Role of Hydroalcoholic and Aqueous leaf extracts of *Murraya koenigii* in Gastroprotection

Hyacinth Highland*, Anal Engineer, Hetal Jethva, Ketaki Desai

Department of Zoology, Biomedical Technology and Human Genetics, Gujarat University, Ahmedabad, India

Corresponding author*
Dr. Hyacinth Highland
Department of Zoology, Biomedical Technology and Human Genetics, Gujarat University, Ahmedabad, India
E-mail: hnhighland@gujaratuniversity.ac.in

Abstract

Objective: *Murraya koenigii* is an extremely common plant and its leaves are regularly used in our diet as a condiment. Hence, our gastrointestinal tract is regularly exposed to its leaf extract and therefore evaluation of its effect on the organs of the gastrointestinal tract is imperative. The present study was therefore focused on the Gastroprotective action of *Murraya koenigii* leaf extracts on certain target tissues (Pancreas and Duodenum) in male Swiss albino mice.

Methods: Fresh curry leaves were dried and powdered followed by soxhlet extraction. Two extracts were prepared (Hydroalcoholic and Aqueous). Male albino mice weighing 35-40 gm were treated with *Murraya koenigii* leaf extracts against Carbon tetrachloride (CCL4) induced toxic model for seven days, using two different dose concentrations. The mice were divided into six groups, including Group I – Untreated Controls and Group I A – Vehicle controls. The negative control (Group II) was administered CCL4 along with vehicle model (olive oil). Group III and IV were given low dose (150 mg/kg body weight) of aqueous and hydroalcoholic extracts after inducing toxicity with CCL4. Similarly, Group V and VI were administered with high dose (250mg/kg body weight) of the extracts. The mice were then monitored for biochemical changes of ATPase, ALKPase, ACPase, SDH, Protein and Cholesterol in both target tissues. In addition, duodenal Triglycerides and Pancreatic amylase were also estimated.

Results: From the experiment, it was observed that *Murraya koenigii* leaf extracts significantly ameliorated the toxicity of carbon tetrachloride. The levels of ATPase, SDH, ALKPase, ACPase and Amylase were lowered after CCL4 exposure. The extracts had a mitigative effect and were able to bring these enzymes to near normal values. The hydroalcoholic extract was seen to be more effective than aqueous extract.

Conclusion: The present study establishes that the *Murraya koenigii* leaf extracts have potent ameliorative action on the CCL4 induced toxicity in the duodenum and pancreas. Hence they do possess potent gastroprotective activity. It was also observed that the effect varied with dose but there was no clear cut beneficial effect of high doses over the low doses. The present study has significant impact since the plant is used extensively in both cuisine and medication.

Keywords: *Murraya koenigii*, Carbon tetrachloride, Gastroprotective, duodenum, pancreas.

1. Introduction

Traditional herbal alternatives are being used more frequently in current times, since synthetic drugs may cause undesirable side effects. The uses of herbal plants have stimulated a new wave of interest in ethnomedicine because of their cost effectiveness and minimal side effects. Hence the current work was focused on studying the ameliorative action of *Murraya koenigii* against CCL4 induced toxicity in certain target tissues of the gastrointestinal tract. *Murraya koenigii* is well known for its extensive use as a popular spice herb in Indian dishes and also for its therapeutic uses in Ayurveda. The bark and leaf extracts have been therapeutically used in folk medicine to control diabetes, pancreatitis, asthma, stomach-ache etc. *Murraya koenigii* also possesses hypoglycaemic and antiulcer activity [1]. Tachibana et al [2] have demonstrated that *Murraya koenigii* leaves increase digestive secretions and relieve nausea, indigestion, and vomiting. Leaves are also used in many of the Indian Ayurveda and Unani prescriptions [3]. An infusion of toasted leaves is used to stop vomiting [4]. According to Saini et al [5] the acute toxicity study of curry leaves showed that their diet does not possess any toxic effect and are safe till the dose level of 9000 mg/kg. *Murraya koenigii has also shown* hepatoprotective activity and considerable free radical scavenging activity [6].

The plant has been also studied for their various pharmacological activities like antioxidant, antibacterial, anti fungal, antiprotozoal, anti-lipid peroxidative, hypoglycemic and hypolipidemic activity. *Murraya koenigii* is an extensively investigated spice for various organic constituents such as coumarins, terpenoids and carbazole alkaloids [7]. Also the extensive use of *Murraya koenigii* for various disorders and ailments shows that this plant has an abundance of bioactive phytochemicals that could prove useful to have ameliorative action. However there is no scientific report on the effect of
**Murraya koenigii** on the gastrointestinal tract.

