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## Original Research Article

Role of *Moringa Oleifera* flower extract in preventing hyperlipidemia and liver lipid peroxidation in male miceYasha J Jha<sup>1</sup>, Anand Kar<sup>1\*</sup>, Durgesh Mahar<sup>1</sup><sup>1</sup>School of Life Sciences, Devi Ahilya Vishwavidyalaya, Indore, Madhya Pradesh, India

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## ABSTRACT

**Background:** Hyperlipidemia leads to coronary artery disease. Although statins are used conventionally, they are often associated with side effects. It has now been attempted to study the role of *Moringa oleifera* flower (MOF) extract in the regulation of tyloxapol-induced hyperlipidemia and tissue lipid peroxidation (LPO) in male mice.

**Materials and Methods:** Six groups of animals were taken. Group I acted as control and group II, receiving tyloxapol (300 mg/kg, single dose on 14<sup>th</sup> day) served as hyperlipidemic control. Group III received simvastatin (200 mg/kg), while group IV, V and VI received MOF extract at 400, 200 and 100 mg/kg every day respectively for 15 days. These four groups (III- VI) also received same amount of tyloxapol on 14<sup>th</sup> day. On day 16<sup>th</sup> changes in the serum total cholesterol, triglycerides, high-density lipoprotein, low-density lipoprotein and very low-density lipoprotein; hepatic LPO, super oxide dismutase, catalase, glutathione peroxidase and histological changes in liver were analyzed. Total phenolic and flavonoid contents were also estimated. The in-vitro antioxidative property was checked through DPPH and H<sub>2</sub>O<sub>2</sub> assays.

**Result:** Results showed significant reduction in all the serum lipids except HDL, which was increased in MOF treated hyperlipidemic mice, with the parallel decrease in hepatic LPO and increase in antioxidants. Histological studies also showed reduction in hepatic damage with the pre-treatment of MOF. However, the most effective dose was found to be 400 mg/kg of MOF.

**Conclusion:** We suggest that *Moringa oleifera* flower extract may ameliorate hyperlipidemia with antioxidative benefits.

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## 1. Introduction

Coronary artery disease (CAD) is increasing alarmingly leading to premature death in almost all countries.<sup>1</sup> Hyperlipidemia, a condition exhibiting high levels of serum lipids, such as cholesterol, triglycerides, and low-density lipoprotein (LDL).<sup>2,3</sup> is thought to be the primary factor for CAD. Currently, some conventional medicines including statins are in use for the treatment of hyperlipidemia.<sup>4,5</sup> However, statins are known to cause side effects.<sup>6,7</sup> It

is, therefore imperative to find an alternative drug for its treatment. Since plant, based drugs are thought to be effective and free of adverse effects, quite a few investigations have been made on plant extracts with respect to the regulation of hyperlipidemia.<sup>8</sup> However, using flowers, available on this aspect are meager,<sup>9,10</sup> despite the fact that the use of flowers in diet and in medicine has played an important role in nearly every culture on earth.<sup>11-13</sup> On edible flowers, reports are nearly negligible.<sup>14</sup> In *Moringa oleifera* plant, although few investigations have been made on the regulatory effect on hyperlipidemia,<sup>15,16</sup> these are mostly on its leaves, fruits or bark and not a single report is

\* Corresponding author.

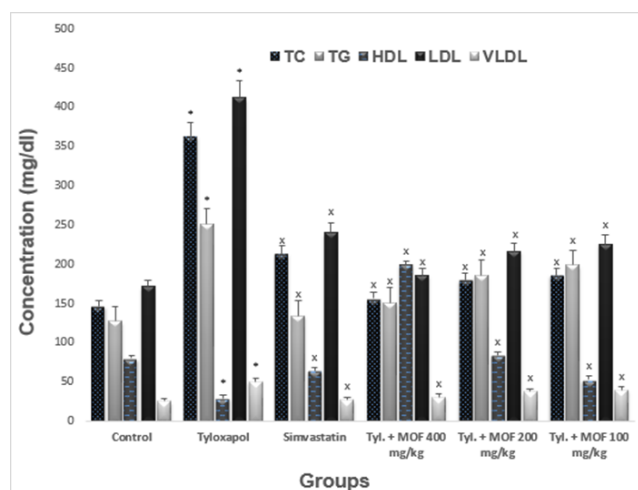
E-mail address: [yashajitenjha@gmail.com](mailto:yashajitenjha@gmail.com) (A. Kar).

there on its flower. The present investigation has attempted to find out the hypolipidemic action of *Moringa oleifera* flower, if any, in tyloxapol-induced hyperlipidemic male mice. Simultaneously, its role on the regulation of tissue LPO in liver, the major target organ of a drug has also been studied.

