

## Research Article



## In-Vitro Antioxidant Activity of *Feronia limonia* in Rat Testicular Homogenate

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### ABSTRACT

The aim of the present study was to evaluate the antioxidant effects of methanol fruit extract of *Feronia limonia* on Carbon tetrachloride (CCl<sub>4</sub>)-induced oxidative stress and to examine its protective effects on testicles of male Wistar rats. The extract was tested on antioxidant enzyme activities and lipid peroxidase enzyme in the rat testicular homogenate. Activity of antioxidant enzymes; catalase (CAT), superoxide dismutase (SOD) and phase II metabolizing enzymes viz; glutathione reductase (GSR), glutathione-S-transferase (GST) and reduced glutathione (GSH) was decreased while hydrogen peroxide contents and thiobarbituric acid reactive substances (TBARS) were increased with CCl<sub>4</sub> treatment. Treatment with MEFL effectively ameliorated the alterations in the biochemical markers. These findings suggest the antioxidant properties of MEFL that could be attributed to phenolic compounds which is revealed by phytochemical studies of the extract. The results also suggest that the *F. limonia* is having antioxidant activity with associated deleterious effects on testes. To our understanding, this is the first report on antioxidant activity of *Feronia limonia* fruit pulp methanolic extract on testicles.

**Keywords:** CCl<sub>4</sub>, *Feronia limonia*, Phenolic, Testis.

### INTRODUCTION

Carbon tetrachloride (CCl<sub>4</sub>) has been used as a model toxic agent and has been the focus of many *in vitro* and *in vivo* toxicological studies. The primary site of CCl<sub>4</sub> toxicity is the liver resulting in fatty degeneration, cellular necrosis, fibrosis and cirrhosis in multiple species and through multiple routes of exposure. CCl<sub>4</sub> also causes structural and functional damages in other organs of body such as kidney, lung, reproductive organs and brain. The free radicals initiate lipid peroxidation (LPO) by attacking polyunsaturated fatty acids (PUFAs) in membranes, setting off a free radical chain reaction sequence. LPO is known to cause membrane disruption, resulting in the loss of membrane integrity and the leakage of microsomal enzymes. By-products of LPO can form protein and DNA adducts and may contribute to toxicity and carcinogenicity.<sup>1</sup> Spermatozoa require a high PUFA content to provide the plasma membrane with the fluidity essential at fertilization. However, this makes spermatozoa particularly vulnerable to attack by reactive oxygen species (ROS) that have clear associations with reduced fertility.<sup>2</sup> In addition, as the testis, prostate and germ cells contain CYP isozymes, it is possible that CCl<sub>4</sub> causes oxidative damage in lipids of these tissues and cells.<sup>3-5</sup> Acute or chronic CCl<sub>4</sub> administration has been reported to cause morphological, structural and functional damages in reproductive system through oxidative toxicity in male laboratory animals.<sup>6-8</sup> In the present study, an attempt has been made to study the effect of MEFL in CCl<sub>4</sub> induced testicular damage pertaining to biochemical marker enzymes & histopathology. The result of this study will support the plant *Feronia limonia* as a good herbal antioxidant agent.

### MATERIALS AND METHODS

#### Plant material and extraction

Fruits of *Feronia limonia* L were collected from local market Bellary, Karnataka, India during the month of March, 2012. It was authenticated by the Department of P.G studies and Research in Botany, Gulbarga University, Gulbarga. Crude fruit pulp extract was prepared by Soxhlet extraction method, as described earlier by Jaya shree and Londonkar.<sup>9</sup> Solvent free methanolic extract dissolved in 1% Dimethyl sulphoxide (DMSO) was used for the studies.