Hence, in the present study, two specific extracts (aqueous and hydro-alcoholic) of *Murraya koenigii* leaves, were selected for evaluation of its possible action in mitigating the induced toxicity of carbon tetrachloride on target gastrointestinal organs, such as the duodenum and pancreas. These tissues are often the target site of most toxic activity. Damage to these tissues results in adverse effect on physiological processes of digestion and assimilation and indirectly influences several other vital functions. In addition, it is well known that in our country *Murraya koenigii* is extensively used as a condiment in cuisine and hence the population consumes appreciable quanta of *Murraya koenigii* leaf extracts, on a daily basis. The study of its action on duodenal and pancreatic tissues would consequently have added significance.

## 2. Materials and methods

The present study was focused on the Gastroprotective action of *Murraya koenigii* in certain target tissues (Pancreas, Duodenum) in male Swiss albino mice. Male albino mice weighing 35-40 gm were treated with *Murraya koenigii* against Carbon tetrachloride induced toxic model for seven days using different dose concentration i.e., Low dose (150 mg/kg body weight) and High dose (250 mg/kg body weight).

### 2.1 Animals

Healthy, adult, pathogen free, male albino mice (*Mus musculus*) of Swiss strain, weighing between 35-40 gm were obtained from a recognised supplier. The animals were housed in air-conditioned animal house with 12h light and 12h dark cycles at a temperature of 26 ± 2 °C and relative humidity 30-70%. Animals of different experimental groups were caged separately and maximum of five animals per cage were maintained on a standard chow obtained from Pranav Agro Industry, distilled water was given *ad libitum*. All the animals were acclimatized seven days prior to the commencement of the treatment. Experiments were conducted in accordance with the guidelines set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India and experimental protocols were approved by Institutional animal ethics committee; CPCSEA Registration No. 167/1999/CPCSEA.

### 2.1.1 Collection of plant material

The selected plant material (*Murraya koenigii* leaves) was collected from Junagarh, Gujrat, India, during the month of December-2014. Leaves were carefully selected and washed thoroughly with running tap water, followed by rinsing with distilled water were shade dried at room temperature and then pulverized into powder and stored in air tight container till further use.

### 2.1.2 Preparation of Extract

The crude powder of the plant material (*Murraya koenigii* leaves) was then defatted with petroleum ether for about 24h. After defattation, extraction was carried out using a soxhlet apparatus. Two extracts were prepared: 1) Hydro-alcoholic extracts (Methanol: Water - 70:30) 2) Aqueous extract

### 2.1.3 Dose Administration

The treatment was administered for seven days. Toxicity was induced in animals using Carbon tetrachloride. The toxicant was given intraperitoneally using a 26 gauge needle. Carbon tetrachloride (1ml/kg) was given in a vehicle (olive oil) (1ml/kg) at every 48hrs during course of treatment. After inducing toxicity in the target tissues of Swiss albino male mice, the animals were given an oral dose of 150 mg/kg body wt. (low dose) and 250 mg/kg body wt. (high dose) of aqueous and hydro-alcoholic *Murraya koenigii* leaf extracts. The animals were grouped as mentioned below:

- **Group I:** Control Animal (Control animals were only given standard food pellets and distilled water).
- **Group I-A:** Vehicle Control
  - Treated with Olive oil (vehicle) at 1ml/kg body weight.
- **Group II:** Negative Control Animals (Animals were injected Carbon tetrachloride (1ml/kg body weight) with Figaro olive oil as a vehicle model, intraperitoneally to induce toxicity in the target tissue at every 48 hours for seven days).
- **Group III:** CCl₄ + Aqueous Curry leaf extract (Low dose 150 mg/kg body weight)
- **Group IV:** CCl₄ + Hydro alcoholic Curry leaf extract (Low dose 150 mg/kg body weight)
- **Group V:** CCl₄ + Aqueous Curry leaf extract (High dose 250 mg/kg body weight)
- **Group VI:** CCl₄ + Hydro alcoholic Curry leaf extract (High dose 250 mg/kg body weight)

Animals were injected with Carbon tetrachloride (1ml/kg body weight) along with low dose (150 mg/kg body weight) and high dose (250 mg/kg body weight) aqueous and hydro-alcoholic extracts (Groups III, IV, V, VI).