## 2. Materials and Methods

### 2.1. Chemicals

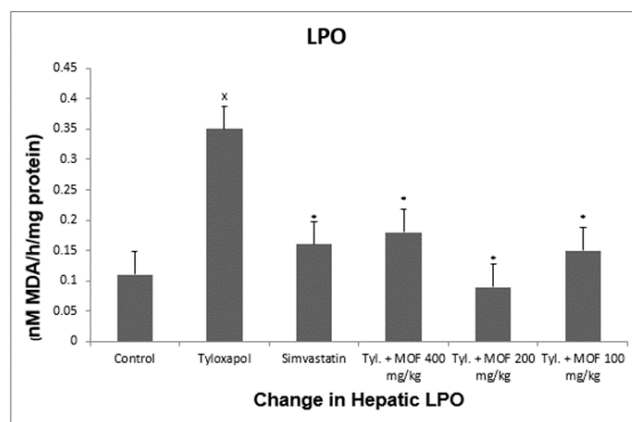
Acetic acid, Aluminum chloride (AlCl<sub>3</sub>) Metaphosphoric acid, Sodium dodecyl sulphate, Pyrogallol, Diethylene-triamine-penta acetic acid (DTPA), EDTA, Sodium citrate, 1, 1 Diphenyl-2-picryl hydrazyl (DPPH), Hematoxylin, Eosin, Gallic acid (GA), Quercetin, Sodium nitrite (NaNO<sub>2</sub>), Thiobarbituric acid (TBA), and Tyloxapol were purchased from E. Merk (India), Bombay, India. Kits for the estimations of Total cholesterol (TC), Triglyceride (TG), and High-density lipoprotein (HDL), were purchased from Erba diagnostics Mannheim, Germany. Loba Chemie Pvt. Ltd., India, provided all other routinely used chemicals that were of reagent grade.



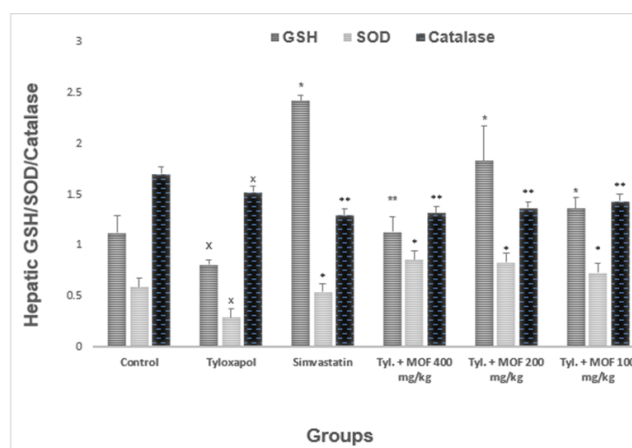
**Figure 1:** Alteration in different serum lipids following the treatment of tyloxapol and tyloxapol + three different concentrations of MOF extracts (400, 200 and 100 mg/kg). Data are in mean  $\pm$  S.E.M. <sup>x</sup>p<0.001 as compared to the value of tyloxapol. \*p<0.001 as compared to the respective control value. TC, Total cholesterol; TG, Triglyceride; HDL, High-density lipoprotein; LDL, Low Density lipoprotein and VLDL, Very Low Density Lipoprotein. MOF, aqueous extract of *Moringaoleifera* flower.

### 2.2. Plant extracts preparation

Fresh flowers of *Moringa oleifera* were collected locally in Indore, India. They were thoroughly washed with water, dried in the shade at room temperature for 15 days and the dried flowers were pulverized using a mechanical grinder



**Figure 2:** Alterations in hepatic LPO (nM MDA/h/mg protein), in Tyloxapol induced and MOF flower extract treated mice. Values are in mean  $\pm$  S.E.M. <sup>x</sup>p<0.001, as compared to respective control value; \*p<0.001, as compared to respective value of the tyloxapol group.



**Figure 3:** Alterations in hepatic SOD (units/mg protein), CAT ( $\mu$ M H<sub>2</sub>O<sub>2</sub> decomposed/min./mg protein  $\times$  10), and GSH ( $\mu$ M GSH/mg protein). Following the treatment of Tyloxapol (300 mg/kg); Tyloxapol + Simvastatin (200 mg/kg); and Tyloxapol + MOF (400, 200 and 100 mg/kg). Data are in Mean  $\pm$  S.E.M. <sup>x</sup>p<0.05 as compared to control. \*\*p<0.05, \*p<0.001, as compared to tyloxapol + MOF extract/simvastatin doses administration in hepatic tissue of mice.

to form a powder, which was utilized in different extract preparations as mentioned below.

