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Effect of geraniol on high fat diet induced vascular endothelial dysfunction in rats

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ABSTRACT

Geraniol is a monoterpenoid and an alcohol. It is the primary part of rose oil, palmarosa oil, and citronella oil. It also occurs in small quantities in geranium, lemon, and many other essential oils. Geraniol has been used for many years in traditional medicine as anti-asthmatic, anti-allergic, anti-oxidant, antidiarrhoeic, antihepatotoxic, diuretic, tonic, and haemostatic, stomachic and anti-diabetic. In the present study, the animals were fed with HFD for 12 weeks to induce pathogenesis and the investigation was done whether oral administration of Gr on HFD fed rats could show protective effect on Vascular Dysfunction. The studies associated with HFD could cause hyperlipidemia, a risk factor for nephropathy. After 12 weeks, the lipid levels of HFD group were altered i.e., TG, TC, LDL levels increased and HDL levels decreased. Administration of Gr, for 6 weeks lowered the levels of TC, TG, LDL, while the levels of HDL was elevated. The results indicated that the Gr could attenuate the hyperlipidemia.

Keywords: Hyperlipidemia, Geraniol, Vascular Endothelial Dysfunction, Palmarosa oil

INTRODUCTION

Changing life style has turned food habits towards western diet, i.e., atherogenic diet [1-3]. Excess energy consumption and low physical exercises result in over-body weight and obesity [5, 6]. Risk factors associated to obesity made public awareness to diet. High-fat diet to induce obesity was descripted by a nutritional intervention in 1959 [7]. Obesity may also be due to dyslipidemia (abnormal lipid metabolism) or genetic factors. High-fat diet induced obesity produce oxidative stress. Oxidative stress is involved in the pathogenesis of endothelial dysfunction, hypertension, inflammation and atherosclerotic cardiovascular disease. Obesity, endothelial dysfunction, hypertension, type-II diabetes, dyslipidemia, inflammation together collectively known as metabolic syndrome, also called X syndrome [8]. Approximately, 20-40% populations get affect to metabolic syndrome in the industrialized nations and expected to increase [9].

Endothelium is majorly located in the intima of blood vessels. High fat diet contains high amounts of free fatty acids that circulate in the blood. In the vessels, these free fatty acids inhibits the Na^+/K^+-ATPase and Sodium pump, followed by changes in the interaction of enzyme with neighbouring membrane proteins, formation of multiple signaling modules resulting epidermal growth factor receptor

activation and reactive oxygen species (ROS, such as superoxide anion, oxygen radical, peroxide radicals) production, finally causing oxidative stress in the vessels [10]. Inturn, high amounts of ROS reacts with the vascular NO, the vascular relaxing hormone in stress, forming peroxynitrate species, a fatal compound. The ROS produce cell injury, including peroxidation of membrane lipids, proteins denaturation and DNA damage in the vessels, macro and micro vessels [11]. This leads to the dysfunction of vascular endothelium. Endothelial dysfunction, defined as reduction of bioavailability of vasodilators, mainly NO, is also characterized by a state of endothelial activation where a pro-inflammatory, proliferative and pro-coagulant milieu predominates [12-15].

Geraniol is a monoterpenoid and an alcohol. It is the primary part of rose oil, palmarosa oil, and citronella oil. It also occurs in small quantities in geranium, lemon, and many other essential oils. Geraniol has been used for many years in traditional medicine as anti-asthmatic, anti-allergic, anti-oxidant, antidiarrhoeic, antihepatotoxic, diuretic, tonic, and haemostatic, stomachic and anti-diabetic.

Geraniol has antioxidant property (Research in Pharmacy 3(1):01-06, 2013). The chemopreventive effect of Geraniol was evaluated in N-nitrosodiethylamine-induced experimental liver tumor in Wistar albino rats (Fouzia Banu, Nausheen Dawood, Yamini Sudha Lakshmi, S. Gopalakrishnan and [16-18]. The present study aimed to study the effect of geraniol on high-fat diet induced vascular dysfunction in male wistar rats.

DRUG PROFILE

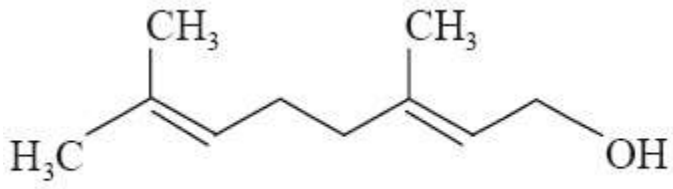
Geraniol

Biological Source

Geraniol is a primary part of rose oil, palmarosa oil and citronella oil.

Description & Chemical structure

It appears as a clear to pale-yellow oil that is insoluble in water, but soluble in most common organic solvents.



Geraniol

Fig. 1

Uses

Geraniol has been used for many years in traditional medicine as anti-asthmatic, anti-allergic, anti-oxidant, antidiarrhoeic, antihepatotoxic, diuretic, tonic, and haemostatic, stomachic and anti-diabetic [19, 20]. Geraniol is used internally in folk medicine and in aromatherapy.

MATERIALS

Experimental animals

Male wistar rats weighing 180-220g were used and were procured from National Centre for Lab Animal Sciences, National Institution of Nutrition, Hyderabad, India. The rats were maintained at a stable temperature (22±2 °C) and humidity (55±5%) under a 12-h light/dark cycle and had free access to food and water. After seven days of acclimatization period, they were randomly selected for different experimental groups.

All the experimental procedures were carried out in accordance with Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA) guidelines. All the experimental procedures were approved by the Institutional Animal Ethical Committee.