**Rationale for dose selection:**

From literature survey it was found that the most commonly used dosage of *Murraya koenigii* was 200mg. Hence a dose lower than the existing dose i.e, 150mg and a dose higher than the existing dose i.e, 250mg were selected for the study.
2.1.4 Data Collection

At the end of each treatment, the animals were first weighed on digital balance (Reptech) and necropsied according to CPCSEA specification. Vital tissues pancreas and duodenum were dissected out carefully and blood was collected for serum to further analysis. For histological studies, a suitable amount of the vital tissue was placed immediately in appropriative fixative. All the chemicals used were of analytical grade (AR).

2.2 Biochemical Parameters

2.2.1 Adenosine Triphosphatase (ATPase) (E.C.3.6.1.3)

The ATPase activity in Duodenum and Pancreas of control and all treated groups of animals was assayed by the method of Quinn and White [8]. The enzyme adenosine triphosphatase (ATPase) hydrolyses the substrate ATP into adenosine diphosphate (ADP) and inorganic phosphate (ip). Readings were taken at 660 nm on a Systronics Digital Spectrophotometer 167.

2.2.2 Alkaline Phosphatase (ALKpase) (E.C.3.6.1.3)

Alkaline phosphatase activity was estimated in Duodenum and Pancreas by the method of Bessey et al [9]. The enzyme alkaline phosphatase hydrolyses the substrate p-nitrophenyl phosphate into inorganic phosphate and p-nitrophenol. The quantity of p-nitrophenol released under standardized condition is measured at 410 nm. The enzyme activity was expressed as µ moles p-nitrophenol released / 30 min/ mg protein by further dividing it with protein concentration.

2.2.3 Acid Phosphatase (ACPase) (E.C.3.1.3.2)

Activity of Acid phosphatase was determined in Duodenum and Pancreas of all treated and control mice by the method of Bessey et al [9]. Acid Phosphatase, orthophosphoric monoester phosphohydrolase catalyses the hydrolysis of p-nitrophenyl phosphate at pH 4.8, liberating paranitrophenol and inorganic phosphate. The liberated p-nitrophenol combines with NaOH to form a yellow coloured complex, which is measured at 420 nm and is directly proportional to the enzyme activity.

2.2.4 Succinate Dehydrogenase (SDH) (E.C.1.3.99.1)

The activity of SDH was estimated in Duodenum and Pancreas of control and all treated groups of animals according to the method of Beatty et al [10] using 2(4-bydophenyl) 3-(4-Nitrophenyl) 5-phenyl tetrazolium chloride (INT) as an electron acceptor. The electrons released by the enzyme SDH from the substrate are taken up by an electron acceptor i.e. INT which is reduced to red coloured formazan. After extracting it in ethyl acetate, the colour intensity is measured at 420 nm.

2.2.5 Protein

Protein levels in Duodenum and Pancreas of control and all treated groups of animals were estimated by the method of Lowry et al [11]. Protein containing preparation when treated with phenol reagent of Folin-Ciocalteau results in a blue colouration which was read at 540 nm.

2.2.6 Cholesterol

The levels of cholesterol in Duodenum and Pancreas of control and all treated groups of mice were estimated by the method of Zlatkis et al [12]. In the presence of concentrated sulphuric acid and glacial acetic acid, cholesterol forms a coloured complex with ferric chloride (FeCl₃) which can be measured on Systronics Digital Spectrophotometer 167 against blank.

2.2.7 Triglyceride

Triglycerides were estimated from the duodenum by Trinder’s method [13] using reagents in Merill Laboratories Triglyceride kit and the results obtained on automated Biochemical Analyser.

2.2.8 Pancreatic α-Amylase [E.C.3.2.1.1.]

Amylase was estimated from pancreatic tissue, using reagents in kits from Merill Laboratories and read on an automated Biochemical analyser- Merilyzer ClinQuant [14].

2.2.9 Statistical Analysis

For each biochemical parameter, a minimum of 6 replicates were done. Values are expressed as Mean ± S.E. The Student’s’ t’ test was used to verify level of significance at the p<0.05 value.

3. Results

The freshly prepared extracts were subjected to preliminary phytochemical screening test for various constituents. This revealed the presence of alkaloids, tannins, flavonoids, glycosides, terpenoids and steroids.

3.1 Survival and Mortality

No mortality occurred in any of the treatment groups during the treatment period.

3.2 Duodenum

An increase in the organ weight of Duodenum was recorded after Carbon tetrachloride treatment. Administration
of both aqueous and hydro-alcoholic leaf extracts brought about an insignificant recovery in the weight of Duodenum, as compared to control (Table 2).