In order to obtain the aqueous extract, 40 grams of the flower powder was mixed with 100 ml of d/w and then kept for 24 hrs at 37<sup>o</sup> C.<sup>17</sup> Following this, the solution was filtered using Whatman no. 1 filter paper and subsequently centrifuged at a speed of 5000 rpm. The supernatant was collected and allowed to dry at room temperature (RT). The aqueous extract yield was found to be 3.5 %.

For solvent extractions, 3 gms of the test powder was mixed with 20 ml of 70% methanol<sup>18</sup> and 70%

**Table 1:** DPPH radical scavenging activity (RSA), expressed in percentage inhibition by different concentrations of ascorbic acids and solvents of *M. oleifera* flower extract.

Concentration ( $\mu\text{g/ml}$ )	Inhibition of DPPH %			
	Methanolic extract	Aqueous extract	Ethanollic extract	Ascorbic acid
0.1	4.81 $\pm$ 0.20	11.50 $\pm$ 2.13	16.65 $\pm$ 0.46	87.81 $\pm$ 0.54
0.2	6.77 $\pm$ 0.11	18.74 $\pm$ 4.77	22.12 $\pm$ 3.20	90.20 $\pm$ 0.10
0.4	16.09 $\pm$ 1.15	31.24 $\pm$ 2.07	23.70 $\pm$ 0.74	90.72 $\pm$ 0.10
0.6	19.53 $\pm$ 0.11	34.37 $\pm$ 4.65	31.27 $\pm$ 2.11	91.87 $\pm$ 0.17
0.8	24.02 $\pm$ 0.46	36.45 $\pm$ 2.75	33.04 $\pm$ 0.79	93.43 $\pm$ 2.52
1	45.83 $\pm$ 4.53	57.23 $\pm$ 0.19	41.94 $\pm$ 0.45	98.43 $\pm$ 0.00
3	8.04 $\pm$ 0.41	9.37 $\pm$ 3.60	7.50 $\pm$ 1.46	98.43 $\pm$ 0.00

Data are in mean  $\pm$  SEM.

**Table 2:** H<sub>2</sub>O<sub>2</sub> radical scavenging activity (RSA), expressed in percentage inhibition by different concentrations of ascorbic acid and solvents of *M. oleifera* flower extract.

Concentration ( $\mu\text{g/ml}$ )	Inhibition of H <sub>2</sub> O <sub>2</sub> %			
	Methanolic extract	Aqueous extract	Ethanollic extract	Ascorbic acid
0.1	3.12 $\pm$ 1.21	5.23 $\pm$ 0.45	19.33 $\pm$ 2.79	21.32 $\pm$ 4.80
0.2	11.48 $\pm$ 2.60	18.64 $\pm$ 0.40	20.10 $\pm$ 0.10	25.13 $\pm$ 5.58
0.4	23.45 $\pm$ 1.58	34.69 $\pm$ 0.65	22.34 $\pm$ 1.74	37.25 $\pm$ 2.79
0.6	30.89 $\pm$ 0.99	45.72 $\pm$ 0.69	32.80 $\pm$ 0.34	51.11 $\pm$ 2.81
0.8	42.48 $\pm$ 0.86	50.85 $\pm$ 0.22	33.40 $\pm$ 0.72	58.22 $\pm$ 0.10
1	69.29 $\pm$ 0.91	70.89 $\pm$ 0.96	69.79 $\pm$ 0.18	69.85 $\pm$ 0.48
3	29.15 $\pm$ 1.35	32.67 $\pm$ 0.92	18.70 $\pm$ 1.08	70.79 $\pm$ 0.44

Data are in mean  $\pm$  SEM.

**Table 3:** Total Phenolic content (TPC) and Total Flavanoid content (TFC) in *M. oleifera* flower extract.

M. Oleifera	TPC	TFC
Aqueous extract	537 $\pm$ 0.001	515 $\pm$ 0.001
Methanolic extract	523 $\pm$ 0.011	432 $\pm$ 0.0005
Ethanollic extract	578 $\pm$ 0.060	244 $\pm$ 0.032

Data are in mean  $\pm$  SEM.

**Table 4:** Percent increase or decrease in different serum lipids following the administration of Simvastatin (200 mg/kg) and MOF extract (400, 200, and 100 mg/kg) in tyloxapol induced mice.

