

Original Article

LIPID LOWERING ACTIVITY OF *FERONIA LIMONIA* LEAF IN TRITON WR-1339 (TYLOXAPOL) INDUCED HYPERLIPIDEMIC RATS

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Received: 25 Jun 2014 Revised and Accepted: 26 Jul 2014

ABSTRACT

**Objectives:** The present study was undertaken to assess the lipid lowering activity of methanolic extract from *Feronia limonia* leaf in Triton WR-1339 induced hyperlipidemic rats.

**Methods:** Hyperlipidemia was induced in rats by a single intraperitoneal (i.p) injection of Triton WR 1339 (300 mg/kg b.w.) and it showed sustained elevated levels of serum cholesterol and triglyceride levels. Methanolic extract of *Feronia limonia* at dose of 125, 250 and 500 mg/kg b.w. was administered to triton induced hyperlipidemic rats. The serum was analysed for lipid profile and the result was compared to the triton control group. The results of the study were expressed as mean  $\pm$  S.D. and data was analysed by using one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests. Values with  $P < 0.05$  were considered as significant.

**Results:** Oral administration of methanolic extract of *Feronia limonia* to hyperlipidemic rats at a dose of 500 mg/kg b.w. exhibited significant reduction in serum lipid parameters like total cholesterol, triglycerides, low density lipoprotein, very low density lipoprotein. Significant elevation ( $p < 0.001$ ) in high density lipoprotein and fecal fat content was observed in treated group when compared to Triton WR-1339 control group.

**Conclusion:** The present study demonstrates that methanolic extract of *Feronia limonia* has significant lipid lowering activity in Triton WR-1339 induced hyperlipidemia in rats.

**Keywords:** Lipid lowering effect, Hyperlipidemia, *Feronia limonia*, Tyloxapol, Serum lipid parameters.

INTRODUCTION

Hyperlipidemia is a secondary metabolic dysregulation usually associated with diabetes. Elevated serum levels of triglycerides (TG), total cholesterol (TC) and very low density lipoprotein (LDL) along with decrease in high density lipoprotein (HDL) is known to cause hyperlipidemia. Also, these are some of the major risk factors which contribute to the development of cardiovascular diseases like hypertension, coronary heart disease, atherosclerosis<sup>[1]</sup> etc. In many cases, hyperlipidemia is also caused due to over-ingestion of alcohol, abnormal diet and in disease condition; hence more attention has been paid for its treatment and prevention along with the use of strict dietary management<sup>[2]</sup>. A large number of synthetic drugs for hyperlipidemia are currently available in the market, but they lack the desired features such as safety on prolonged use, effectiveness, cost<sup>[3,4]</sup> etc. Therefore, attention is being directed towards plants as a medicinal source for lipid lowering activity. *Feronia limonia* Linn, is a moderate sized deciduous tree found throughout India. Traditionally, the leaves of *Feronia limonia* have been used to treat diarrhoea, urinary disorders, ringworm and other chronic skin infections in Charak and Shushrut Samhita<sup>[5]</sup>. Some ethnic communities of Gujarat and Maharashtra (Western India) also use *Feronia limonia* leaves in the treatment of piles or haemorrhoids<sup>[6]</sup> and for treating acidity, ulcers<sup>[7]</sup> etc. The leaf extracts of *Feronia limonia* has been reported to possess antioxidant<sup>[8]</sup>, larvicidal<sup>[9]</sup>, antidiabetic<sup>[10]</sup> and hepatoprotective<sup>[8]</sup> activities. Although, the medicinal value of *Feronia limonia* is applied for the treatment and management of several pathologic challenges, their application as an agents for lipidemic control are yet to be investigated and exploited. The present study was undertaken to investigate the potential of methanolic leaf extract of *Feronia limonia* (MFLE) to ameliorate hyperlipidemia elicited by Triton WR-1339 (Tyloxapol) induced animal model.

MATERIALS AND METHODS

Collection of plant material and extraction

*F. limonia* fresh leaves were collected from Mumbai, Maharashtra in the month of October. The plant leaves were authenticated by

Blatters Herbarium, Xaviers College Mumbai (Voucher specimen No ANH 245 of A. N. Henry). The leaves of *F. limonia* were shade dried and coarsely powdered. The coarse powder (50 g) was subjected to extraction with methanol at 60-70 °C for 30 hrs in a Soxhlet apparatus. The methanolic extract of *F. limonia* was filtered and concentrated. The amount of residue was measured and stored at 4 °C for further use.

Chemicals

Triton WR-1339 (Tyloxapol) was procured from Sigma Aldrich, USA, while Atorvastatin was obtained from Cipla Ltd. Diagnostic kits for HDL, TC and TG were purchased from Span Diagnostics India Ltd. All other chemicals used for the present study were of analytical grade (AR) and obtained locally.

Animals

Adult albino wistar rats of both sexes, weighing 150-200 g were procured from Bharat Serums and Vaccines Ltd., Thane, Mumbai. The animals were housed in standard environmental conditions and fed with food and water *ad libitum*. The rats were acclimatised to the laboratory conditions for 10 days prior to initiation of the experiment. The experimental protocol was approved by the Institutional Animal Ethics Committee (CPCSEA/IAEC/SPTM/P-35/2011) and all experiments were carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, India.

