Full Length Article

ALLEVIATIVE EFFECT OF ETHANOLIC EXTRACT OF *Mirabilis jalapa* L. ON THE HAEMATO-BIOCHEMICAL PARAMETERS OF HIGH-FAT DIET-INDUCED ATHEROSCLEROSIS IN WISTAR RATS

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ABSTRACT

Atherosclerosis is one of the risk factors for coronary artery disease. The present study was conducted to find out the alleviative effect of ethanolic extract of Mirabilis jalapa L. against haemato-biochemical alterations of high-fat diet-induced atherosclerosis in Wistar rats. Fifty-four Wistar rats (male – 27 and female – 27) were randomly divided into six different groups: Group I (normal pellet feed), group II (high-fat diet), group III (root extract of Mirabilis jalapa L.), group IV (atorvastatin), group V (HFD + root extract of Mirabilis jalapa L.) and group VI (HFD + atorvastatin). The treatments were given from the 8thday to the 30th day in group V and group VI. Haematological parameters, hepatic enzymes, serum lipid profile and atherogenic indices were studied in HFD induced atherosclerosis. Ethanolic extract of M. jalapa L. (EEM) showed a highly significant reduction in lipid profile, atherogenic indices, hepatic enzymes (ALT and AST) compared with the HFD group. No significant differences were observed in the haematological values of all the groups. The results indicated that M. jalapa L. has a partial alleviative effect on HFD induced biochemical changes in the atherosclerotic rat.

Keywords: Atherosclerosis, haemato-biochemical parameters, high fat diet

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INTRODUCTION

Atherosclerosis is a complex multifactorial inflammatory disease characterized by the accumulation of lipids in the wall of arteries producing lesions and atherosclerotic plaque formation due to

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genetic, metabolic and environmental factors (Leiva *et al.,* 2015). This disease is detected by the accumulation of lipid deposits (mainly cholesterol) in the macrophages of large as well as medium-sized arteries (Leiva *et al.,* 2015). Hypercholesterolemia is a well-known risk factor in human atherosclerosis that mainly occurs due to the elevation of total cholesterol and low-density lipoprotein cholesterol (Pearson *et al.,* 2002). Atherosclerotic cardiovascular diseases are to be treated by using allopathic drugs of statins and non-statins leads to a reduction in the low-density lipoprotein (LDL) concentration (Hegele *et al.,* 2015). Lipid peroxidation reduction might be one of the important features for the favourable effect of statins in preventing or reducing atherosclerosis (Franzoni *et al.,* 2003).

Mirabilis jalapa L. is commonly known as the four O'clock flower (Family: *Nyctaginanaceae*). It is found in India, tropical South America, the Philippines and France (Shaik *et al.,* 2012) and the whole plant is used as a traditional medicine which is used for the treatment of muscular pain, diarrhoea, inflammation, boils and purgative (Saha *et al.,* 2020). *Mirabilis jalapa* L. root showed the hypoglycemic and hypolipidemic effects on streptozotocin-induced diabetic Wistar albino rats (Piyali *et al.,* 2011).

The present study was undertaken to find out the alleviative effect of *Mirabilis jalapa* L. on the haemato-biochemical changes in hypercholesterolemia induced atherosclerotic Wistar rats.

MATERIALS AND METHODS

Animal study

Fifty-four Wistar rats of 27 male and 27 female (weighing around 120 to 150 g and of age six to eight weeks) were randomly distributed into six different groups based on their body weights. Group I received normal pellet feed. Group II received a high-fat diet (HFD) that consisted of 4% cholesterol, 1% cholic acid and 0.5% thiouracil from day one till the end of the experimental trial. Group III received an only ethanolic extract of *Mirabilis jalapa* L. root (EEM @ 20 mg/Kg BW) by oral gavage from the 8th day to the 30th day of the experiment. Simultaneously group IV received only atorvastatin (STORVAS 10 @ 10 mg/Kg BW) by oral gavage from the 8th to 30thday of the experiment. Group V received HFD from day one and EEM @ 20 mg/Kg BW by oral gavage from day 8th to the 30th day of the experiment. Group VI received HFD from day one and atorvastatin (STORVAS 10 @ 10 mg/Kg BW) by oral gavage from the 8th day to the 30th day of the experiment. The study was approved by IAEC (Institutional Animal Ethical Committee, Lr. No.1039/DFBS/IAEC/2020; dated: 14/12/2020). The animals were monitored daily for clinical signs and mortality. Four and five animals from each group were euthanized by using isoflurane gas on the 15th and 30th day of the experiment before which, blood samples were collected by retrobulbar plexus method in EDTA and plain vacutainers for haematological and serum biochemical studies respectively.