Chemicals and drugs

- Geraniol was procured from sigma aldrich
- Glucose, Cholesterol, HDL and Triglycerides kits were purchased from Erba Diagnostics, USA.
- Acetylcholine, Sodium nitroprusside, L-phenylephrine, was procured from Sigma-Aldrich, St. Louis, USA.
- Vitamin mix, mineral mix and cellulose were procured from MP Biomedicals India Pvt Ltd.
- Casein was purchased from Lact'l, Manufacturers of casein, Ahmedabad.

Instruments

- Invasive blood pressure apparatus (Power lab-AD Instruments)
- Semi-autoanalyzer (Lab India)
- Micro centrifuge-REMI
- Micropipettes
- UV Spectrophotometer (Shimadzu UV-1800 ENG 240V)

METHODS

Experimental animal models and study design

In order to study the effect of geraniol on vascular dysfunction in the present study, high fat diet induced vascular dysfunction in wistar rat is used as animal model.

Induction of Obesity

Vascular dysfunction was induced in rats by feeding with high fat diet for 12 weeks [21].

STATISTICAL ANALYSIS

All data are presented as Mean ±S.E.M. The significance of difference among the groups were assessed using one way analysis of variance(ANOVA) followed by Dunnet's test using Graph pad PRISM software and $p \leq 0.05$ was considered significant.

RESULTS

Body weight and food intake

From the data in Table 1, there was a significant increase ($p<0.01$) in body weight gain in HFD group when compared to control group. Treatment with Geranium Oil at 200mg/kg and 400mg/kg dose level showed a significant reduction ($p<0.05$, $p<0.01$ respectively) in body weight gain when compared to HFD group. From Table 2, there wasn't remarkable significance with respect to food intake in all groups.

Blood pressure

HFD feeding for 56 days in rats lead to significant ($p<0.001$) increase in mean arterial pressure when compared to control group (Table 3).

Treatment with GR at 200mg/kg & 400 mg/kg dose levels showed significant decrease ($p<0.05$, $p<0.001$) in mean arterial pressure in dose dependent manner when compared with the HFD fed rats.

Electrocardiograph

HFD feeding in rats lead to significant increase in QT interval ($p<0.001$), QTc interval ($p<0.001$), RR interval ($p<0.001$) and heart rate ($p<0.001$) when compared to control group (Table 3).

Treatment with GR 200mg/kg showed significant decrease in QT interval ($p<0.01$), QTc interval ($p<0.01$), RR interval ($p<0.05$) and heart rate ($p<0.01$) when compared to HFD group. Treatment with GR 400mg/kg showed significant decrease in QT interval ($p<0.001$), QTc interval ($p<0.001$), RR interval ($p<0.01$) and heart rate ($p<0.001$) when compared to HFD group.

Glucose and Lipid profiles

Rats fed with HF diet showed significant increase in glucose ($p<0.001$), triglycerides ($p<0.001$), cholesterol ($p<0.001$), LDL ($p<0.01$) levels and exhibited significant decrease in HDL ($p<0.01$) level when compared to control group (Table 5).

The group treated with GR 200mg/kg displayed significant decrease in triglyceride ($p<0.01$) and cholesterol ($p<0.05$) levels and showed no significant decrease seen in glucose, LDL levels and no significant increase in HDL level when compared with HFD group.

The group treated with GR 400mg/kg showed significant decrease in plasma glucose ($p<0.01$), triglyceride ($p<0.001$), cholesterol ($p<0.01$), LDL ($p<0.01$) levels and showed increase in HDL ($p<0.01$) levels significantly when compared to HFD group.

Antioxidant levels

HFD fed rats showed significant decrease in SOD ($p<0.001$) and GSH ($p<0.01$) activity levels and exhibited no significant decrease in Catalase activity level when compared with control group (Table 6).

Treatment group, GR 200mg/kg, showed no significant increase in SOD, Catalase and GSH activity levels when compared to HFD group. Treatment group, GR 400mg/kg showed significant increase in SOD ($p<0.01$), Catalase ($p<0.01$) and GSH ($p<0.05$) activity levels when compared to HFD group.

Vascular reactivity

The arteries from all groups were contracted by phenylepherine ($1X10^{-6}M$) and then exposed to different concentrations of either acetylcholine or sodium nitroprusside (SNP). Compared with the control rats the endothelium-dependent relaxation, induced by acetylcholine, was strongly ($p<0.01$) decreased in the HFD group. The maximum relaxation (Emax) induced by acetylcholine was 96.57 ± 0.2814% in the control group while, the relaxation induced by acetylcholine was only 48.62 ± 0.424% in aorta isolated from the HFD fed rats. This dysfunction in aorta was improved by both the doses of the drug (Table 7).

The maximum relaxation in thoracic aorta isolated from the rats treated by 200 and 400 mg/kg of the GR were 69.11 ± 9.11% and 90.16 ± 0.1716%, respectively. Contrary to the case of endothelium-dependent relaxation, the maximum relaxation response (Emax) induced by endothelium-independent vasodilator sodium nitroprusside were not significantly different among the groups.