#### Animals

Animals used for the preparation of testicular homogenate were 2 month-old male Wistar albino rats weighing about 150-175 g body weight. The protocol was approved by the Institute's Animal Ethical Committee (IAEC Reg No. 34800/ CPCSEA Dated: 19.08.2001). Animals were kept in animal house at an ambient temperature of 25°C and 45-55% relative humidity, with 12 h each of dark and light cycles. They were fed with a balanced diet as described by Central Food and Technological Research Institute (CFTRI, Mysore) and water ad libitum. OECD guidelines were followed.<sup>10</sup>

#### Chemicals

6-hydroxy-2, 5, 7, 8- tetramethylchromane- 2-car- boxylic acid (trolox) was purchased from Sigma Aldrich, Bangalore. All other chemicals and reagents were of analytical grade, and they were used as received.



## Acute toxicity experiment

Albino rats were divided into control and test groups (6 animals each). Control group received the vehicle (3% Tween 80) while the test groups got graded doses (100–4000 mg/kg) of MEFL orally and were observed for mortality till 48 h and the LD<sub>50</sub> was calculated.

## Experimental design

30% CCl<sub>4</sub> was prepared in olive oil. Animals of group 2, 3, 4 and 5 were given single dose of CCl<sub>4</sub> at 1ml/kg body weight (bw) intraperitoneally (i.p). Methanolic extract of *Feronia limonia* at the dose level of 100 mg/kg bw and 400 mg/kg bw as low dose and high dose were administered to animals of group 4 and 5 orally with the aid of an intragastric catheter for 7 days. Silymarin (50 mg/kg bw) was used orally as a standard drug to group 3 at a single dose. Rats were divided into five groups, each group consisting of six animals.

Group 1: Normal control group received saline 1ml/kg body weight.

Group 2: Toxic control treated with CCl<sub>4</sub> at 1ml/kg body weight.

Group 3: Positive control received CCl<sub>4</sub> + Silymarin for 7 days.

Group 4: The CCl<sub>4</sub> treated animals were given MEFL low dose for 7 days.

Group 5: The CCl<sub>4</sub> treated animals were given MEFL high dose for 7 days.

At the end of the experimental period, blood sample from each rat (2 ml) was withdrawn by cardiac puncture, collected in previously labeled centrifuge tubes, and allowed to clot for 30 min at room temperature. Serum was separated by centrifugation at 10,000 rpm for 5 min.

## Assessment of testicular lipid peroxidation and antioxidant activities

50 mg of testis was homogenized in 10 volume of KH<sub>2</sub>PO<sub>4</sub> (100 mmol) buffer containing EDTA (1 mmol, pH 7.4) and centrifuged at 12,000 × g for 30 min at 4°C. The supernatant was collected and used for enzymatic studies. Protein concentration of tissue supernatant was determined by the method of Lowry et al using BSA as standard.<sup>11</sup> Various antioxidant enzymes including CAT and SOD (Mishra and Fridovich)<sup>12</sup>, TBARS (Yagi)<sup>13</sup>, GST (Mohandas et al)<sup>14</sup>, GSR (Wu et al)<sup>15</sup> and GSH (Turner and Lysiak)<sup>16</sup> were carried out.

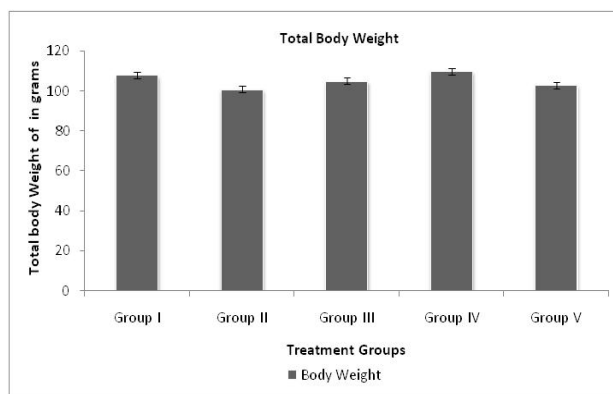
## Statistical analysis of data

To determine the effects of treatment, the data was calculated by applying one way analysis of variance using software SPSS 13.0. Level of significance among the various treatments was determined by LSD at 0.01% level of probability.