3.3 Pancreas
Carbon tetrachloride treatment caused an insignificant decline in the weight of Pancreas. Treatment after the Carbon tetrachloride induced toxicity with both doses (low dose + high dose) of aqueous extracts of Murraya koenigii resulted in a recovery in the weight of Pancreas. High dose of hydro-alcoholic extract proved beneficial in reducing the body weight which returned to control values (Table 2).

3.4 Enzyme activity in Duodenum:
Carbon tetrachloride treatment for 7 days brought about an insignificant decrease (p<0.001, p<0.01, p<0.01) in ACPase, ATPase and SDH activity in the Duodenum of animals of Group II as compared to the control Group I. While extract treatment Group III, IV, V, VI resulted in a significant (p<0.001, p<0.01) recovery in ACPase, ATPase and SDH levels in the Duodenum of animals as compared to the Carbon tetrachloride treated Group II (Table 3). The hydroalcoholic extract was seen to be more effective in bringing back the levels of ACPase.

3.5 Metabolic markers in Duodenum:
Carbon tetrachloride treatment for 7 days caused a significant increase (p<0.001) in protein and cholesterol levels in the Duodenum of animals of Group II as compared to control Group I. However a significant decline was observed in the triglyceride content of the duodenum Whereas, extract treated group recorded a recovery, and was observed to decrease the protein and cholesterol level in Group III, IV (p<0.001) and Group V, VI (p<0.01) as compared to CCl4 Group II, restoring protein levels to near normal values (Table 4). The triglyceride content was also ameliorated with treatment in both doses of Murraya koenigii (Table 4).

3.6 Enzyme activity in Pancreas:
Activity of ATPase, ALPase, ACPase, and SDH in Pancreas of Carbon tetrachloride treated mice for 7 days was found to be reduced significantly in Group II as compared to control Group I. However, a significant recovery in ATPase, ALPase (Group VI) (p<0.001, p<0.01) was observed after treatment with the hydroalcoholic extract. The ACPase and SDH activity was also recovered in Pancreas of mice in Groups III, IV, V (p<0.01) and Group VI (p<0.001) after treatment with both doses of both extracts (Table 5, Fig 2).

3.6.1 Amylase activity in Pancreas:
Toxicity induced due to Carbon tetrachloride treatment for 7 days reduced the Amylase activity of Pancreas significantly (p<0.001) as compared to control Group I. Extract treatment resulted in a significant increase as seen in Group III (p<0.01), IV (p<0.001), V and VI (p<0.001) as compared to the CCl4 intoxicated Group II (p<0.001) causing an ameliorative effect, bringing the values close to control levels (Table 6). The hydroalcoholic extract was seen to have more potency in ameliorating CCl4 toxicity in all extract treated groups.

3.7 Metabolic markers in Pancreas
Protein content as well cholesterol content was increased significantly (p<0.01) in Pancreas of mice following CCl4 administration for 7 days as compared to the control Group I. Extract treatment of both low dose and high dose showed a decrease in the level of protein (Group III, V (p<0.001) and IV, VI (p<0.01) and cholesterol (Group III (p<0.001), IV (p<0.01), V (p<0.001), Group II (p<0.001)), resulting in a mitigative effect (Table 6).

Table 1: showing body weight in control and treated mice, with aqueous and hydro-alcoholic extracts of Murraya koenigii.

<table>
<thead>
<tr>
<th>No.</th>
<th>Groups</th>
<th>Body weight (B.T.) (gm)</th>
<th>Body Weight (A.T.) (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control (N.T.)</td>
<td>40±2.89</td>
<td>42.66±2.33</td>
</tr>
<tr>
<td>I-A</td>
<td>Vehicle Control (1ml/kg)</td>
<td>41.33±3.1 *NS</td>
<td>41.57±2.9 *NS</td>
</tr>
<tr>
<td>II</td>
<td>CCl4 (1ml/kg)</td>
<td>46.66±1.67 *NS</td>
<td>42±1.11 *NS</td>
</tr>
<tr>
<td>III</td>
<td>CCl4 + LDaq (150 mg/kg)</td>
<td>48.33±7.27 *NS</td>
<td>49.66±7.89 *NS</td>
</tr>
<tr>
<td>IV</td>
<td>CCl4 + HDaq (250 mg/kg)</td>
<td>42.33±5.05 *NS</td>
<td>43±4.94 *NS</td>
</tr>
<tr>
<td>V</td>
<td>CCl4 + LDha (150 mg/kg)</td>
<td>39.33±4.7 *NS</td>
<td>37±3 *NS</td>
</tr>
<tr>
<td>VI</td>
<td>CCl4 + HDha (250 mg/kg)</td>
<td>34.66±2.6 *</td>
<td>38.66±2.03 *NS</td>
</tr>
</tbody>
</table>

N=4 /group; N.T. = No Treatment; *p<0.01 **p<0.001; B.T. = Before Treatment; Values are Mean ± S.E; A.T. = After Treatment; NS=Non-Significant
Table 2: showing organ weight of Duodenum and Pancreas in control and treated mice, with aqueous and hydro-alcoholic extracts of *Murraya koenigii*.