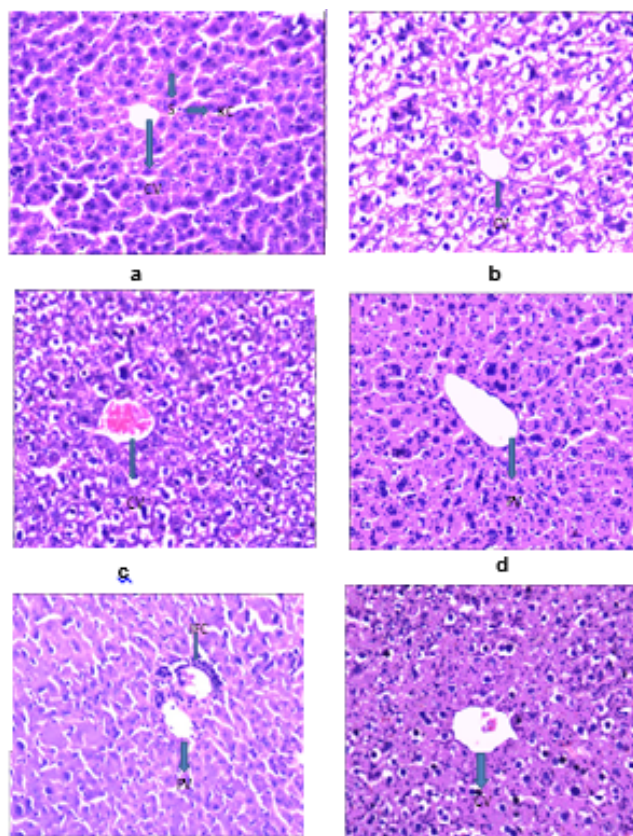
Groups	TC (mg/dl)	TG(mg/dl)	HDL(mg/dl)	LDL(mg/dl)	VLDL(mg/dl)
Tyloxapol	$\uparrow$ 59.66	$\uparrow$ 49.26	$\downarrow$ 63.71	58.42	$\uparrow$ 49.28
Tyl. + Simvastatin	$\downarrow$ 41.15	$\downarrow$ 46.70	$\uparrow$ 54.58	$\downarrow$ 41.84	$\downarrow$ 46.71
Tyl. + MOF (400 mg/kg)	$\downarrow$ 56.96	$\downarrow$ 39.79	$\uparrow$ 84.52	$\downarrow$ 54.90	$\downarrow$ 39.79
Tyl. + MOF (200 mg/kg)	$\downarrow$ 50.43	$\downarrow$ 26.01	$\uparrow$ 65.48	$\downarrow$ 47.50	$\downarrow$ 26.02
Tyl. + MOF (100 mg/kg)	$\downarrow$ 48.68	$\downarrow$ 20.76	$\uparrow$ 45.30	$\downarrow$ 45.28	$\downarrow$ 20.76

$\uparrow$ , increase;  $\downarrow$ , decrease. Change in Tyloxapol percentage as compared to control animals. Change in percentage of Tyl. +Simvastatin and Tyl. + MOF extract doses as compared to tyloxapol treated animals. MOF, *Moringa oleifera* flower aqueous extract, Tyl., Tyloxapol, TC, Total cholesterol, TG, Triglyceride, HDL, High-density lipoprotein, LDL, Low Density lipoprotein, VLDL, Very Low Density Lipoprotein.

ethanol<sup>19</sup> separately. In RT, this mixture was kept for a period of 3 days with occasional shaking to facilitate the extraction process. The resulting solution was then filtered and dried at 37<sup>0</sup> C. The yield of the flower extract was determined to be 3.1% and 3.2% for ethanolic and methanolic extracts respectively.

### 2.3. Animals

Colony bred healthy Swiss albino male mice (30  $\pm$  2 gm) were selected for the study. Animals were kept under standard environmental conditions (14 h light and 10 h dark cycles, 25 $\pm$  2<sup>0</sup>C and 35-50% humidity). Commercially purchased mice feed and boiled water were provided ad libitum. Standard ethical guidelines of Government of India were followed as prescribed by the



**Figure 4:** **a:** Liver section of control animal showing the central vein (CV) surrounded by the radiating cords of hepatocytes, Kupffer cells (KC) intervening the walls of the sinusoids. **b:** Histological changes in liver section of Tyloxapol-induced liver, showing degeneration of hepatocytes near central vein. **c:** Liver section of Tyloxapol-induced and Simvastatin treated mouse hepatocytes with cytoplasmic vacuolation is seen. **d:** Histological changes in liver section of Tyloxapol-induced MOF at 400mg/kg, treated mice liver, near normal histological structure is seen with normal hepatocytes. Only moderate degree of liver damage is found. **e:** Liver section of Tyloxapol-induced MOF of dose 200 mg/kg treated mouse liver cytoplasmic vacuolation is found. **f:** Liver section of Tyloxapol-induced and MOF at dose of 100 mg/kg treated mouse liver the hepatocytes have seen to be vacuolated.

Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, (Our institutional registration No. is 779/PO/Re/S/03/CPCSEA).