Haematology

The haematological parameters such as packed cell volume (PCV), haemoglobin

(Hb), total erythrocyte count (RBC), total leukocyte count (TLC), differential leukocyte count (DLC) and platelet count (PLTs) were enumerated in the EDTA samples analyzed by auto analyzer BC vet 2800 (Mindray).

Serum biochemistry

The blood collected in plain vacutainer with clot activator was allowed to settle for 30 minutes and centrifuged at 2500 rpm for five minutes. The separated serum was collected and subjected to serum biochemical evaluation in A15 BioSystem auto biochemical analyzer **(**BioSystem**).** The parameters analyzed were liver-specific enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and the lipid profile which included estimation of triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C). The very low density lipoprotein cholesterol (VLDL-C), atherogenic indices (AI, CRR and AC), TC/HDL ratio and LDL/ HDL ratio were calculated as described by Sadiq *et al.*(2018) as follows: VLDL= TG/5, atherogenic index (AI) = [$\log_{10}$ (TG/HDL)], cardiac risk ratio (CRR) = TC/HDL-C and atherogenic coefficient (AC) = (TC - HDL-C) / HDL-C.

Statistical analysis

The data generated from the different parameters of the experimental study were subjected to one-way analysis of variance (ANOVA) test using statistical package for the social sciences (SPSS) software version 20 for the Window (Snedecor and Cochran, 1983).

RESULTS AND DISCUSSION

Hypercholesterolemia has been closely associated with the pathogenesis of atherosclerotic cardiovascular diseases (Borén and Williams, 2016). In the experimental study period of 30 days with 23 days of treatment with EEM and standard drug atorvastatin, no appreciable clinical signs and mortality could be recorded in any of the groups. The results of haematological parameters assessed such as hemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC), platelet count, neutrophil count, lymphocyte count, monocyte count and eosinophil count indicated no significant differences between control and treated groups at both the time points (15th day and 30th day) studied (Table 1 and 2). The HFD group revealed no significant ($p<0.05$) difference compared with other groups.

The haematological values in the present study are in agreement with earlier reports of Naik *et al.* (2018) and Ragavan *et al*. (2017).

The liver function test parameters, ALT level in Group II (HFD) showed a highly significant increase ($P<0.01$) compared with treatment Groups V, VI and control Group I (Table 3). The AST values of Group II HFD group was non-significant compared with other treatment groups on the 15th day of sacrifice but the numerical elevation was observed in Group II and it was numerically reduced in treatment groups of V and VI whereas on the 30th day of sacrifice, significant ($P<0.05$) elevation in AST levels were observed in the HFD group compared with other groups.

Table 1. Haematological values (mean ± SE) of HFD induced aortic atherosclerotic Wistar rat model on 15th day (n=4)