Table 1: Effect of Geraniol on %body weight gain in high fat diet fed male wistar rats

Groups/Weeks	% Body weight gain											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Control	6.99 ±0.85	10.13 ±0.94	16.24 ±1.83	21.60 ±2.28	27.25 ±2.58	35.74 ±3.81	39.60 ±3.12	43.90 ±2.88	46.90 ±0.94	49.43 ±3.81	51.25 ±3.81	53.89 ±2.28
HFD	15.72* ±1.52	21.00* ±2.50	31.41* ±3.40	39.42* ±5.36	49.90** ±4.51	55.45** ±3.80	63.59** ±4.13	68.92** ±4.45	72.42 ±2.28	75.08 ±2.50	78.00 ±1.52	80.09 ±2.28
HFD + GR 200	16.98 ±2.11	22.70 ±2.72	31.42 ±3.50	40.67 ±3.84	46.52 ±4.04	50.04 ±4.20	53.60 ±4.21	55.30$ ±3.99	59.28 ±0.85	63.90 ±3.40	65.89 ±1.27	67.23 ±2.11
HFD + GR 400	15.82 ±1.27	21.07 ±2.02	30.58 ±2.89	39.38 ±1.38	42.78 ±1.97	44.35 ±1.30	45.83# ±1.27	47.20## ±1.61	49.76 ±2.72	52.38 ±1.30	56.06 ±1.38	58.12 ±1.30

Table 2: Effect of Geraniol on food intake in High fat diet fed male wistar rats

Groups/Days	Cumulative food intake (g)											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Control	100.9 ±3.23	174.1 ±3.87	271.5 ±4.42	338.6 ±3.97	446.2 ±5.07	532.6 ±5.06	658.1 ±7.41	733.1 ±5.78	756.2 ±5.07	802.0 ±3.97	843.7 ±3.97	876.8 ±7.41
HFD	114.4 ±3.02	165.9 ±5.69	256.4 ±8.57	307.3 ±13.37	385.1 ±13.09	452.9 ±15.12	565.7 ±13.9	642.7 ±15.4	698.1 ±13.3	722.4 ±13.3	754.7 ±8.57	799.1 ±8.57
HFD+GR 200	113.9 ±3.36	162.9 ±4.23	250.4 ±3.88	302.7 ±3.74	379.4 ±2.80	446.3 ±5.38	559.7 ±8.37	635.2 ±12.0	675.2 ±5.38	706.6 ±2.80	767.8 ±5.38	819.7 ±2.80
HFD+GR 400	112.7 ±4.11	161.7 ±2.17	251.0 ±5.04	300.9 ±2.03	380.2 ±2.67	442.8 ±10.84	554.0 ±9.84	626.2 ±8.24	669.3 ±5.04	699.5 ±2.17	734.0 ±2.67	782.4 ±10.84

*p<0.05 Control v/s HFD, **p<0.01 Control v/s HFD
$p<0.05 HFD v/s HFD+GR 200
#p<0.05 HFD v/s HFD+GR 400, ##p<0.01 HFD v/s HFD+GR 400

Table 3: Effect of Geraniol on ECG and mean arterial pressure (MAP) in High-fat diet fed male wistar rats

Parameters/Groups	Control	HFD	HFD+GR200	HFD+GR400
QT interval	0.0498	0.0728***	0.0620$$	0.0527###
(s)	±0.002	±0.002	±0.001	±0.003
QTc interval	0.1195	0.1540***	0.1386$$	0.1297###
(s)	±0.004	±0.002	±0.003	±0.003
RR interval	0.1159	0.1449***	0.1318$	0.1265##
(s)	±0.005	±0.002	±0.001	±0.003
Heart rate	397.8	467.0***	437.7$$	413.7###
(BPM)	±5.91	±8.03	±5.67	±4.57
MAPmm of Hg)	104.5	133.7***	125.5$	112.0###
	±1.06	±2.08	±1.71	±2.56

Data are expressed as Mean ± S.E.M
***p<0.001 Control v/s HFD
$p<0.05 HFD v/s HFD+GR 200, $$p<0.01 HFD v/s HFD+GR 200
##p<0.01 HFD v/s HFD+GR 400, ###p<0.001 HFD v/s HFD+GR400

Table 4: Effect of Geraniol on SOD, Catalase, GSH in High Fat diet fed wistar rats

Parameters/Groups	CONTROL	HFD	HFD+GR	HFD+GR
SOD	3.82	9.68**	4.06$$	5.27#
(U/100 mg protein)	±1.20	±0.85	±0.63	±1.04
Catalase	3.628	9.73***	5.66$	6.30#
(µmol of H_2O_2 consumed/mg of protein)	±0.61	±0.96	±0.60	±1.39
GSH	6.00	1.64**	5.33$$	5.63##
(U/mg protein)	±0.74	±0.42	±0.63	±0.90

Data are expressed as Mean ± S.E.M of six animals. Superscript symbols represent the statistical significance done by ANNOVA, followed by Tukey's multiple comparision tests
**p<0.01 Control v/s HFD, ***p<0.001 Control v/s HFD,
$p<0.05 HFD v/s HFD+GR 200, $$p<0.01 HFD v/s HFD+GR 200
##p<0.01 HFD v/s HFD+GR 400, ###p<0.001 HFD v/s HFD+GR 400

Table 5: Effect of Geraniol on Plasma glucose, triglycerides, cholesterol, HDL and LDL in High Fat diet fed male wistar rats

Parameters/Groups	CONTROL	HFD	HFD+GR	HFD+GR
Glucose	98.88	284.0***	113.0##	95.50$$$
(mg/dL)	±7.22	±10.46	±5.06	±4.21
Triglycerides	33.91	78.61**	46.96#	35.22$$
(mg/dL)	±3.20	±6.85	±6.16	±3.38
Cholesterol	72.82	138.6***	98.86#	80.80$$$
(mg/dL)	±4.68	±12.06	±6.68	±5.54
HDL	49.31	35.40***	39.49	49.62$$
(mg/dL)	±2.20	±2.55	±3.60	±1.37

Data are expressed as Mean ± S.E.
**p<0.01 Control v/s HFD, ***p<0.001 Control v/s HFD,

$p<0.05 HFD v/s HFD+GR 200, $$p<0.01 HFD v/s HFD+GR 200
##p<0.01 HFD v/s HFD+GR 400, ###p<0.001 HFD v/s HFD+GR 400