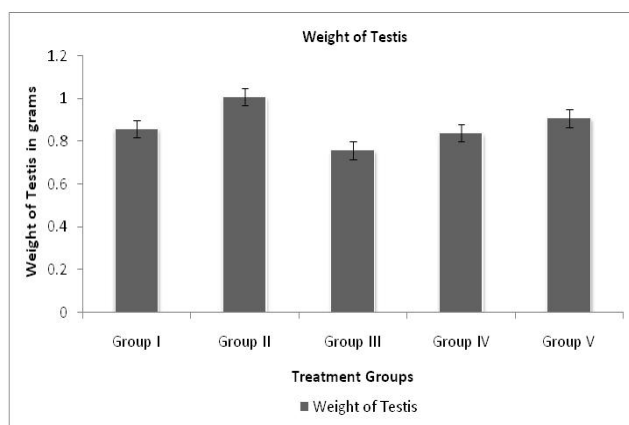
## RESULTS

### Effect of MEFL on body and testis weight

The mean values of body weight shown in Figure 1 indicate that no significant difference of the body weights in MEFL treated mice and the control. Similarly, no significant differences were found in the weight of testis after MEFL treatment with 100 and 400 mg/kg bw for 7 days period (Figure 2).



**Figure 1:** Effect of MEFL administration on body weight; data are expressed as Mean ± SEM (n=6). (Group I: Normal Control, Group II: CCl<sub>4</sub> treated, Group III: CCl<sub>4</sub>+Silymarin treated, Group IV: CCl<sub>4</sub>+MEFL 100mg/kg treated, Group V: CCl<sub>4</sub>+MEFL 400mg/kg treated)



**Figure 2:** Effect of MEFL administration on reproductive organ weight; data are expressed as Mean ± SEM (n=6). (Group I: Normal Control, Group II: CCl<sub>4</sub> treated, Group III: CCl<sub>4</sub>+Silymarin treated, Group IV: CCl<sub>4</sub>+MEFL 100mg/kg treated, Group V: CCl<sub>4</sub>+MEFL 400mg/kg treated)

### Effect of MEFL on GST, GSR, GSH, TBARS

The effects of methanol extract of *Feronia limonia* at dose levels of (100 and 400 mg/kg bw) on tissue phase II metabolizing enzymes viz; GST, GSR, GSH and TBARS are shown in Table 1. Testicular injury induced by CCl<sub>4</sub> has caused significant rise in GST, GSR and GSH activities and decrease in TBARS level ( $P < 0.01$ ). Administration of methanol extract of *Feronia limonia* at two different dose levels attenuated the increased levels of the enzymes and decrease in TBARS produced by CCl<sub>4</sub>, and resulted in subsequent recovery towards normalization almost like that of standard drug treatment Silymarin.

**Table 1:** Effect of MEFL on testis GSH, GST, GSR and TBARS in rat

Treatment group	GSH (mg/g prot)	GST (nM/min/mg prot)	GSR (nM/min/mg prot)	TBARS(nM/min/mg prot)
Control	1.36 ± 0.33+	23.12 ± 1.83+	62.44 ± 2.33+	16.87 ± 1.14+
CCl <sub>4</sub> 30%	1.09 ± 0.27*	14.25 ± 1.67*	45.2 ± 1.17*	25.17 ± 1.93*
CCl <sub>4</sub> +Silymarin 50mg/kg	1.28 ± 0.24+	22.58 ± 1.42+	59.63 ± 1.25+	17.0 ± 1.12+
CCl <sub>4</sub> +MEFL 100mg/kg	1.11 ± 0.22+	19.02 ± 1.74*+	56.41 ± 2.66*+	12.30 ± 1.19+
CCl <sub>4</sub> +MEFL 400mg/kg	1.20 ± 0.35+	21.14 ± 1.55+	59.95 ± 2.21+	16.03 ± 1.20+

Mean ± SEM (n = 6 number), \* indicate significance from the control group at P < 0.01 probability level, + indicate significance from the MEFL group at P < 0.01 probability level.

