: Effect of alcoholic extract of flowers of plant *Tagetes erecta* on ALP in CCl₄ treated rats.
The microscopic examination of liver of this group revealed that the test drug when used in 100mg/kg body wt. was not able to provide proper protection from fatty change in liver. However 200 and 400 mg /kg body wt. group revealed almost normal hepatocytes with only occasional fine fat vacuoles and mild inflammation.

CONCLUSION

Pretreatment with extract of flowers of plant Tagetes erecta (200 and 400 mg/kg) significantly reduced the elevated levels of biochemical marigers like SGPT, SGOT, ALP and bilirubin. Histopathological observations reveal that CCl₄ treatment has damaged the liver architecture, pretreatment Tagetes erecta. Prevented/reversed the CCl₄ induced liver damage in a dose dependant manner. Since, quercetin and related flavonoids are present in flowers of plant Tagetes erecta and flavonoids are reported to possess hepatoprotective properties. However, further studies are needed for confirmation.

REFERENCES