Table no. 6: Effect of Geraniol on OGTT

Group	0 min	30 min	60 min	120 min
I (vehicle)	99.9±3.969	126.1±4.19	130.6±5.82	97.93±1.73
II (HFD)	259.8***±19.4	450.4***±22.57	443.6***±8.96	399.1***±19.16
III (HFD+ Gr 200mg/kg)	162.9###±9.56	184.4###±6.15	180.2###±13.24	155.3###±12.19
IV (HFD+ Gr 400mg/kg)	125$$$±8.62	189.9$$$±6.95	140.9$$$±56.83	130.1$$$±6.57

Data are expressed as Mean ± S.E.
**p<0.01 Control v/s HFD, ***p<0.001 Control v/s HFD,

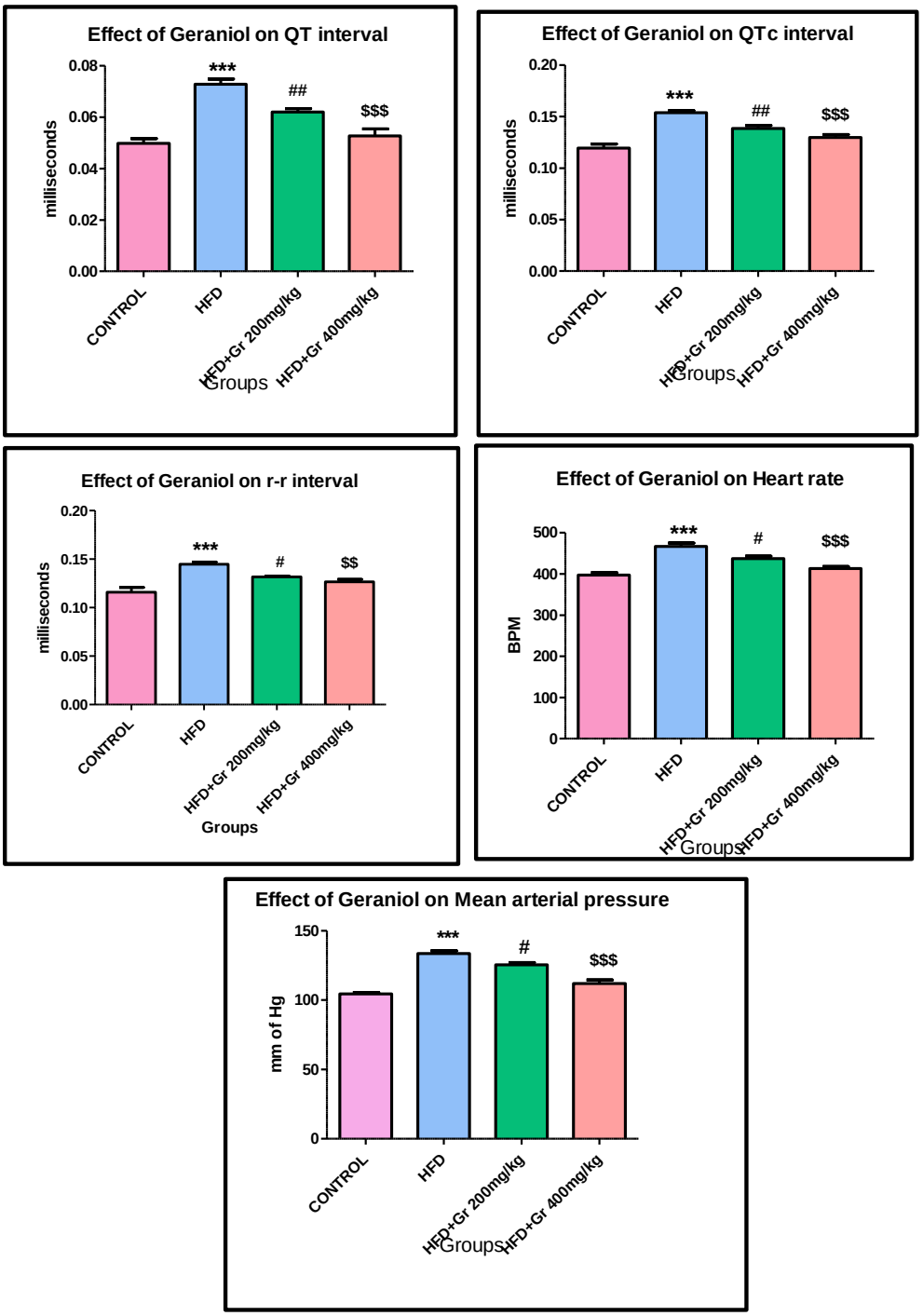
$p<0.05 HFD v/s HFD+GR 200, $$p<0.01 HFD v/s HFD+GR 200
##p<0.01 HFD v/s HFD+GR 400, ###p<0.001 HFD v/s HFD+GR 400

Table 7: Effect of Geraniol on vascular reactivity (endothelium dependent and independent relaxation) in high fat diet fed rats

Groups	Acetylcholine	Sodium nitroprusside
	Emax	**Emax**
Control	96.57 ± 0.28	97.27 ± 0.44
HFD	48.62*** ± 0.42	93.94 ± 0.53
HFD+GR 200mg/kg	69.11$ ± 9.11	95.3 ± 0.46
HFD+GR400 mg/kg	90.16## ± 0.17	96.37 ± 0.29

Data are expressed as Mean ± S.E.
**p<0.01 control v/s HFD, ***p<0.001 control v/s HFD

