

## ALLEVIATION OF FLUORIDE-INDUCED HEPATIC AND RENAL OXIDATIVE STRESS IN RATS BY THE FRUIT OF *LIMONIA ACIDISSIMA*

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**SUMMARY:** We report an investigation into the antioxidative effect of *Limonia acidissima* (LA) fruit powder on fluoride-induced hepatic and renal oxidative stress in rats administered 100 ppm NaF (45.2 ppm F ion) in their drinking water. LA fruit powder was mixed with the diet at 2.5, 5.0, and 10.0 gram percent levels for four weeks, after which the antioxidant status of the liver and kidneys was assessed by measuring the levels of lipid peroxidation by thiobarbituric reactive substances (TBARS), total ascorbic acid (TAA), and reduced glutathione (GSH), along with decreased activities of catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx). LA produced a significant ( $p < 0.05$ ) dose-dependent decrease in the levels of TAA and GSH, and also the activities of CAT, SOD, and GPx, along with an elevation in the TBARS concentration. These results indicate that LA fruit powder has considerable anti-oxidant activity as a nutritional supplement and offers protection against F-induced hepatic and renal oxidative stress.

Keywords: Antioxidants; Fluorotic rats; Kidney enzymes; *Limonia acidissima*; Lipid peroxidation; Liver enzymes; Oxidative stress.

### INTRODUCTION

Dietary supplements such as calcium, selenium, and vitamins C, D, and E have been found to be useful in countering and ameliorating toxic effects of fluoride (F).<sup>1</sup> Recent studies on certain herbal plants with antioxidant potential have indicated their efficacy in reducing oxidative stress caused by F.<sup>2-4</sup> This investigation deals with the fruit of *Limonia acidissima* L (LA) (synonym: *Feronia limonia* C), commonly known as wood apple, elephant apple, monkey fruit, and curd fruit. The LA tree is a deciduous tree native to India, Myanmar, Sri Lanka, and Malaysia (Figures 1 and 2).

The fruit of the LA tree was known as a medicinal plant in ancient histories of Greek and Roman times and is an important plant in Ayurveda, the traditional system of Indian medicine. LA fruit is available in the market seasonally and used for various food purposes. The sweetened pulp of the ripe fruit is used as a dessert and in the preparation of jam, marmalade, syrup, jelly, squash, and toffee. The pulp is mixed with sugar or jaggery (solidified sap of cane sugar) and consumed. It is also used to make temporary pickles and sometimes mixed with yoghurt for consumption. This fruit is recommended in Ayurveda for treatment of tumors, asthma, wounds, diarrhea, dysentery, cardiac dysfunction, hepatitis, sore throat, etc., and is considered a tonic, astringent (when unripe), antiscorbutic, and alexiformic agent.<sup>5,6</sup> LA fruit has also been reported to possess hypoglycemic and hypolipidaemic properties, antioxidant, and hepatoprotective activities, and to exhibit significant wound healing properties.<sup>7-9</sup> Additionally, the ethanol extract of

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this fruit was found to be a potent anti-ulcer agent for treatment of gastric ulcers induced by indomethacin.<sup>10</sup>

**Figure 1 (on right).** Mature *Limonia acidissima* (LA) tree in fruiting season. The tree has sharp spines and reaches an average height of 9-12 m. The fruit is harvested with curved knives (sickles) attached to the ends of long bamboo poles.



**Figure 2 (below).** Ripe LA fruit is round to oval, 5-12 cm in diameter. The fruit is hard, woody, and grayish-white in color with a 6-mm thick scurfy rind. The pulp in the ripe fruit is brown and separates readily from the rind (inset).



Thus, although the medicinal value of LA has been reported from time to time in the literature, there are no sources of information regarding the use of LA fruit pulp as a food supplement to relieve or ameliorate any ailment. The literature refers to traditional uses of LA, and the modern scientific literature deals primarily with the effectiveness of LA fruit pulp to treat certain ailments like diabetes and gastric ulcers. Therefore the present investigation was undertaken using arbitrary dosages of LA fruit powder in the diet of F-intoxicated rats to evaluate its antioxidant potential in the liver and kidneys.

## MATERIALS AND METHODS

*Fruit powder preparation and analysis:* Ripe LA fruit was purchased at a local food market, and the pulp was extracted, air dried, ground to a powder, and stored in an airtight container. A quantitative phytochemical analysis for saponins, polyphenols, flavanoids, and total ascorbic acid was carried out as described in the literature.<sup>11-13</sup> The total antioxidant capacity was determined using TPTZ reagent.<sup>14</sup>

*Animals:* Colony-bred Charles Foster strain male albino rats (3 months old) were provided a standard diet (Pranav Agro Industries, Vadodara, India), normal water *ad libitum*, and were housed individually in a well-ventilated animal unit ( $26 \pm 2^\circ\text{C}$ , humidity 62%, and 12-hr light/dark cycle). The design of the study was approved by the Institutional Animal Ethics Committee (MoEF/CPCSEA/Reg.337).