<table>
<thead>
<tr>
<th>No.</th>
<th>Groups</th>
<th>Organ weight (Duodenum)(mg)</th>
<th>Organ weight (Pancreas)(mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>227.56±9.77</td>
<td>446.8±35.23</td>
</tr>
<tr>
<td>I-A</td>
<td>Vehicle Control (1ml/kg)</td>
<td>231±10.2**</td>
<td>448.02±32.21 NS**</td>
</tr>
<tr>
<td>II</td>
<td>CCl4 (1ml/kg)</td>
<td>279.93±37.06 NS**</td>
<td>304.66±2.9*</td>
</tr>
<tr>
<td>III</td>
<td>CCl4 + LDAQ (150 mg/kg)</td>
<td>212±11.41 NS**</td>
<td>355.86±66.54 NS</td>
</tr>
<tr>
<td>IV</td>
<td>CCl4 + HDaq (250 mg/kg)</td>
<td>231.06±25.47 NS</td>
<td>392.2±34.62 NS**</td>
</tr>
<tr>
<td>V</td>
<td>CCl4 + LDha (150 mg/kg)</td>
<td>240.36±56.31 NS</td>
<td>254.76±36.21 NS</td>
</tr>
<tr>
<td>VI</td>
<td>CCl4 + HDha (250 mg/kg)</td>
<td>238.16±58.81 NS</td>
<td>368.5±19.69*</td>
</tr>
</tbody>
</table>

N=4 /group; *p<0.01 **p<0.001; NS=Non-Significant; Values are Mean ± S.E.

Table 3: showing ATPase, ACPase and SDH activity in Duodenum of control and treated mice, with aqueous and hydro-alcoholic extract of *Murraya koenigii*.

<table>
<thead>
<tr>
<th>No.</th>
<th>Groups</th>
<th>ATPase*a</th>
<th>ACPase*b</th>
<th>SDH*c</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>37.04±5.6</td>
<td>19.24±0.27</td>
<td>498.98±22</td>
</tr>
<tr>
<td>I-A</td>
<td>Vehicle Control (1ml/kg)</td>
<td>37.2±4.1 NS</td>
<td>18.6±0.3 NS</td>
<td>492.4±15 NS</td>
</tr>
<tr>
<td>II</td>
<td>CCl4 (1ml/kg)</td>
<td>28.58±2.9*</td>
<td>7.06±0.08 **</td>
<td>401.94±26.5*</td>
</tr>
<tr>
<td>III</td>
<td>CCl4 + LDAQ (150 mg/kg)</td>
<td>44.20±.04 **</td>
<td>19.39±0.55 **</td>
<td>459.02±23.7*</td>
</tr>
<tr>
<td>IV</td>
<td>CCl4 + HDaq (250 mg/kg)</td>
<td>31.87±6.5**</td>
<td>13.26±0.47 **</td>
<td>457.12±23.8*</td>
</tr>
<tr>
<td>V</td>
<td>CCl4 + LDha (150 mg/kg)</td>
<td>32.65±4.8**</td>
<td>13.23±0.31**</td>
<td>463.67±28.1*</td>
</tr>
<tr>
<td>VI</td>
<td>CCl4 + HDha (250 mg/kg)</td>
<td>27.63±3.13**</td>
<td>19.36±0.38**</td>
<td>471.04±25.2*</td>
</tr>
</tbody>
</table>

*p<0.01 **p<0.001; a=μmoles of inorganic phosphate released/mg/min; N=4 /group; b=μmoles of p-nitro Phenol released/100 mg tissue; Values are Mean ± S.E.; c=μg formazon formed/min/tissue wt

Table 4: showing Proteins, Cholesterol and Triglyceride levels in Duodenum of control and treated mice, with aqueous and hydro-alcoholic extract of *Murraya koenigii*.