Preliminary phytochemical screening of MFLE

The air dried powder of *F. limonia* leaf was tested for the presence of carbohydrates, glycosides, tannins, alkaloids, flavonoids, saponins, sterols and triterpenes<sup>[11,12]</sup>.

Acute toxicity studies

The methanolic extract of MFLE was studied for the acute oral toxicity according to the guideline No 423 of Organisation for Economic Co-operation and Development (OECD). Wistar rats of

both sexes, weighing 150-200 g were used for the study and single dose of 2000 mg/kg b.w. was orally administered to the test animals (n=6). The treated animals were monitored for 14 days for toxicological effects, if any, on the basis of mortality and behavioural changes.

### Induction of hyperlipidemia

A single intraperitoneal injection of Triton WR-1339 (300 mg/kg b.w.) dissolved in 0.9% saline was injected to overnight fasted albino wistar rats to induce hyperlipidemia. One hour following Triton injection, the animals were given feed *ad libitum*. Blood was collected from these animals and serum was separated and used to determine cholesterol concentration 24 h after Triton injection to confirm hyperlipidemia.

### Lipid lowering activity of MFLE in Triton induced hyperlipidemia

Rats were divided into six groups I-VI, each consisting of six rats. All animals except the normal control group (group I) were injected intraperitoneally with Triton WR-1339 (Sigma, USA) at a dose of 300 mg/kg b.w. to induce hyperlipidemia. The normal control group was injected with normal saline (NS). Twenty four hours following the Triton WR-1339 administration, animals from group IV, V and VI were treated with methanolic *F. limonia* extract (MFLE) at doses of 125, 250, and 500 mg/kg b.w. respectively, by gastric intubation using gavage needle. Simultaneously, the standard drug Atorvastatin at a dose of 30 mg/kg b.w. was orally administered to group III which served as a positive control. 0.5% carboxy methyl cellulose (CMC) was used as the vehicle to administer MFLE and Atorvastatin. Twenty four hours following administration with Atorvastatin and MFLE blood was withdrawn by retro-orbital sinus puncture and serum was separated by centrifugation at 5000 rpm for 10 minutes and stored at -20 °C.

### Fecal fat content

Fecal matter was collected at the end of the experimental period. The powdered fecal matter was extracted with chloroform:methanol (2:1) mixture. The resultant extract was analyzed for fecal fat content [13].

### Biochemical analysis

The amount of serum TC and HDL were estimated by the enzymatic cholesterol oxidase-phenol + aminophenazone (CHOD- PAP) method [14]. TG was estimated by the enzymatic glycerol-3-phosphate oxidase-phenol + aminophenazone (GPO-PAP) method [15]. LDL and VLDL were calculated using the Friedwald formula [16].

### Statistical Analysis

The results have been expressed as mean  $\pm$  SD (n=6 per group). One way analysis of variance (ANOVA) was performed followed by Dunnett's multiple comparison tests. The significance of all the differences among treatment groups, to all the statements of significance were based on the probability of  $p < 0.05$ .

## RESULTS

### Preliminary phytochemical screening of MFLE

Qualitative phytochemistry tests of MFLE indicated the presence of alkaloids, carbohydrates, phytosterols, tannins, proteins and flavonoids.

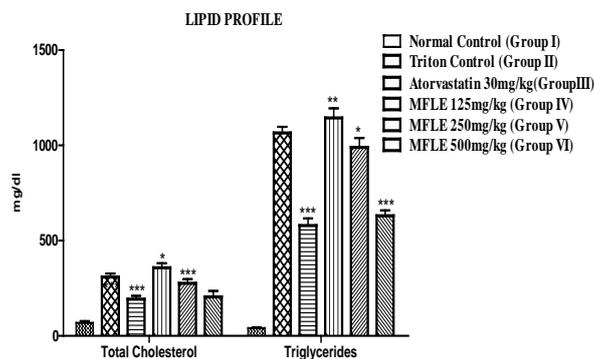
### Acute toxicity studies

The methanolic extract of *F. limonia* was found to be non-toxic at dose of 2000 mg/kg b.w. It did not cause any mortality and no behavioral changes were observed in the tested animals.

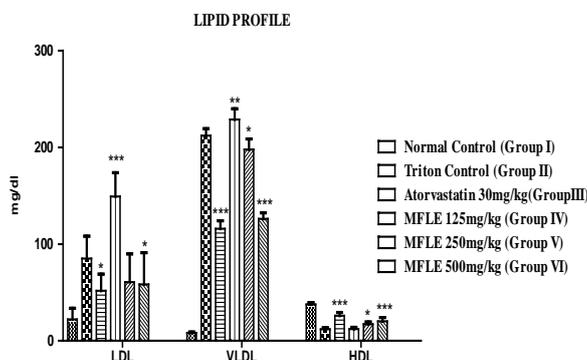
### Effect of MFLE on Triton induced hyperlipidemia

The effect of different doses of MFLE on serum lipid profile in experimental rats is shown in Fig. 1 and 2. The injection of Triton WR-1339 caused a significant elevation ( $P < 0.001$ ) of lipids and lipoprotein level (Fig. 1 and 2.) when compared with normal control group (Group I). Administration of MFLE at doses of 500 mg/kg b.w.