*Experimental protocol:* After a 10-day adaptation period, 30 rats were randomly segregated into 5 groups of 6 animals each: Control (C)—normal animals without any treatment; Fluoride control (FC)—45.24 ppm F (100 mg NaF/L) in the drinking water; and three groups with F water plus LA powder—F+LA 2.5, F+LA 5.0, and F+LA 10.0 gram % LA powder in the diet. The composition of the diet is given in Table 1.

Table 1. Composition of the laboratory diet in gram %

	C	FC	F LA 2.5	F LA 5.0	F LA 10.0
Moisture	8.75	8.75	8.53	8.31	7.88
Crude protein	22.12	22.12	21.57	21.01	19.91
Crude carbohydrates	55.67	55.67	54.28	52.89	50.10
Crude fat	4.06	4.06	3.96	3.86	3.65
Crude fiber	3.76	3.76	3.67	3.57	3.38
Mineral mixture	5.64	5.64	5.50	5.35	5.08
LA fruit powder	---	---	2.50	5.0	10.0

At the end of the four-week period, the rats were fasted overnight and sacrificed under mild ether anesthesia. Liver and kidney weights were recorded, and tissues were kept frozen until analyzed.

*Biochemical Analyses:* Hepatic and renal tissue total ascorbic acid (TAA), superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6), glutathione peroxidase (GPx; EC 1.11.1.9) activities, and reduced glutathione

(GSH) levels and lipid peroxidation (by increased malondialdehyde [MDA] concentration) were determined according to standard methods.<sup>13,15-19</sup>

*Statistical Analysis:* Data are presented as mean±SEM. One-way analysis of variance (ANOVA) with Tukey's significant difference post hoc test was used to compare differences among groups. Data were analyzed statistically by Graph Pad Prism 3.0 statistical software. P values <0.05 were considered significant.

## RESULTS AND DISCUSSION

Excess intake of F causes fluorosis, a slow progressive degenerative disorder. Chronic exposure to high F in water, food, and air during early developmental stages has been shown to increase oxidative stress and weaken the antioxidant defense systems in liver with reduced activities of SOD, CAT, GPx, and glutathione transferase<sup>20-22</sup> and to decrease the levels of GSH and TAA. In the present context, too, NaF-treated rats exhibited higher levels of hepatic and renal lipid peroxidation and a significant decline in antioxidant profiles. Administration of LA fruit powder to the F-exposed animals resulted in a significant decrease in tissue lipid peroxidation. As seen in Tables 2 and 3, this reduction was dose-dependent, i.e., the 10 gram % dose was more potent than the 2.5 and 5 gram % doses. As an important antioxidant in plasma and tissues, ascorbic acid promotes elimination of reactive oxygen species, thereby helping to reduce oxidative stress. In the present context, the TAA content of hepatic and renal tissues decreased significantly in the F-exposed rats and was increased with administration of LA fruit powder (Tables 2 and 3).

**Table 2.** Effects of *Limonia acidissima* on hepatic lipid peroxidation and antioxidant profiles

Parameter	C	FC	F LA 2.5	F LA 5	F LA 10
TBARS nM MDA/g	10.78±0.32	15.01±0.43 <sup>a</sup>	14.35±0.87 <sup>a</sup>	13.06±0.63 <sup>a</sup>	12.35±0.71 <sup>ab</sup>
TAA µg/g	131.12±0.85	99.89±0.47 <sup>a</sup>	107.36±0.54 <sup>a</sup>	109.50±0.76 <sup>ab</sup>	125.00±0.92 <sup>ab</sup>
SOD U/mg protein	4.66±0.12	1.63±0.74 <sup>a</sup>	2.94±0.82	3.15±0.17	4.59±0.96 <sup>ab</sup>
CAT H <sub>2</sub> O <sub>2</sub> decomposed/sec/g	17.70±0.15	8.66±0.42 <sup>a</sup>	8.96±0.38 <sup>a</sup>	9.60±0.61 <sup>a</sup>	10.82±0.27 <sup>ab</sup>
GSH mg/100 g	40.75±0.32	26.38±0.25 <sup>a</sup>	26.85±0.77 <sup>a</sup>	29.38±0.11 <sup>ab</sup>	33.03±0.62 <sup>ab</sup>
GPx U/mg protein	7.41±0.14	4.11±0.37 <sup>a</sup>	4.29±0.58 <sup>a</sup>	4.77±0.16 <sup>a</sup>	6.35±0.19 <sup>ab</sup>

Values are means±SEM; n=6; p<0.05 was considered significant; superscripts a are for comparison with non-F controls and superscripts b for comparison with F controls.