$p<0.05 HFD v/s HFD + GR 200 mg/kg
##p<0.01 HFD v/s HFD + GR 400 mg/kg



Effect of Gr on ECG and Mean Arterial Pressure in High-fat-diet fed rats

Fig. 2

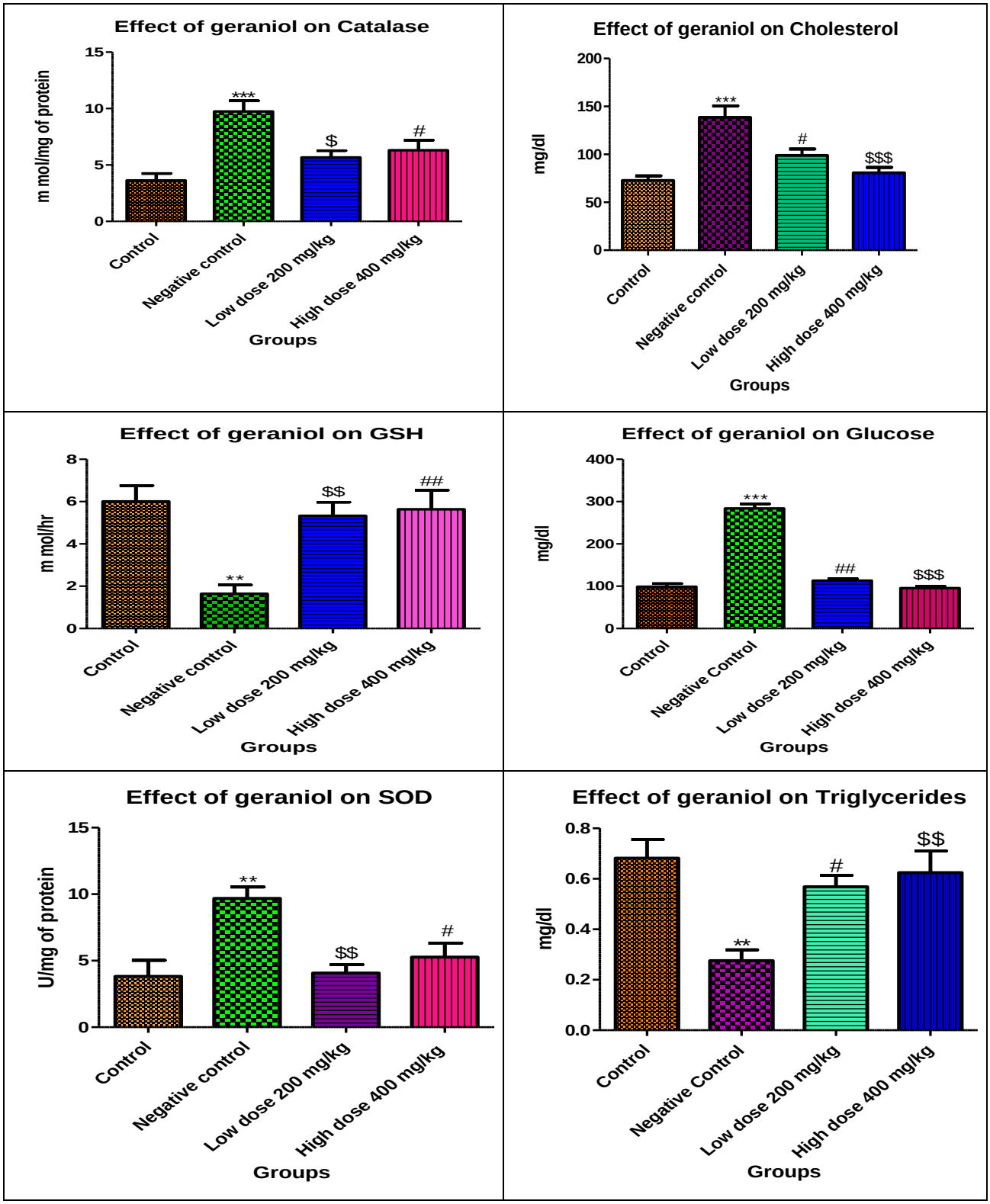

Effect of geraniol on Catalase
m mol/mg of protein
15
10
5
0
***
$
#
Control
Negative control
Low dose 200 mg/kg
High dose 400 mg/kg
Groups
Effect of geraniol on Cholesterol
mg/dl
200
150
100
50
0
***
#
$$$
Control
Negative control
Low dose 200 mg/kg
High dose 400 mg/kg
Groups
Effect of geraniol on GSH
m mol/hr
8
6
4
2
0
**
$$
##
Control
Negative control
Low dose 200 mg/kg
High dose 400 mg/kg
Groups
Effect of geraniol on Glucose
mg/dl
400
300
200
100
0
***
##
$$$
Control
Negative Control
Low dose 200 mg/kg
High dose 400 mg/kg
Groups
Effect of geraniol on SOD
U/mg of protein
15
10
5
0
**
$$
#
Control
Negative control
Low dose 200 mg/kg
High dose 400 mg/kg
Groups
Effect of geraniol on Triglycerides
mg/dl
0.8
0.6
0.4
0.2
0.0
**
#
$$
Control
Negative Control
Low dose 200 mg/kg
High dose 400 mg/kg
Groups