#### 2.4. *In vitro* studies

Total antioxidant activity study

#### 2.5. DPPH assay

Antioxidant compounds such as polyphenols, flavonoids, and phenolics actively scavenge free radicals, thereby hindering the oxidative mechanism responsible for

degenerative diseases. The DPPH radical scavenging activity, frequently employed as *in vitro* method, was utilized in the evaluation of antioxidant activities of *Moringa oleifera* flower extract. For this assay, an earlier method<sup>20</sup> was employed with slight modifications. For this assay, 20  $\mu$ l of different concentrations of plant extract were mixed with 1 ml of 0.4 mM DPPH solution prepared in methanol. With subsequent addition of 3 ml of methanol in the reaction tube, the mixture was incubated in darkness at RT for duration of 30 minutes following which; the absorbance was taken at 517 nm. Ascorbic acid served as the positive control. The following formula was applied in order to determine the % inhibition.

DPPH scavenging effect (%) = (A of control – A of sample) / A of control X100, Where, A stands for absorbance.

#### 2.6. Hydrogen peroxide assay

Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), a feeble oxidizing agent, possesses the capability to deactivate certain enzymes through the process of thiol group oxidation. When hydrogen peroxide permeates the cellular membrane, it undergoes a reaction with metal ions such as Fe<sup>+2</sup> and Cu<sup>+2</sup>, consequently forming –OH radicals that elicit toxic consequences. Hence, it is of paramount biological significance to maintain a delicate equilibrium of hydrogen peroxide in order to avert the deleterious impact of radicals. The hydrogen peroxide examination was carried out following the methodology devised by Ruch et al,<sup>21</sup> with certain modifications.

A solution of H<sub>2</sub>O<sub>2</sub> (40 mM/l) was prepared in PBS (50 mM/l). Different concentrations of plant extract in PBS were added to H<sub>2</sub>O<sub>2</sub> and the absorbance at 230 nm was taken after 10 minutes against a blank solution. Ascorbic acid was used as positive control. The percentage scavenging of H<sub>2</sub>O<sub>2</sub> was calculated according to following formula:

$$\% \text{ scavenging (H}_2\text{O}_2) = (A_0 - A_1 / A_0) \times 100$$

#### 2.7. Determination of total phenolic content

The total phenolic content assay was carried out by employing the Folin-Ciocalteu method<sup>22</sup> with some modifications. Initially, 1 ml of test extract (1mg/ml) was introduced into a test tube that contained 5 ml of phenol reagent. The resulting mixture was thoroughly mixed, after which 4 ml of a 10% Na<sub>2</sub>CO<sub>3</sub> solution was added and mixed well and then subjected to incubation at a temperature of 40°C in a water bath for duration of 30 minutes. The absorbance of the reaction mixture was determined at a wavelength of 760 nm using a UV-Vis spectrophotometer, and the obtained values were compared to a known concentration of gallic acid to establish a standard curve. Finally, the average of three readings was expressed in terms

of mg gallic acid equivalent /100 gms dry weight of the plant extract.

## 2.8. Determination of total flavonoid content

For quantification of total flavonoids, a well-established calorimetric method was followed as described earlier.<sup>23</sup> One ml of plant extract (concentration of 4 mg/ml in methanol) was placed in a test tube, followed by 1 ml of d/w. Then, 150  $\mu$ l of a 5% solution of NaNO<sub>2</sub> was added, and after 6 minutes, the same volume of a 10% solution of AlCl<sub>3</sub> was introduced. The reaction mixture was thoroughly mixed, and after an additional 6 min, 2 ml of 1M NaOH and 70  $\mu$ l of d/w was added. The resulting solution was incubated at RT for 15 min and then the absorbance was measured at 510 nm using a UV-Vis spectrophotometer. The absorbance was compared to a known concentration of quercetin. The average value of three readings was calculated and expressed in mg of quercetin equivalent /100 grams of the dry weight of the test extract.

## 2.9. In vivo studies

### 2.9.1. Experimental design

Mice were randomly divided into 6 groups of 5 animals each. Group I animals received normal saline (0.1 ml /animal) and served as control, while the animals in-group II were administered with tyloxapol (300 mg/kg, single dose) on 14<sup>th</sup> day. Group III mice received 200 mg/kg of simvastatin once per day orally. Animals in Group IV, V and VI were administered orally MOF extract, at 400, 200 and 100 mg/kg, respectively once per day. All the animals of group III to VI were injected single dose of 300 mg/kg of 3% tyloxapol solution intra-peritoneal on 14<sup>th</sup> day of experiment to induce hyperlipidemia. Animals were sacrificed on day 16<sup>th</sup> and the liver of each one was taken out and stored at -20<sup>o</sup>C for the evaluation of LPO and different antioxidants. The blood was collected and allowed to clot and centrifuged and the serum was used to estimate the different lipids.