Groups	Group I	Group II	Group III	Group IV	Group V	Group VI	Sig
Hb (g/dL)	13.55 ± 0.35	12.80 ± 0.88	11.85 ± 0.69	11.03 ± 1.65	11.08 ± 2.26	12.75 ± 0.62	NS
PCV (%)	39.88 ± 1.88	37.58 ± 2.99	36.83 ± 3.13	33.20 ± 5.86	32.68 ± 6.57	37.85 ± 2.72	NS
RBC (× 10^6/µL)	7.14 ± 0.25	6.86 ± 0.54	6.45 ± 0.48	5.80 ± 1.03	5.95 ± 1.18	6.55 ± 0.38	NS
WBC (× 10^3/µL)	10.65 ± 1.27	12.45 ± 2.1	11.60 ± 1.56	11.25 ± 1.25	9.85 ± 1.06	12.30 ± 1.89	NS
Platelet (× 10^3/µL)	934.50 ± 112.62	885.50 ± 271.31	715.25 ± 111.27	795.75 ± 97.01	906.70 ± 213.10	1098.25 ± 77.51	NS
Neutrophils (%)	16.33±1.31	11.15±1.02	13.48± 1.90	11.35 ± 0.84	15.93 ± 2.39	12.63 ± 0.29	NS
Lymphocyte (%)	79.43 ± 1.44	84.75 ± 1.62	82.63 ± 2.40	84.85 ± 0.63	78.55 ± 3.80	83.55 ± 0.18	NS
Monocytes (%)	2.88 ± 0.14	2.63 ± 0.28	2.55 ± 0.31	2.50 ± 0.18	2.70 ± 0.51	2.63 ± 0.15	NS
Eosinophils (%)	1.13 ± 0.05	1.20 ± 0.25	1.20 ± 0.20	1.13 ± 0.16	1.13 ± 0.13	1.05 ± 0.12	NS

Means bearing similar superscripts within a row do not differ significantly (P<0.05); One-way ANOVA; Duncan's test; NS – Non-significant

Table 2. Haematological values (Mean± SE) of HFD induced aortic atherosclerotic Wistar rat model on 30th day (n=5)

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI	Sig
Hb (g/dL)	15.12 ± 0.59	14.40 ± 0.62	13.40 ± 0.38	13.34 ± 0.17	13.66 ± 0.52	13.44 ± 0.47	NS
PCV (%)	45.26 ± 1.64	42.60 ± 1.84	41.18 ± 1.21	40.88 ± 0.77	40.10 ± 1.36	40.48 ± 1.12	NS
RBC (× 10^6/µL)	7.94 ± 0.25	8.12 ± 0.32	7.53 ± 0.24	7.33 ± 0.16	7.67 ± 0.31	7.59 ± 0.28	NS
WBC (× 10^3/µL)	11.60 ± 1.21	14.32 ± 1.83	13.46 ± 1.00	14.22 ± 6.25	11.67 ± 3.18	12.00 ± 3.40	NS
Platelet (× 10^3/µL)	907.28 ± 228.11	1235.60 ± 06.05	1013.00 ± 37.39	1074.40 ± 27.92	1191.00 ± 35.81	1147.00 ± 94.02	NS
Neutrophils (%)	17.54 ± 2.04	17.00 ± 1.99	12.60 ± 2.04	14.16 ± 0.49	16.58 ± 1.76	12.82 ± 1.02	NS
Lymphocyte (%)	77.98 ± 2.49	80.06 ± 1.17	82.46 ± 1.63	81.74 ± 0.68	80.24 ± 1.89	83.48 ± 1.10	NS
Monocytes (%)	3.16 ± 0.35	3.14 ± 0.24	2.76 ± 0.19	2.84 ± 0.15	3.04 ± 0.19	2.94 ± 0.11	NS
Eosinophils (%)	1.14 ± 0.14	1.26 ± 0.10	1.10 ± 0.07	1.12 ± 0.14	1.06 ± 0.14	1.02 ± 0.07	NS

Means bearing similar superscripts within a row do not differ significantly (P<0.05); One-way ANOVA; Duncan's test; NS – Non-significant

A similar significant elevation of hepatic marker enzyme in the HFD group as per the observations of the earlier workers (Deepa and Varalakshmi, 2005; Vijayabaskar *et al.,* 2008). The result indicated that significant damage to the liver as shown in the liver function test due to the HFD diet (Suliman *et al.,* 2020) and it was reduced in the case of treatment groups. The elevation of hepatic enzymes activity observed in response to oxidative stress caused by HFD (Demori *et al.,* 2006) is in agreement with the present study also revealed elevation of the hepatic enzymes. The levels of ALT and AST were reduced in *M. jalapa* L. and atorvastatin groups compared to the HFD group indicated a partial alleviative effect of *M. jalapa* L. and atorvastatin can be due to the antioxidant effect of EEM against hypercholesterolemia (Selvakumar *et al*., 2012).