<table>
<thead>
<tr>
<th>No.</th>
<th>Groups</th>
<th>Proteins*a</th>
<th>Cholesterol*b</th>
<th>Triglyceride*b</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>8.26±0.09</td>
<td>96.2±3.81</td>
<td>110.73±6.4</td>
</tr>
<tr>
<td>I-A</td>
<td>Vehicle Control (1ml/kg)</td>
<td>8.51±0.06**</td>
<td>97.1±2.9 NS</td>
<td>107.52±7.1</td>
</tr>
<tr>
<td>II</td>
<td>CCl4 (1ml/kg)</td>
<td>17.49±1.74**</td>
<td>151.3±7.32**</td>
<td>93.56±7.4</td>
</tr>
<tr>
<td>III</td>
<td>CCl4 + LDAQ (150 mg/kg)</td>
<td>8.70±1.06**</td>
<td>61.14±0.25**</td>
<td>88.41±5.8</td>
</tr>
<tr>
<td>IV</td>
<td>CCl4 + HDaq (250 mg/kg)</td>
<td>9.93±2.02**</td>
<td>84.40±0.74**</td>
<td>118.20±9.2</td>
</tr>
<tr>
<td>V</td>
<td>CCl4 + LDha (150 mg/kg)</td>
<td>12.5±2.31*</td>
<td>65.34±5.44**</td>
<td>113.88±7.5</td>
</tr>
<tr>
<td>VI</td>
<td>CCl4 + HDha (250 mg/kg)</td>
<td>14.42±1.85*</td>
<td>92.11±3.92**</td>
<td>114.64±6.1</td>
</tr>
</tbody>
</table>

*p<0.01 **p<0.001; a=mg / 100mg tissue wt; N= 4 / group; b=mg/dl; Values are Mean ± S.E.

Table 5: showing ATPase, ALPase, ACPase and SDH activity in Pancreas of control and treated mice, with aqueous and hydro-alcoholic extract of *Murraya koenigii*.

<table>
<thead>
<tr>
<th>No.</th>
<th>Groups</th>
<th>ATPase*a</th>
<th>ALPase*b</th>
<th>ACPase*b</th>
<th>SDH*c</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>14.02±2.8</td>
<td>13.54±1.03</td>
<td>10.37±1.2</td>
<td>444.86±32.9</td>
</tr>
<tr>
<td>I-A</td>
<td>Vehicle Control (1ml/kg)</td>
<td>13.96±1.3 NS</td>
<td>13.28±1.06 NS</td>
<td>10.53±1.1 NS</td>
<td>447.5±28.2 NS</td>
</tr>
<tr>
<td>II</td>
<td>CCl4 (1ml/kg)</td>
<td>2.66±0.03**</td>
<td>7.23±0.46**</td>
<td>8.37±0.3**</td>
<td>320.13±35.0**</td>
</tr>
<tr>
<td>III</td>
<td>CCl4+LDAQ (150 mg/kg)</td>
<td>8.30±0.071*</td>
<td>9.57±0.33*</td>
<td>10.56±1.09**</td>
<td>493.27±28.4**</td>
</tr>
<tr>
<td>IV</td>
<td>CCl4+HDaq (250 mg/kg)</td>
<td>10.19±1.03*</td>
<td>12.43±2.01*</td>
<td>11.82±1.05*</td>
<td>404.00±24.1**</td>
</tr>
<tr>
<td>V</td>
<td>CCl4+LDha (150 mg/kg)</td>
<td>9.25±0.85*</td>
<td>5.07±0.19</td>
<td>11.58±0.87*</td>
<td>485.66±31.6**</td>
</tr>
<tr>
<td>VI</td>
<td>CCl4+HDha (250 mg/kg)</td>
<td>12.07±1.17**</td>
<td>12.38±1.4*</td>
<td>8.48±0.28</td>
<td>376.59±29.9*</td>
</tr>
</tbody>
</table>

*p<0.01 **p<0.001; a=μmoles of inorganic phosphate released/mg/min; N= 4 /group; b=μmoles of P-nitro Phenol released/100mg tissue wt; Values are Mean ± S.E.; c=μg formazon formed/min/tissue wt
Table 6: Showing Proteins and Cholesterol level, Amylase activity in Pancreas of control and treated mice, with aqueous and hydro alcoholic extract of *Murraya koenigii*