## 2.10. Evaluation of oxidative stress and antioxidants:

### 2.10.1. Lipid peroxidation in liver

To assess the level of lipid peroxidation (LPO) in the tissue, the quantification of thiobarbituric acid substances (TBARS) was conducted using a modified version of a previously established method,<sup>24</sup> as per the standard practice in our laboratory.<sup>25</sup> For this, liver homogenate was prepared in a phosphate buffer solution (pH 7.4) followed by centrifugation at 15000 Rpm for 15 minutes at 4<sup>o</sup>C. Afterwards, 1 ml of the resulting supernatant was taken and mixed with 0.2 ml of 8.1% SDS, 1.5 ml of 20% acetic acid (pH 3.5), and 1.5 ml of 1% TBA. The obtained reaction mixture was then heated for 1 hour at 95<sup>o</sup>C, and cooled. After cooling the reaction mixture, 4 ml of 10% TCA

was added, following with centrifugation at 3000 Rpm for 5 minutes. The absorbance of the resulting pink-colored solution was measured at a wavelength of 532 nm.

## 2.11. Superoxide dismutase (SOD) assay

The determination of SOD test was conducted using a standardized approach, the pyrogallol auto-oxidation inhibition assay as outlined in a previous study.<sup>26</sup> In this assay, auto-oxidation rate is determined by measuring the absorbance at 420 nm. To ensure accurate results, the interference of Fe<sup>++</sup>, Ca<sup>++</sup>, and Mn<sup>++</sup> was prevented by DTPA as a chelator. A cuvette of 3-ml containing Tris-HCl buffer, pyrogallol and tissue supernatant was utilized, and the absorbance of the sample was taken at 420 nm at regular intervals of 30-second. The final quantification of SOD activity was expressed in units per mg of protein.

## 2.12. Catalase (CAT) assay

The decomposition of H<sub>2</sub>O<sub>2</sub> was used for this assay. To summarize, 20 $\mu$ l of 50-mM phosphate buffer (pH 7), 20 $\mu$ l tissue supernatant and 0.1 ml of 30 mM H<sub>2</sub>O<sub>2</sub> were combined and the reduction in absorbance was monitored at every 5 seconds interval for duration of 30 sec. at 240 nm. The CAT activity was quantified as  $\mu$ mol of H<sub>2</sub>O<sub>2</sub> decomposed /min/ mg of protein.

## 2.13. Reduced glutathione (GSH) assay

The method of Ellman<sup>27</sup> was used for the estimation of GSH in which DTNB and 5, 5-dithio-bis-2-nitrobenzoic acid reagents were used. GSH reacted with DTNB and produced a yellow-colored 2-nitro-5-mercapto-benzoic acid. The absorbance was taken at 412 nm. Tissue supernatant (0.5 ml) was precipitated with 2.0 ml of 5% TCA, subsequently centrifuged at 3000rpm, and to the supernatant Ellman's reagent and phosphate buffer were added. The yellow color, so developed was read at 412 nm and expressed in  $\mu$ mol of GSH/mg protein.

## 2.14. Histological analyses of liver tissues

Liver tissues were taken out and washed in PBS and then fixed in Bouin's fluid for 24 h. Afterwards, dehydrated in ascending grades of ethanol (30–100%), cleared in xylene and embedded in paraffin wax (58–60<sup>o</sup>C). The sections were cut into (5-7  $\mu$ m thickness), staining was done with hematoxylin-eosin (H & E) and was observed under light microscopy (10-x magnification).

## 2.15. Statistical analysis

Data were expressed in mean  $\pm$  S.E.M. Analysis of variance (ANOVA), followed by student's "t" test were used. P < 0.05, 0.01 & 0.001 were considered for determination of differences between groups as significant.

### 3. Results

#### 3.1. In-vitro studies

##### 3.1.1. DPPH assay

From Table 1, it was observed that *M.oleifera* flower extract inhibited the DPPH free radicals by all 3 types of extracts in dose dependent manner. However, the highest inhibition was observed in 1 mg/ml concentration of each solvent extract, which was 45.83%, 57.23%, and 41.94% in methanolic, aqueous and ethanolic extracts respectively.

#### 3.2. Hydrogen peroxide assay

The antioxidant properties of aqueous, ethanolic and methanolic extracts of *M. oleifera* flower as estimated using H<sub>2</sub>O<sub>2</sub> assay, indicated that the H<sub>2</sub>O<sub>2</sub> inhibitory activity was highest in its aqueous extract (70.89%, Table 2), that was nearly similar in methanolic (69.29%) and in ethanolic extract (69.79%).