In lipid profile values, TG, TC, LDL-C, VLDL-C, TC/HDL-C and LDL-C/HDL-C showed highly significant ($P<0.01$) elevation in the HFD group compared to treatment groups (group V and group VI) and the control (Group I) in 15th and 30thday of sacrifice (Table 4 and 5). The lipid profile values of group V indicated that significant ($P<0.01$) reduction in the values compared to group II could be due to the partial alleviative effect of *M. jalapa* L. Zhao *et al.* (2017) reported that significant ($P<0.01$) increases were observed in the values of serum TC, non-HDL-C, LDL-C and LDL/HDL of high cholesterol high-fat diet in miniature pigs (Subramani *et al.,* 2017; Ibrahim *et al.,* 2019). Dyslipidemia is one of the major risk factors for atherosclerosis which stimulates oxidized LDL accumulation in the arterial wall and it helps in the initiation and progression of atherogenesis. Atherogenesis is mainly concerned with high oxidative stress. Oxidative modification of LDL-C is an initial event in the conversion of LDL-C into atherogenic form (Stocker and Keaney, 2004). The detection of atherosclerosis based on LDL/HDL is used as an important indicator (Kunutsor *et al.,* 2017) agrees with the present study.

The HDL-C levels of Group II showed a highly significant reduction ($P<0.01$) compared to treatment groups (group V and group VI) and the control (group I) on the 15th and 30th-day sacrifice (Table 4 and 5). Group V and VI revealed a highly significant ($P<0.01$) elevation in HDL-C levels compared to group II but compared with the control group showed a slight reduction. It indicated that *M. jalapa* L. has a partial alleviative effect on hypercholesterolemia in the 15th day and 30th-day sacrifice. Elevated levels of TC, TG and LDL-C and reduced levels of HDL-C in serum are commonly observed with atherosclerosis (Srinivas *et al.,* 2008). In addition to that HDL-C concentration is inversely related to the levels of TC and reduced levels of HDL-C might increase the incidence of development of atherosclerosis by impairment of cholesterol clearance from the arterial wall (Stocker and Keaney, 2004). In the present study elevation of HDL-C was observed in the treatment groups suggested the alleviative effect of *M. jalapa* against HFD induced atherosclerosis. The improvement of lipid profile values indicated that the *M. jalapa* L is having hypolipidemic activity by the presence of the bioactive compound of trigonelline (Zhou *et al*., 2012).

Table 3. Serum hepatic enzyme (Mean ± SE) values in HFD induced aortic atherosclerotic Wistar rat model on 15th (n=4) and 30th (n=5) days

Groups	15th day		30th day	
	ALT (U/L)	AST (U/L)	ALT (U/L)	AST (U/L)
Control	$69.00^{a} \pm 4.18$	272.25 ± 6.09	$72.80^{a} \pm 5.58$	$181.80^{a} \pm 19.93$
HCD	$112.00^{c} \pm 8.20$	342.50 ± 8.29	$117.40^{c} \pm 5.08$	$243.00^{b} \pm 8.00$
EEM	$71.50^{ab} \pm 2.96$	275.00 ± 37.19	$74.60^{a} \pm 3.70$	$183.40^{a} \pm 8.83$
Atorvastatin	$74.25^{ab} \pm 7.44$	279.75 ± 16.71	$80.80^{ab} \pm 3.09$	$188.20^{a} \pm 17.10$
HCD + EEM	$86.50^{ab} \pm 3.86$	301.25 ± 8.62	$91.40^{b} \pm 7.63$	$204.40^{ab} \pm 16.05$
HCD + Atorvastatin	$89.50^{b} \pm 6.09$	304.50 ± 6.86	$94.40^{b} \pm 4.61$	$211.00^{ab} \pm 6.80$
F value	7.748	2.243	10.362	2.867
Sig	**	NS	**	*

Means bearing similar superscripts within a row do not differ significantly (P<0.05); One-way ANOVA; Duncan's test; ** Significant (P<0.01), * Significant (P<0.05), NS – Not significant