(Group VI) showed a significant reduction ( $P < 0.001$ ) in the serum TC levels by 34%, serum TG level by 41%, serum LDL levels by 33% and serum VLDL levels by 41% and increase of HDL levels by 43.17% compared to the triton control group.



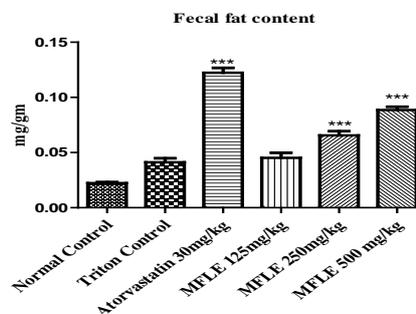
**Fig. 1: Effect of methanolic extract of *Feronia limonia* on TC and TG levels in serum of control and experimental rats. Each bar indicates the Mean  $\pm$  S.D. of six animals per group. MFLE and drug treated group was compared with Triton control group. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .**



**Fig. 2: Effect of methanolic extract of *Feronia limonia* on LDL, VLDL and HDL levels in serum of control and experimental rats. Each bar indicates the Mean  $\pm$  S.D. of six animals per group. MFLE and drug treated group was compared with Triton control group. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .**

### Effect of MFLE on fecal fat contents

The effect of different doses of MFLE on fecal fat content in animals is shown in Fig 3. Treatment with MFLE at doses of 500 mg/kg b.w. induced 114% increase in fecal fat content respectively, when compared with Triton control. Atorvastatin showed an increase of 197% in fecal fat content.



**Fig. 3: Effect of methanolic extract of *Feronia limonia* on fecal fat content in control and experimental rats. Each bar indicates the Mean  $\pm$  S.D. of six animals per group. MFLE and drug treated group was compared with Triton control group. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .**

## DISCUSSION

In the current study, Triton WR-1339 non-ionic surfactant was used to induce hyperlipidemic state in rats [17]. As reported by Schurr *et al* [18], Triton WR-1339 is known to cause structural modification in circulatory lipoproteins, suppress the action of lipases and thereby block uptake of circulating lipids by extra hepatic tissues, resulting in increased blood lipid concentration.

Administration of Triton WR-1339 to wistar rats induced hyperlipidemia as reflected by the increased serum lipid levels. Administration of MFLE in doses of 250 mg/kg b.w. and 500 mg/kg b.w. effectively lowered the elevation of lipid parameters. Among the three doses that were administered, 500 mg/kg b.w.

was found to be most effective in lowering lipid levels. The lipid lowering effect of MFLE is possibly associated with the decrease in intestinal absorption of cholesterol resulting in an increase in fecal excretion of neutral lipids [19].

A number of bioactive compounds [20] such as stigmasterols, orientin, bergapten, tannins, triterpenoids (lupeol and limonin) and steroids (sitosterol and sitosterol- $\beta$ -D-glucoside) [21] are reported to amend lipid levels. These changes in lipid levels after MFLE treatment may be attributed to the presence of bioactive compounds such as alkaloids, phytosterols, flavonoid, tannins and substantial level of  $\beta$ -sitosterol (results not shown) that were detected in the phytochemical screening they might have contributed in lipid lowering effect in similar manner.

Cholesterol in the intestine can arise both from the diet and hepatic secretions. Further, inhibition of cholesterol absorption from intestine also decreases the delivery of cholesterol to the liver, thereby lowering serum as well as hepatic cholesterol. This, in turn, accelerates the uptake of LDL from plasma via LDL receptors and an increase in the clearance of plasma cholesterol [22].

Plant sterols are also reported to decrease cholesterol absorption but through a different mechanism. Phytosterols, specifically,  $\beta$ -sitosterol competes with dietary and biliary cholesterol for incorporation into mixed micelles in the intestinal lumen thus inhibiting their uptake [23]. It was also reported that the intake of plant materials rich in  $\beta$ -sitosterol caused reduction in total serum cholesterol levels in the experimental animals [24, 25].

A significant increase in fat content of fecal matter in hyperlipidemic rats after MFLE administration indicates interference in absorption of intestinal cholesterol. The results strongly suggest that sterols found in MFLE could have decreased cholesterol absorption and increased fat excretion thereby contributing to the lipid lowering activity.

## CONCLUSION

It can be concluded from the current study that methanolic extract of *F. limonia* leaf possess potent lipid lowering activity. Pronounced lipid lowering activity was observed at a dose of 500 mg/kg b.w. of MFLE.

## CONFLICT OF INTERESTS

Declared None.

## ACKNOWLEDGEMENTS

The authors are grateful to Dr. Aparna Khanna, Dean, School of Science, SVKM's NMIMS (deemed-to-be University), Mumbai, for providing the necessary facilities to perform the experiments.

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