#### 3.3. Total phenolic content

As shown in Table 3, the total phenolic content of *M. oleifera* flower was found to be  $523 \pm 0.011$  mg/100gm of dry weight in methanolic extract,  $578 \pm 0.060$  in ethanolic extract and  $537 \pm 0.001$  mg/100 gm of the dry weight of the aqueous extract. The total phenolic value was calculated using the following linear equation based on the calibration curve of gallic acid:  $Y = 0.001x + 0.396$ ,  $R^2 = 0.818$

#### 3.4. Total flavonoid content

The total flavonoid content of *M. oleifera* flower was found to be  $432 \pm 0.0005$  mg,  $244 \pm 0.032$  and  $515 \pm 0.001$  mg in methanolic, ethanolic and aqueous extract respectively (Table 3). The calculation was made using the linear equation based on the calibration curve of quercetin:  $Y = 0.001x + 0.476$ ,  $R^2 = 0.86$

#### 3.5. In-vivo studies

##### 3.5.1. Changes in lipid profile

In the tyloxapol-induced animals, there was a significant increase in the serum TG, TC, VLDL and LDL along with a decrease in HDL ( $p < 0.001$  for all) (Figure 1). The pretreatment of MOF extract caused a significant decrease in the levels of serum TC, TG, LDL, and VLDL, and an increase in HDL concentration ( $p < 0.001$  for all). On calculation of % increase or decrease (Table 4), TC decreased in statin treated animals by 41.15%, and with MOF treatment it decreased by 56.96%, 50.43% and 48.68% by 400, 200 and 100 mg/kg of MOF respectively. Statin decreased TG by 46.70% and MOF decreased it by 39.79%, 26.01% and 20.76% with the dose of 400, 200 and 100 mg/kg respectively. With the treatment of statin, the HDL increased with 54.58% while with the treatment of MOF

it increased with 84.52%, 65.48% and 45.30% with the dose of 400, 200 and 100 mg/kg respectively. VLDL was decreased by 46.71% in statin treated animals, while with MOF treatment it decreased by 39.79%, 26.02% and 20.76% at 400, 200 and 100 mg/kg respectively. LDL decreased by statin at 41.84% and with MOF doses, 54.90%, 47.50% and 45.28% at its 400, 200, and 100 mg/kg of concentrations respectively.

#### 3.6. Changes in hepatic LPO, SOD, CAT and GSH

As observed in Figure 2, in comparison to tyloxapol-treated group, statin administration decreased the hepatic LPO by 54.28% ( $p < 0.001$ ). MOF could also decrease the hepatic LPO by all its three different doses. However, maximum inhibition was observed at its 200 mg/kg (74.28%). Different antioxidants such as SOD, CAT and GSH were significantly increased ( $p < 0.001$ ) by the treatment of MOF extract by all 3 doses (Figure 3).

#### 3.7. Histological changes in Liver tissue

The liver of control mouse showed a normal histological structure with cords of polyhedral hepatocytes radiating from the central vein, while the tyloxapol induced liver showed hepatic damage with cytoplasmic vacuolation of hepatocytes and inflammation near portal vein (Figure 4 a-f). However, with the treatment of MOF improved the liver architecture to more or less similar to that of control by preventing the cellular damage and inflammation. Tyloxapol treated animals showed cytoplasmic vacuolation and Inflammation, found near portal vein (PV) region. However, there is decrease in inflammatory cells in MOF treated animals.

### 4. Discussion

Our results clearly indicate the preventive effects of the test drug, MOF in the tyloxapol-induced hyperlipidemia. This is for the first time, the flower of *Moringa oleifera* has been found to reduce hyperlipidemia. Earlier, only its leaf, bark, and pod were reported to be lipid lowering in nature.<sup>28–30</sup> Similar type of hypolipidemic effects were also shown by few other flower extracts.<sup>31–33</sup> This may be noted that with our results *Moringa oleifera* plant appears to be helpful in regulating hyperlipidemia with all of its parts. Interestingly, when a comparison was made between the test flower extract and the conventional medicine, simvastatin, the hypolipidemic effect was found to be little better in 400 mg/kg of the test plant extract treated animals, particularly with respect to the decrease in total cholesterol and LDL values.

Lipid peroxidation increases because of oxidative stress. This occurs when there is a loss of dynamic equilibrium between the Peroxidative and antioxidant mechanisms. As a result, lipoprotein peroxidation in dyslipidemic states can

change the physical properties of the cell membranes. This can activate the NADH oxidase and allow free radicals to escape from the mitochondrial electron transport chain. Many oxygenated compounds, particularly aldehydes such as malondialdehyde (MDA) and conjugated dienes, are produced during the attack of free radicals on membrane lipoproteins and polyunsaturated fatty acids.<sup>34,35</sup> The formation of MDA equivalents was lower in the mice treated with all the doses of MOF and it was maximum at 400 mg/kg.