Fig. 3

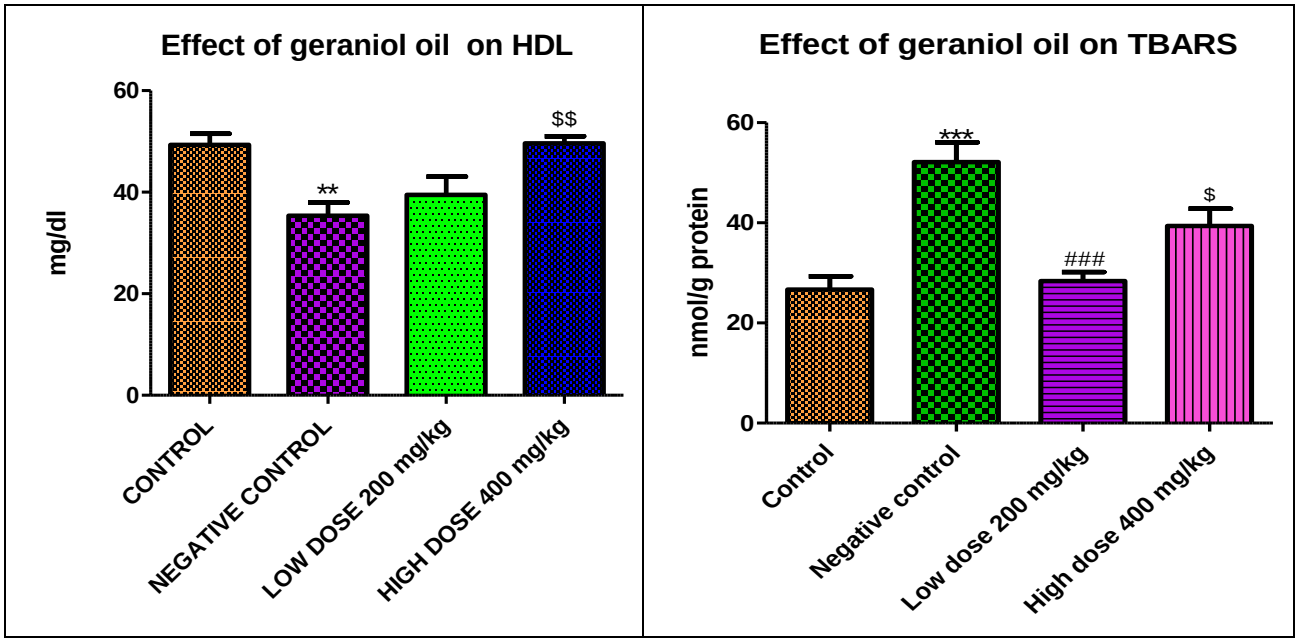


Fig. 4

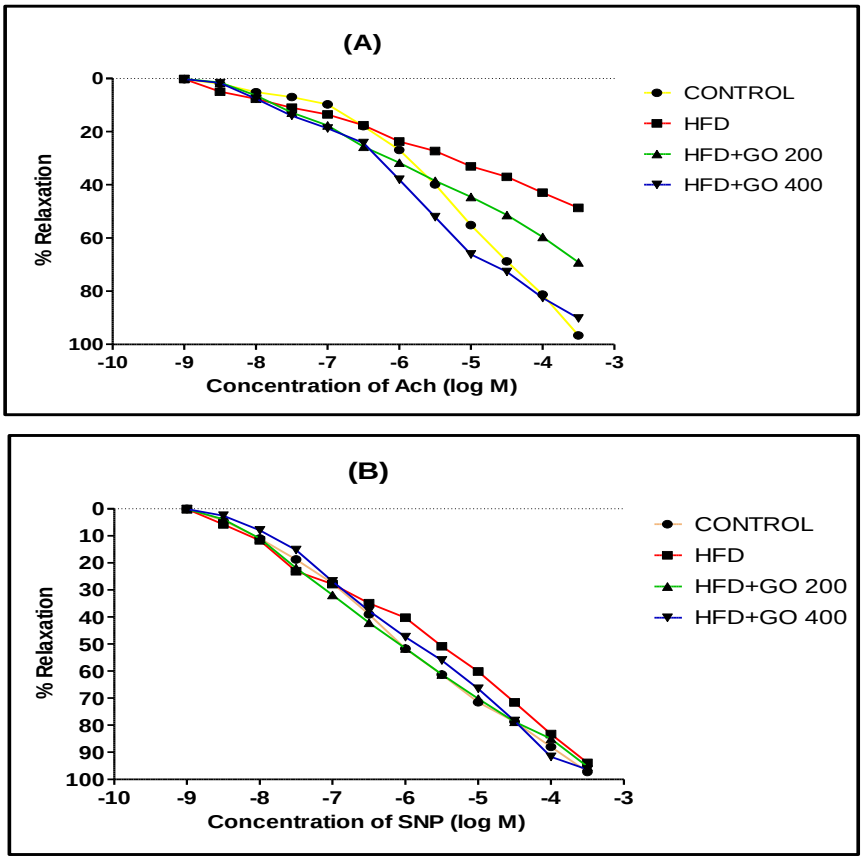


Fig. No. 4.2 Effects of GO on the vascular relaxant responses induced by Acetylcholine (A) and Sodium nitroprusside (SNP)