**Table 2:** Effect of MEFL on tissue protein, activity of CAT and SOD

Treatment group	Protein (µg/mg tissue)	CAT (U/min)	SOD (U/mg prot)
Control	2.07 ± 0.53+	5.09 ± 1.43+	21.23 ± 2.33+
CCl <sub>4</sub> 30%	1.95 ± 0.82 *	3.16 ± 1.27*	14.00 ± 1.57*
CCl <sub>4</sub> +Silymarin 50mg/kg	1.98 ± 0.17+	4.58 ± 1.32+	19.03 ± 2.15+
CCl <sub>4</sub> + MEFL 100mg/kg	1.94 ± 0.22*+	4.20 ± 1.04*+	18.61 ± 1.66*+
CCl <sub>4</sub> + MEFL 400mg/kg	2.1 ± 0.26 +	4.41 ± 1.05+	20.95 ± 2.21+

Results are expressed as Mean ± SEM (n = 6), \* indicate significance from the control group at P < 0.01 probability level, + indicate significance from the MEFL group at P < 0.01 probability level.

### MEFL and antioxidant profile

CCl<sub>4</sub> treatment resulted in the depletion (P<0.01) of the testicular antioxidant enzymes. The activities of SOD, CAT and amount of tissue protein were depleted to 14.00 ± 1.57, 3.16 ± 1.27 and 1.95 ± 0.82 respectively of the toxic control group (Table 2). The decline in the activities were noticeably attenuated (P<0.01) by administration of 100 mg/kg bw and 400 mg/kg bw of MEFL in rats. Treatment with MEFL enhanced testicular antioxidant enzymes even after CCl<sub>4</sub> treatment thus proving the antioxidant potential of the MEFL.

### DISCUSSION

The results of the present study revealed that the CCl<sub>4</sub> treatment caused a slight decrease in the body and decrease in the testis weight of toxic control group. Administration of methanol extract of *Feronia limonia* at two different dose levels attenuated the increased body weight and decrease in testis weight produced by CCl<sub>4</sub>, and resulted in subsequent recovery towards normalization compared to the normal control group. The results are in accordance to the previous report of Castilla-Cortazar et al.<sup>17</sup> The increase in the testis weight may be due to the increase in lipid peroxidation resulting from the oxidative damage induced in rat testis. Oxidative stress may result in overproduction of oxygen free-radical precursors and decreased efficiency of the antioxidant system. CCl<sub>4</sub> and oxygen free-radical generation is associated with impaired glutathione metabolism, alterations in the antioxidant status.<sup>18</sup> The results of our present investigation showed that CCl<sub>4</sub> administration in rats caused significant reduction in the activity of antioxidant enzymes, GSH, GSR, GST and increased TBARS. Reduction of antioxidant enzymes activity in

testicular tissue might be due to the accumulation of free radicals leading to enhanced lipid peroxidation or inactivation of the antioxidant enzymes.<sup>19</sup> Recover of increased testicular enzyme levels in CCl<sub>4</sub> treated group towards the normal levels by the administration of *Feronia limonia* methanolic extracts at 100 mg/kg bw and 400 mg/kg bw demonstrated the antioxidant potential of the plant. Improvement in the levels of antioxidant enzymes may be due to the presence of phenolic and polyphenolic constituents which may have different functional properties such as scavenging of active oxygen species, inhibition of the generation of free radicals and chain breaking activity.<sup>20</sup>

Several conditions associated with male infertility are inducers of oxidative stress, results in germ cell apoptosis and subsequent hypo spermatogenesis. The hazardous effects caused by the CCl<sub>4</sub> is minimized by the administration of *Feronia limonia* methanolic extract, which exerts many health-promoting effects, including the ability to increase intercellular antioxidant levels, scavenge oxidants and free radicals.<sup>21</sup>

### CONCLUSION

This study shows that the 30% CCl<sub>4</sub> at single dose per day was sufficient to significantly induce oxidative stress in rat testicles. The result shows that the *Feronia limonia* has protective nutraceuticals capacity to overcome the oxidative stress induced by CCl<sub>4</sub>. To our knowledge this is the first report on antioxidant activity of *Feronia limonia* fruit pulp methanolic extract on the testicles.



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