<table>
<thead>
<tr>
<th>No.</th>
<th>Groups</th>
<th>Proteins(^{a})</th>
<th>Cholesterol(^{a})</th>
<th>Amylase(^{b})</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>12.3±1.07</td>
<td>85.9±6.2</td>
<td>21.38±1.07</td>
</tr>
<tr>
<td>I-A</td>
<td>Vehicle Control (1ml/kg)</td>
<td>12.7±0.93(^{NS})</td>
<td>85.6±4.9(^{NS})</td>
<td>21.92±1.04(^{NS})</td>
</tr>
<tr>
<td>II</td>
<td>CCl(_4) (1ml/kg)</td>
<td>16.5±0.28(^*)</td>
<td>162.5±9.8(^{**})</td>
<td>8.43±0.03(^{**})</td>
</tr>
<tr>
<td>III</td>
<td>CCl(_4) + LDaq (150 mg/kg)</td>
<td>11.15±2.1(^{**})</td>
<td>75.98±0.24(^{**})</td>
<td>13.06±1.24(^*)</td>
</tr>
<tr>
<td>IV</td>
<td>CCl(_4) + HDaq (250 mg/kg)</td>
<td>13.4±0.71(^*)</td>
<td>114.35±13.86(^*)</td>
<td>14.05±0.93(^**)</td>
</tr>
<tr>
<td>V</td>
<td>CCl(_4) + LDha (150 mg/kg)</td>
<td>11.27±2.4(^{**})</td>
<td>103.22±6.19(^{**})</td>
<td>9.47±0.81(^{NS})</td>
</tr>
<tr>
<td>VI</td>
<td>CCl(_4) + HDha (250 mg/kg)</td>
<td>14.31±0.05(^*)</td>
<td>73.51±9.15(^{**})</td>
<td>16.45±1.15(^{**})</td>
</tr>
</tbody>
</table>

\(^{a}\)=mg/ 100mg tissue wt.; \(^{b}\)=U/L; \(^{NS}\)=Not significant; \(^{*}\)\(^{p}<0.01\); \(^{**}\)\(^{p}<0.001\); Values are Mean ± S.E.

**Figure 1:** Showing ATPase, ACPase and SDH activity in Duodenum of control and treated mice, with aqueous (aq) and hydroalcoholic (ha) extract of *Murraya koenigii*.

**Figure 2:** Showing ATPase, ALPase, ACPase and SDH activity in Pancreas of control and treated mice, with aqueous (aq) and hydroalcoholic (ha) extract of *Murraya koenigii*.
4. Discussion

The organs of the digestive tract are amongst the most susceptible vital organs, imperative for our wellbeing and lifelong health. A functional impairment of the gastrointestinal tract can lead to chronic health issues and thereby deteriorate the quality of life.

*Murraya koenigii* is an essential component of Asian cuisine and hence a vast majority of the population consumes extracts of this leaf inadvertently as a part of their daily intake. Thus, the gastrointestinal tract and associated glands are exposed to the extracts of *Murraya koenigii* leaves which therefore, Warrents investigation into understanding the effects of *Murraya koenigii* leaf extracts on the gastrointestinal tract.

The present investigation was focused on *Murraya koenigii* leaf extract’s (both hydroalcoholic as well as aqueous) ameliorative potential against CCl$_4$ induced toxicity in male mice. Administration of CCl$_4$ caused insignificant change in body weight probably due to loss of appetite as a result of CCl$_4$ toxicity. The results indicated that appetite recovered with administration of low and high doses of aqueous and hydroalcoholic extracts of *Murraya koenigii* leaves leading to recovery of body weight, suggesting an ameliorative effect.

Further, the present study also revealed that the increase in duodenum weight after CCl$_4$ intoxication may be correlated with an increase in protein and cholesterol content due to an accumulation of these metabolites. The accumulation of cholesterol with a concomitant reduction in triglyceride levels as indicated by biochemical analysis suggests poor breakdown of metabolites due to impaired digestion in this part of the gastrointestinal tract. Further this impaired digestion and the increase in cholesterol could be attributed to the blockage of bile ducts causing reduction/cessation of bile secretion to duodenum. Fatima [15] has showed that CCl$_4$ induced toxicity caused inhibition in liver synthetic function resulting in a depletion of bile synthesis and its secretion into the duodenum. Thus CCl$_4$ hepatotoxicity inhibits digestion in the duodenum, which supports the findings in our investigation. Administration of *Murraya koenigii* extracts proved to be effective in the recovery of duodenal weight.

A decrease in pancreatic weight after CCl$_4$ administration possibly results from impaired secretory, synthetic and metabolic activities of the said organ. Several researchers [16-18] have also shown that CCl$_4$ caused decrease in organ weights such as liver and kidney. These findings corroborate with our results, however it was observed that both aqueous and hydroalcoholic extracts of *Murraya koenigii* leaves manifested excellent ameliorative action on organ weight recovery, even over the aqueous extract.