Fig. 5

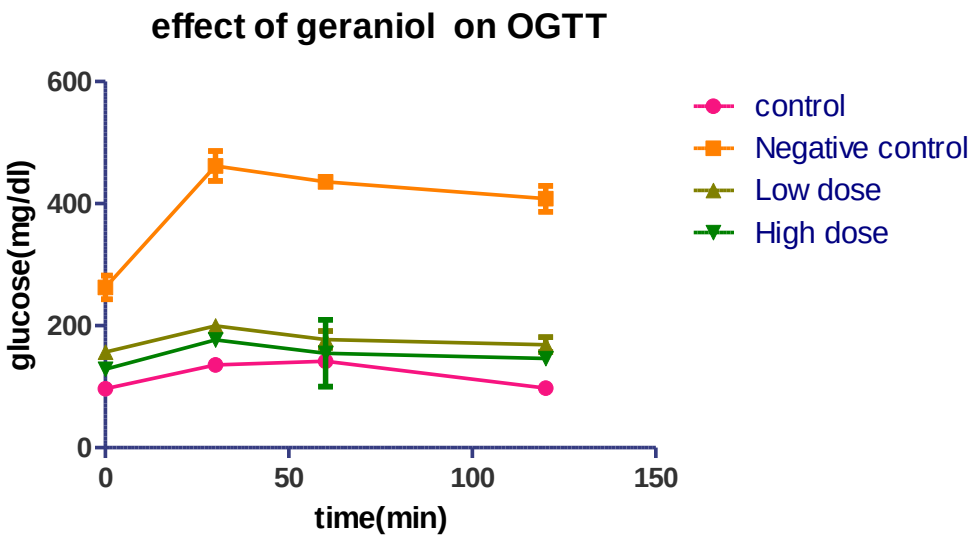


Fig. 6

DISCUSION

Food habits play an important role in the metabolic functions of the body. Life style progress turned food consumption western diet. These diets are rich in fatty acids(mostly saturated fats).Studies revealed that prolonged consumption of fat diet leads to obesity, dyslipidema, endothelial dysfunction, inflammation, hypertension, type II diabetes and end stage renal disease and indicate a risk factor them [22-29].

In the present study, the animals were fed with HFD for 12 weeks to induce pathogenesis and the investigation was done whether oral administration of Gr on HFD fed rats could show protective effect on Vascular Dysfunction. It is of note that the HFD used in study was formulated to resemble diets commonly consumed in westernized societies. As said earlier, Fat Diet consumption could increase body weight, an index of obesity. In the study, results showed a remarkable increase in body weight gain in HFD group when compared to control group. But the treatment with Gr shown a significant decrease in body weight gain when compared to HFD group. Further, the food intake was assessed and has no significance in comparison [30].

Obesity is the risk factor for type-II diabetis, hence, the blood glucose levels were assessed after 12 weeks. An alarming level of glucose in plasma was seen in HFD group. Gr treatment for 6 weeks reduced the plasma glucose levels remarkably. It suggests the hypoglycemic property of GR.

The studies associated with HFD could cause hyperlipidemia, a risk factor for nephropathy [31]. After 12 weeks, the lipid levels of HFD group were altered i.e., TG, TC, LDL levels increased and HDL levels decreased. Administration of Gr, for 6 weeks lowered the levels of TC, TG, LDL, while the levels of HDL was elevated. The results indicated that the Gr could attenuate the hyperlipidemia [31].

Taken in to account, obesity is accompained by oxidative stress. Since, SOD, CAT, GSH contribute the body's antioxidant defence system, therefore, the changes in activities of anti-oxidant enzymes in blood was investigated to elevate the levels of oxidative stress. The activities of SOD, CAT and GSH in the HFD group were reduced. Results from treatment group reported increased levels of anti-oxidant enzymes. It can be hypothesized that Gr could modulate the antioxidant enzyme activities and alleviate oxidative stress [32].

As studies have shown, the diet high in fat content causes ED and Hypertension, which may be in part due to oxidative stress [32]. In brief, as reviewed by Rodriguez-Itrube et al., oxidative stress adversely effects renal and cardiovascular function and structure by promoting inflammation, endothelial injury, smooth vascular muscle cell/fibroblast/mesangial proliferation, matrix accumulation, and generation of isoprostanes from

non-enzymatic oxidation of arachidonic acid. Hence, Ach and SNP- mediated vaso-relaxton response in the aortic rings were assessed. The data from study report that acetylcholine mediated vasorelaxation in treatment groups was significantly higher in compared to HFD group, in contrast, the vasodilatory response to the NO donor, SNP, was similar in all the groups with insignificant variation.

A significant raise in blood pressure was accompanied with enhanced oxidation in the rats fed with HFD. The mean arterial blood pressure measured by invasive technique at the end of the study in the HFD group was higher than the control group. These results suggest a probable role of oxidative stress on the genesis and the maintenance of hypertension. The treated groups showed a significant reduction in blood pressure when compared to the HFD group. Previous studies have indicated that human obesity promotes ECG changes such as lengthening of QTc interval and increase RR interval [32]. The present study shows a significant increase QT interval, QTc interval, RR interval and heart rate in HFD group. The rats treated with Gr showed a significant reduction in QTc interval, RR interval and heart rate when compared to HFD group. The observations confirm that the Gr descends mean arterial pressure (MAP), normalizes ECG and reduces heart rate.

CONCLUSION

In conclusion from the pharmacological screening it's revealed that the treatment with Gearaniol exerted protective property against Endothelial Dysfunction. It was understood from the biochemical parameters, that the drug possesses hypoglycemic effect. Moreover the ability to enhance LDL levels have proved to be protective effect but, further studies have to be done on the HDL parameter, as it shown increased levels in treated group. The ECG and MAP are considered to be normal. The treated groups showed a significant reduction in blood pressure when compared to the HFD group. Hence, from conclusion it's revealed that the drug can be used in treatment of High fat Diet induced Vascular Endothelial Dysfunction.