SOD is an enzyme responsible for the conversion of superoxide radicals into less harmful products like hydrogen peroxide, while CAT brings about the reduction of hydrogen peroxide and protects tissues from the highly reactive hydroxyl radicals.<sup>23,24</sup> Here a significant reduction was noted in the activities of both hepatic and renal SOD and CAT in the F-exposed animals. However, as seen in Tables 2 and 3, the levels of these enzymes increased significantly when LA fruit powder was added to the diet.

GPx is a selenium-containing enzyme that utilizes glutathione in decomposing H<sub>2</sub>O<sub>2</sub> or other organic hydroperoxides to non-toxic products.<sup>25</sup> A significant decrease in GPx activity was found in the F-exposed rats along with a decline in GSH content. Again, as seen in Tables 2 and 3, LA fruit powder elevated the GPx activity in a dose-dependent manner that coincided with increased GSH content in both hepatic and renal tissues.

**Table 3.** Effects of *Limonia acidissima* on renal lipid peroxidation and antioxidant profiles

Parameter	C	FC	F LA 2.5	F LA 5	F LA 10
TBARS nM MDA/g	2.62±0.65	5.80±0.18 <sup>a</sup>	5.54±0.62 <sup>a</sup>	4.99±0.33 <sup>a</sup>	2.17±0.76 <sup>ab</sup>
TAA µg/g	67.30±0.32	50.17±0.45 <sup>a</sup>	52.91±0.18 <sup>a</sup>	54.57±0.51 <sup>ab</sup>	58.69±0.26 <sup>ab</sup>
SOD U/mg protein	3.96±0.28	2.02±0.91 <sup>a</sup>	2.07±0.37 <sup>a</sup>	2.21±0.13 <sup>a</sup>	3.73±0.14 <sup>b</sup>
CAT H <sub>2</sub> O <sub>2</sub> decomposed/sec/g	5.94±0.12	2.79±0.24 <sup>a</sup>	2.90±0.11 <sup>a</sup>	4.01±0.69 <sup>a</sup>	4.29±0.51 <sup>ab</sup>
GSH mg/100 g	11.28±0.16	7.89±0.58 <sup>a</sup>	8.45±0.86 <sup>a</sup>	9.16±0.27 <sup>a</sup>	11.08±0.48 <sup>b</sup>
GPx U/mg protein	3.42±0.45	1.98±0.17 <sup>a</sup>	2.01±0.63 <sup>a</sup>	2.24±0.34 <sup>a</sup>	2.98±0.71 <sup>ab</sup>

As in Table 2, values are means±SEM; n=6; p<0.05 was considered significant; superscripts a are for comparison with non-F controls and superscripts b for comparison with F controls.

Foods rich in proteins, vitamins, essential amino acids, minerals, and antioxidants such as polyphenols and flavanoids are reported to afford better protection against F-induced oxidative stress.<sup>26,27</sup> Administration of tamarind pulp to F-intoxicated female rats has been reported to significantly attenuate hepatic oxidative stress<sup>28</sup> that was attributable to the antioxidant property of tamarind. Ghosh et al.<sup>29</sup> reported that arjunolic acid, a saponin from the bark of *Terminalia arjuna*, enhances the cellular antioxidant potential and protects the hepatocytes from F-induced cytotoxicity and necrotic death. Although LA is reported to possess hypoglycemic, hypolipidaemic potential, antioxidant, and hepatoprotective activities,<sup>7-9</sup> no reports we know of describe LA's usefulness to relieve F-induced oxidative stress.

Our quantitative analyses of phytochemicals in *L. acidissima* fruit revealed the presence of polyphenols (6.74 g %), flavanoids (0.06 g %), saponins (0.018 g %), ascorbic acid (0.054 g %) with a total antioxidant potential of 0.367 mmole/g,

thereby indicating that LA is a rich source for antioxidants. It is noteworthy that when LA fruit powder was added at 5 and 10 g % level as feed supplement to the F-exposed rats, both hepatic and renal lipid peroxidation decreased, and the antioxidant levels in these tissues increased to levels comparable to those of the controls fed on a basal diet without exposure to F in their drinking water.

We conclude that addition of LA fruit powder to the diet of F-exposed rats is beneficial in reducing lipid peroxidation and improving the antioxidant capacity of the liver and kidneys to alleviate the oxidative stress caused by F-intoxication. Further work is needed, however, for the isolation, characterization, and biological evaluation of the active component(s) of LA fruit to obtain the maximum benefits at lower dosages.

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