Table 4. Lipid profile values (Mean ± SE) of HFD induced aortic atherosclerotic Wistar rat model on 15th day (n=4)

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI	Sig
TG	$66.50^{a} \pm 4.17$	$104.25^{c} \pm 7.36$	$65.75^{a} \pm 4.64$	$64.50^{a} \pm 3.30$	$88.25^{b} \pm 3.01$	$82.75^{b} \pm 6.02$	**
TC	$82.75^{a} \pm 10.29$	$152.75^{c} \pm 11.46$	$81.25^{a} \pm 7.03$	$79.50^{a} \pm 8.22$	$124.43^{b} \pm 8.39$	$121.60^{b} \pm 5.19$	**
LDL-C	$25.50^{a} \pm 3.77$	$94.1^{c} \pm 4.36$	$21.70^{a} \pm 1.27$	$23.33^{a} \pm 1.31$	$40.75^{b} \pm 4.14$	$47.15^{b} \pm 3.23$	**
HDL-C	$47.32^{c} \pm 3.31$	$18.70^{a} \pm 2.31$	$47.00^{c} \pm 4.23$	$46.05^{c} \pm 3.20$	$33.35^{b} \pm 3.47$	$32.13^{b} \pm 3.84$	**
VLDL	$13.30^{a} \pm 0.83$	$20.85^{c} \pm 1.47$	$13.15^{a} \pm 0.93$	$12.90^{a} \pm 0.66$	$17.65^{b} \pm 0.60$	$16.55^{b} \pm 1.20$	**
TC/HDL-C	$1.75^{a} \pm 0.19$	$8.38^{c} \pm 0.82$	$1.79^{a} \pm 0.26$	$1.75^{a} \pm 0.24$	$3.85^{b} \pm 0.45$	$3.89^{b} \pm 0.30$	**
LDL-C/HDL-C	$0.54^{a} \pm 0.08$	$5.24^{c} \pm 0.61$	$0.47^{a} \pm 0.06$	$0.51^{a} \pm 0.03$	$1.23^{ab} \pm 0.04$	$1.53^{b} \pm 0.21$	**

Means bearing similar superscripts within a row do not differ significantly (P<0.05); One-way ANOVA; Duncan's test; ** Significant (P<0.01)

Table 5. Lipid profile values (Mean ± SE) of HFD induced aortic atherosclerotic Wistar rat model on 30th day (n=5)

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI	Sig
TG	$78.00^{a} \pm 5.36$	$186.40^{c} \pm 5.45$	$73.80^{a} \pm 4.27$	$70.40^{a} \pm 6.58$	$154.40^{b} \pm 5.98$	$147.20^{b} \pm 6.76$	**
TC	$76.60^{a} \pm 7.44$	$174.20^{c} \pm 11.71$	$78.00^{a} \pm 4.89$	$74.00^{a} \pm 6.53$	$133.80^{b} \pm 6.80$	$135.40^{b} \pm 4.32$	**
LDL-C	$32.62^{a} \pm 4.98$	$104.14^{c} \pm 5.82$	$29.11^{a} \pm 3.53$	$30.49^{a} \pm 2.67$	$59.50^{b} \pm 3.50$	$65.13^{b} \pm 5.44$	**
HDL-C	$52.24^{c} \pm 4.84$	$20.20^{a} \pm 1.87$	$54.06^{c} \pm 2.21$	$53.04^{c} \pm 3.46$	$39.60^{b} \pm 1.42$	$36.58^{b} \pm 3.79$	**
VLDL	$15.60^{a} \pm 1.07$	$37.28^{c} \pm 1.09$	$14.76^{a} \pm 0.85$	$14.08^{a} \pm 1.32$	$30.88^{b} \pm 1.20$	$29.44^{b} \pm 1.35$	**
TC/HDL-C	$1.53^{a} \pm 0.21$	$8.71^{c} \pm 0.26$	$1.45^{a} \pm 0.09$	$1.45^{a} \pm 0.23$	$3.38^{b} \pm 0.16$	$3.87^{b} \pm 0.44$	**
LDL-C/HDL-C	$0.64^{a} \pm 0.08$	$5.27^{c} \pm 0.36$	$0.54^{a} \pm 0.06$	$0.58^{a} \pm 0.06$	$1.50^{b} \pm 0.06$	$1.86^{b} \pm 0.22$	**