Our study also demonstrated a significant increase in cholesterol content in pancreas after CCl$_4$ administration. This elevated cholesterol level may be due to decreased utilization of cholesterol under stress and accumulation of cholesterol in the pancreas due to poor metabolic turnover. Muthumani *et al* [19] have reported that the levels of serum cholesterol were altered in experimental rats due to ingestion of toxicants. Further, these researchers attributed the remarkable increase in the pancreatic weight to increase in lipid content of the pancreatic tissue. This finding is similar to our observation of elevated cholesterol under CCl$_4$ induced toxic stress.

An increase in the protein content in pancreas of treated mice as compared to control mice was also observed after 7 days exposure to CCl$_4$. In a previous study, stimulation of protein synthesis has been advanced as a contributory hepatoprotective mechanism, which accelerates the regeneration process and the production of liver cells [20]. It was observed here that *Murraya koenigii* leaf extracts both aqueous and hydro-alcoholic helped to lower and restore the level of cholesterol and protein to the original optimal value in the carbon tetrachloride affected pancreatic tissue. Kumar *et al*. [21] have demonstrated that *M. koenigii* extracts have pronounced hypolipidemic activity, which explains the cholesterol lowering effect manifested by the extracts used in the present study.

Furthermore the present study also revealed a significant decline in activity of energy metabolism associated enzymes Adenosine triphosphatase (ATPase) and Succinate dehydrogenase (SDH). The activity of Acid phosphatase (ACPase) and Alkaline phosphatase (ALPase) too showed a decline due to CCl$_4$ toxicity induced in duodenum and pancreas. The data obtained revealed however, that oral feeding of both aqueous and hydroalcoholic extracts prevented and reversed the CCl$_4$ induced toxicity by lowering the enzyme activity levels of ALPase and ACPase to near normal values. Specifically the data indicated that the high dose of the hydroalcoholic extract, appeared to be more potent in bringing about a mitigative action on Acid phosphatase activity in the duodenum.

The toxicity noted in enzyme activity levels are also supported by the work of Jadon *et al* [22]. According to them uncoupling of oxidative phosphorylation by CCl$_4$ causes a fall in activity of ATPase while reduction in SDH activity could be due to structural and functional disorganization of the mitochondrial assembly. Damage to the mitochondria curbs the energy generation contributing to the loss in energy production [23]. Moreover, an alteration in the tissue metabolism towards an anaerobic type therefore possibly caused reduction in succinic dehydrogenase enzyme activity in the CCl$_4$ treated mice. The treatment of *Murraya koenigii* extracts, at both doses, proved to exert a beneficial effect on the mitochondrial enzymes restoring the SDH enzyme activity and its metabolic effect to near normal values.
In a previous study, biochemical analyses have shown that toxic doses decrease the ATP concentration inside cells due to the membrane perturbation [24] resulting from toxic influences and release of free radicals. In the present study the ATPase and SDH activity in Pancreas were decreased significantly in CCl₄ treated mice as compared to control after 7 days. *Murraya koenigii* extracts (aqueous/hydro-alcoholic) brought about a reversal in the inhibition of SDH and the associated CCl₄ mediated toxicity. It was evident from the results obtained that the hydro-alcoholic extract was significantly more effective in restoring ATPase and ALPase activity in the pancreas.

Although the results obtained in this study confirmed that both the aqueous and hydro-alcoholic of *Murraya koenigii* had potent ameliorative action, on the CCl₄ induced toxicity in the duodenum and pancreas. It was observed that the hydro-alcoholic extract showed immense efficiency in its ameliorative action. This protective efficacy of the *Murraya koenigii* leaves lies in the phytochemical constituents who are perhaps more effectively extruded by the hydro-alcoholic solvent and hence the CCl₄ toxicity is reduced or fully combated. Ghasemzadeh et al [25] have confirmed the presence of phenolic acids and flavonoids as the main constituents of *Murraya koenigii* leaf extracts. Potent anti-oxidant phytochemicals such as tannins are present in optimum concentrations in *Murraya koenigii* leaf extract, which aid duodenal activity. Zahin et al [26] have also demonstrated the broad spectrum anti-oxidant properties of *Murraya koenigii*. Moreover, the hydroalcoholic extract proved to have greater efficiency in gastro-protection than the aqueous extract. In addition it was observed that the effect varied with dose administered but there was no clear cut beneficial effect of high doses over the low doses. The present study has significant impact since this plant is extensively used by local populations, both in cuisine and for medicinal purposes. Moreover, the outcomes of this study indicates that *Murraya Koennigii* extracts could well be used for reversing the toxic effects on Duodenum and Pancreas, and manifest a protective effect on these vital organs.

References