REFERENCES

[1]. Ross R. Atherosclerosis: an inflammatory disease. N Engl J Med. 340, 1999, 1928–1929.
[2]. Vita JA, Keaney JF. Endothelial function: a barometer for cardiovascular risk Circulation. 106, 2002, 640–642.
[3]. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s.Nature. 362, 1993, 801–809.
[4]. Schechter AN, Gladwin MT. Hemoglobin and the paracrine and endocrine functions of nitric oxide. N Engl J Med. 348, 2003, 1483–1485.
[5]. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature. 288, 1980, 373–376.
[6]. Forstermann U, Munzel T. Endothelial nitric oxide synthase in vascular disease: from marvel to menace. Circulation. 113, 2006, 1708–1714.
[7]. Corson MA, James NL, Latta SE, Nerem RM, Berk BC, Harrison DG. Phosphorylation of endothelial nitric oxide synthase in response to fluid shear stress. Circ Res. 79, 1996, 984–991
[8]. Govers R, Rabelink TJ. Cellular regulation of endothelial nitric oxide synthase. Am J Physiol Renal Physiol. 280, 2001, F193–F206.
[9]. Busse R, Edwards G, Feletou M, Fleming I, Vanhoutte PM, Weston AH. EDHF: bringing the concepts together. Trends Pharmacol Sci. 3, 2002, 374–380.
[10]. Halcox JP, Narayanan S, Cramer-Joyce L, Mincemoyer R, Quyyumi AA. Characterization of endothelium-derived hyperpolarizing factor in the human forearm microcirculation. Am J Physiol Heart Circ Physiol. 280, 2001, H2470–H2477.
[11]. Moncada S, Higgs EA, Vane JR. Human arterial and venous tissues generate prostacyclin (prostaglandin x), a potent inhibitor of platelet aggregation.Lancet. 1, 1977, 18–20.
[12]. Saye JA, Singer HA, Peach MJ. Role of endothelium in conversion of angiotensin I to angiotensin II in rabbit aorta. Hypertension. 6, 1984, 216–221.

[13]. Kinlay S, Behrendt D, Wainstein M, Beltrame J, Fang JC, Creager MA, Selwyn AP, Ganz P. Role of endothelin-1 in the active constriction of human atherosclerotic coronary arteries. Circulation. 104, 2001, 1114–1118.

[14]. Stamler JS, Lamas S, Fang FC. Nitrosylation. The prototypic redox-based signaling mechanism. Cell. 106, 2001, 675–683.

[15]. Ghosh S, Karin M. Missing pieces in the NF-κB puzzle. Cell. 109, 2002, S81–S96.

[16]. Moncada S, Erusalimsky JD. Does nitric oxide modulate mitochondrial energy generation and apoptosis? Nat Rev Mol Cell Biol. 3, 2002, 214–220.

[17]. Gimbrone MA Jr. Vascular endothelium, hemodynamic forces, and atherogenesis. Am J Pathol. 155, 1999, 1–5.

[18]. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med. 352, 2005, 1685–1695.

[19]. Rhee SG. Cell signaling: H2O2, a necessary evil for cell signaling. Science. 312, 2006, 1882–1883.

[20]. Charakida M, Donald A, Terese M, Leary S, Halcox JP, Ness A, Davey Smith G, Golding J, Klein NJ, Deanfield JE, and ALSPAC Study Team. Endothelial dysfunction in childhood infection. Circulation. 111, 2005, 1160–1165.

[21]. Celermajer DS, Sorensen KE, Bull C, Robinson J, Deanfield JE. Endothelium-dependent dilation in the systemic arteries of asymptomatic subjects relates to coronary risk factors and their interaction. J Am Coll Cardiol. 24, 1994, 1468–1474.

[22]. D'Aiuto F, Parkar M, Nibali L, Suvan J, Lessem J, Tonetti MS. Periodontal infections cause changes in traditional and novel cardiovascular risk factors: results from a randomized controlled clinical trial. Am Heart J. 151, 2006, 977–984.

[23]. Li Y, Huang TT, Carlson EJ, Melov S, Ursell PC, Olson JL, Noble LJ, Yoshimura MP, Berger C, Chan PH, Wallace DC, Epstein CJ. Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase.Nat Genet. 11, 1995, 376–381.

[24]. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature. 414, 2001, 813–820.

[25]. Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. Endocr Rev. 23, 2002, 599–622.

[26]. Griendling KK, Sorescu D, Ushio-Fukai M. NAD (P)H oxidase: role in cardiovascular biology and disease. Circ Res. 86, 2000, 494–501.

[27]. Spiekermann S, Landmesser U, Dikalov S, Bredt M, Gamez G, Tatge H, Reepschlager N, Hornig B, Drexler H, Harrison DG. Electron spin resonance characterization of vascular xanthine and NAD (P) H oxidase activity in patients with coronary artery disease: relation to endothelium-dependent vasodilation. Circulation. 107, 2003, 1383–1389.

[28]. Woywodt A, Bahlmann FH, De Groot K, Haller H, Haubitz M. Circulating endothelial cells: life, death, detachment and repair of the endothelial cell layer. Nephrol Dial Transplant. 17, 2002, 1728–1730.

[29]. Mallat Z, Benamer H, Hugel B, Benessiano J, Steg PG, Freyssinet JM, Tedgui A. Elevated levels of shed membrane microparticles with procoagulant potential in the peripheral circulating blood of patients with acute coronary syndromes. Circulation. 101, 2000, 841–843.

[30]. R Buettner, K G Parhofer, M Woenckhaus, CEWrede, L A Kunz-Schughart, J Schölmerich and L C Bollheimer. Defining high-fat-diet rat models: metabolic and molecular effects of different fat types. Journal of Molecular Endocrinology 36, 2006, 485–501.

[31]. Nazia Begum, Vasudha Bakshi, Vijayalaxmi A, Devender Gaud P, K Sunand, K Sharath Kumar, Sana, Protective Role of Piperine and Metformin on Gentamicin Induced Hepatorenal Toxicity. Int. J. Pharm. Sci. Rev. Res., 35(2), 2015, 83-89.

[32]. Nerella Mounika, Afzal Nazneen, P. Shashikanth, K. Baikuntha Prusty and J. Venkateshwar Rao. Evaluation of hepatoprotective and anti-oxidant activity of root extracts of aegle marmelos in carbon tetrachloride induced hepatotoxicity wistar albino rats. International journal of pharmaceutical sciences and research. 8(10), 2017, 4113-4128.