Similar to the present observation, earlier also the treatment with tyloxapol resulted in an increase in hepatic LPO with parallel inhibition in the activities of the antioxidants (GSH, CAT, and SOD) in mice liver.<sup>36</sup> However, this report was on fruit peel and not on flower. This disturbance indicates increased reactive oxygen species (ROS) production.<sup>37</sup> Reduced glutathione is considered as the first line of cellular defense against oxidative injury. The insufficient detoxification of these reactive oxygen species by the antioxidant enzymes might have led to an occurrence of imbalance between antioxidant and oxidant systems in our study also. Low reduced glutathione level might have also attributed to the enzyme inactivation by ROS causing damage to proteins.<sup>38,39</sup> Saxena and Garg<sup>40</sup> showed that tissue LPO is a degradative phenomenon as a consequence of free radical chain reaction and propagation, which affects mainly the polyunsaturated fatty acids (PUFA).

In the current study, with respect to the per oxidative properties, the hepatic lipid peroxidation was enhanced with tyloxapol administration as was observed earlier.<sup>41–43</sup> Notably, the antioxidant properties of MOF were also observed in this study through the reduction of lipid peroxides (TBARS), increased levels of reduced glutathione, and an elevation of the endogenous antioxidant enzymes (GSH, CAT, and SOD). In fact, all the doses of MOF extract inhibited this tyloxapol-induced hepatic LPO with a parallel increase in different antioxidants such as SOD, CAT, and GSH suggesting the safe nature and antioxidative role of the test extract.

From the results of in-vitro antioxidant assays also exhibited the strong antioxidative properties of the test extract, similar to an earlier report on its flower<sup>44</sup> and 2 reports, available on the other parts of *Moringa* such as leaf and pods.<sup>28,45</sup> These antioxidative properties could be the result of its high contents of phenol and flavonoids. In fact, in our study the total phenolic and flavonoid contents were found to be little higher in the test flower as compared to the results mentioned in some earlier findings.<sup>44–46</sup> This antioxidative property was almost similar to that of the lipid lowering test drug, simvastatin. Out of the 3, methanolic extract, ethanolic extract, and aqueous extract, latter was found to be more antioxidative in nature. In fact, because of this, aqueous extract was chosen for *in vivo* study as well.

With respect to liver, the normal liver histology showed cords of polyhedral hepatocytes radiating from the central vein, while the tyloxapol- induced liver showed hepatic damage with cytoplasmic vacuolation of hepatocytes and inflammation near portal vein. However, the treatment of MOF improved the liver architecture to more or less similar to that of control by preventing the cellular damage and inflammation.

Tyloxapol is a well-known agent to induce hyperlipidemia in animal model.<sup>47</sup> Otway and Robinsons<sup>43</sup> have stated that the large increase in serum cholesterol and triglycerides by tyloxapol resulted mostly from an increase of VLDL secretion by the liver, accompanied by a strong reduction of VLDL-C and LDL-C catabolism. In our Swiss albino mice also, it could significantly increase the serum lipid levels exhibiting its hyperlipidemic properties. In the present investigation, the reduction of serum total cholesterol in response to treatment with the extract of MOF is apparently associated with the significant decrease of its LDL fraction and it appears that the *Moringa oleifera* flower extract might have interfered with the cholesterol biosynthesis and thus lowered the serum lipids. (Tables 1 and 2). This result suggests that the cholesterol lowering activity of MOF could possibly be due to a rapid catabolism of LDLc through its hepatic receptors. Whatever may be the mode of action(s) from our results on hyperlipidemia and lipidperoxidation, both *in-vitro* and *in-vivo*, it is clearly found that *Moringa oleifera* flower extract has the potential to inhibit hyperlipidemia with out any hepatotoxic effects. However, further research may be carried out to find out its therapeutic use.

## 5. List of Abbreviations

1. MOF: *Moringa oleifera* flower
2. LPO: Lipid peroxidation
3. CAT: Catalase
4. SOD: Superoxide dismutase
5. Tyl.: Tyloxapol
6. GSH: Reduced glutathione
7. TC: Total cholesterol
8. TG: Triglyceride
9. HDL: High density lipoprotein
10. LDL: Low density lipoprotein
11. VLDL: Very low density lipoprotein
12. CAD: Coronary artery disease
13. TBARS: Thiobarbituric acid substances
14. TBA: Thiobarbituric acid
15. SDS: Sodium dodecyl sulphate
16. EDTA: Ethylenediaminetetraacetic acid
17. DPPH: 1, 1 Diphenyl-2-picryl hydrazyl
18. GA: Gallic acid

## 6. Author Contributions

Yasha Jitendra Jha conducted the experiment and analysis of data. Anand Kar helped in planning, manuscript preparation and editing.

## 7. Source of Funding

None.

## 8. Conflict of Interest

We, the authors declare that we don't have any conflict of interest.

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### Author biography

**Yasha J Jha**, Ph.D. scholar  <https://orcid.org/0009-0009-4304-0196>

**Anand Kar**, Professor  <https://orcid.org/0000-0002-5222-8221>

**Durgesh Mahar**, Ph.D scholar

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