Means bearing similar superscripts within a row do not differ significantly (P<0.05); One-way ANOVA; Duncan's test; ** - P<0.01

Table 6. Atherogenic indices values (Mean ± SE) in HFD induced aortic atherosclerotic Wistar rat model on 15thand 30thday

Parameters		Group I	Group II	Group III	Group IV	Group V	Group VI	Sig
15th day (n=4)	Atherogenic Indices (AI)	$0.15^{a} \pm 0.04$	$0.75^{c} \pm 0.03$	$0.15^{a} \pm 0.06$	$0.15^{a} \pm 0.02$	$0.43^{b} \pm 0.05$	0.42b ± 0.07	**
	Cardiac Risk Ratio (CRR)	$1.75^{a} \pm 0.19$	$8.38^{c} \pm 0.82$	$1.79^{a} \pm 0.26$	$1.75^{a} \pm 0.24$	$3.85^{b} \pm 0.45$	$3.89^{b} \pm 0.30$	**
	Atherogenic coefficient (AC)	$0.75^{a} \pm 0.19$	$7.38^{c} \pm 0.82$	$0.79^{a} \pm 0.26$	$0.75^{a} \pm 0.24$	$2.85^{b} \pm 0.45$	$2.89^{b} \pm 0.30$	**
30th day (n=5)	Atherogenic Indices (AI)	$0.18^{a} \pm 0.06$	$0.97^{c} \pm 0.04$	$0.13^{a} \pm 0.04$	$0.12^{a} \pm 0.05$	$0.59^{b} \pm 0.02$	$0.61^{b} \pm 0.04$	**
	Cardiac Risk Ratio (CRR)	$1.53^{a} \pm 0.21$	$8.71^{c} \pm 0.26$	$1.45^{a} \pm 0.09$	$1.45^{a} \pm 0.23$	$3.38^{b} \pm 0.16$	$3.87^{b} \pm 0.44$	**
	Atherogenic coefficient (AC)	$0.53^{a} \pm 0.21$	$7.71^{c} \pm 0.26$	$0.45^{a} \pm 0.09$	$0.45^{a} \pm 0.23$	$2.38^{b} \pm 0.16$	$2.87^{b} \pm 0.44$	**

Means bearing similar superscripts within a row do not differ significantly (P<0.05); One-way ANOVA; Duncan's test; Significant (P<0.01)

A highly significant increase in the atherogenic indices of AI, CRR and AC values were observed in group II compared with control (Group I) and treatment groups (Group V and Group VI) in 15 and 30 days study (Table 6). A highly significant reduction in the atherogenic indices in the treatment groups (Group V and Group VI) compared to group II suggested that *M. jalapa* L. has reduced the hypercholesterolemic effect. In the present study, atherogenic indices are elevated in group II compared to the treatment groups suggested that *M. jalapa* L. has reduced the hypercholesterolemic effect. Atherogenic indices are might be one of the indicators of atherosclerosis caused by a high-fat diet (HFD) (Subramani *et al.,* 2017). The reversal of atherogenic indices in Group V was due to the hypolipidemic bioactive compound of trigonelline present in the *M. jalapa* L (Zhou *et al*., 2012).

The present study observed that *M. jalapa* L. reduces most of the serum biochemical changes and antherogenic indices values induced by HFD in 15 and 30 days study. There was an improvement in serum lipid profile values of TG, TC, LDL-C, VLDL-C, TC/HDL-C, LDL-C/HDL-C and atherogenic indices were observed in EEM treatment compared with high-fat dietin 15 and 30 days study except HDL-C showed slight reduction compared to the Group II (HFD) indicated that *M. jalapa* L had partially alleviated the early atherogenicity caused by HFD in Wistar rats.

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