Research Article

Comparative Study of Antihyperlipidemic Effect of *Allium tuberosum* Rottler. ex Spreng and *Trigonella foenum graecum* in Experimentally induced Hyperlipidemia in Albino Rabbits

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ABSTRACT

Allium tuberosum Rottler ex Spreng (ATR) and *Trigonella foenum graecum* (TFG) have been used as complementary and alternative medicine (CAM) for the treatment of hyperlipidemia. So, the present study was planned to compare the antihyperlipidemic effect of the alcoholic extract of ATR and TFG in high cholesterol diet (HCD) induced hyperlipidemia in albino rabbits along with their effect on coagulation profile. Forty-two albino rabbits (1.5-2 kg) were divided into 7 groups (n=6). Hyperlipidemia was induced by administration of high cholesterol diet (HCD) 400 mg/kg for 30 days. The group 1 were treated: (2% Gum Acacia) vehicle control, group 2; disease control, group 3 & 4: 200 and 400 mg/kg ATR extract, group 5 & 6: 200 and 400 mg/kg TFG extract and group 7: (rosuvastatin 2 mg/kg). Lipid and coagulation profile were estimated on day 31 & 61 by semi autoanalyser using standard kits. Lipid profile significantly ($p<0.001$) increased in HCD group as compared to the control group. The ATR and TFG at both the dose 200 and 400 mg/kg significantly ($p<0.001$) reduced all the elevated lipid profile parameters. However, the hypolipidemic efficacy of ATR was found significantly ($p<0.01$) higher than TFG at the dose 400 mg/kg. In case of coagulation profile, ATR significantly ($p<0.001$) increased the prothombin and clotting time in a dose-dependent manner. However, TFG only raised the bleeding time. This finding of the present study illustrated that ATR extract was more potent than TFG against HCD induced hyperlipidemia in rabbits.

Keywords: Hyperlipidemia, High cholesterol diet, *Allium tuberosum* Rottler Ex Spreng, *Trigonella foenum graecum,* rabbit.

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INTRODUCTION

Hyperlipidemia is a metabolic disorder in which all lipids and/or lipoproteins are elevated in the blood.[1] It is the most common chronic disease and reason for approx 12 million deaths globally.[2] It is now well established that hyperlipidemia represents a major risk factor for the premature development of atherosclerosis and its cardiovascular complications which can lead to a rise in morbidity and mortality.[3,4] The most common clinical manifestations are stroke, acute myocardial infarction and in both of these events atherosclerosis is the underlying cause.[5] Hyperlipidemia, mainly increased level of total cholesterol (TC), triglyceride (TG) and low density lipoprotein (LDL) along with a decrease in high density lipoprotein (HDL) contributes significantly to the manifestation and development of atherosclerosis and coronary heart diseases.[6] Hypercholesterolemia is often associated with obesity, diabetes mellitus and hypertension, which contributes to the elevated cardiovascular mortality.[7]

The statins (HMG-CoA reductase inhibitors) are the commonly used drugs for the treatment of hyperlipidemia.[8] HMG-CoA reductase is the rate-limiting enzyme in cholesterol synthesis, which catalyses the conversion of HMG-CoA to mevalonic acid. The long term statins therapy is responsible for some serious adverse effects like myopathy, diabetes mellitus and haemorrhagic stroke.[9] Medicinal plants play a key role in prevention and treatment of various chronic diseases.[10] They have always been considered as a healthy source of medicine for all people due to their well-to-do therapeutic properties and safer to use.[11]. The use of herbal drugs and complementary medicines has increased tremendously over the past two decades.[12] The hypolipidemic activity of various medicines plants were well established in the different regions of the world.[13]

Allium tuberosum Rottler ex Spreng and *Trigonella foenum graecum* are well known for their antihyperlipidemic activity.[14,15] In North Eastern states of India, both the medicinal plants were used for treatment of hyperlipidemia. ATR and TFG are also a main ingredient of the polyherbal Ayurvedic formulation for the hyperlipidemia treatment.[16,17] Various experimental and clinical studies also established the hypolipidemic property of ATR and TFG.[18,19] The antidiabetic effect, hypocholesterolemic influence, antioxidant potency, antiplatelet and hepatoprotective effect of TFG and ATR were also reported.[20-25] In view of the present orientation towards the effective indigenous drug development, this

study was undertaken to compare the antihyperlipidemic effect of ethanolic extracts of *Allium tuberosum* Rottler ex Spreng and *Trigonella foenum graecum*.

MATERIALS AND METHODS

Animal

In the present study, healthy albino rabbits of either sex weighing between 1 to 2 kg were used for the experiment. The rabbits were obtained from the Central Animal House, Regional Institute of Medical Sciences (RIMS), Imphal. They were kept in separate cages and acclimatized in room temperature with maintenance of 12:12 hour light and dark cycle. During the whole period of experiment, the rabbits were maintained on a normal diet and water *ad libitum*. Approval of this study was taken from the "Institutional Animal Ethics Committee (IAEC)" of RIMS, Imphal (1596/GO/a/12/CPCSEA).

Test Drugs

Both the medicinal plants (*Allium tuberosum* and *Trigonella foenum graecum*) were collected from the local farm in valley areas of Imphal West District, Manipur and authenticated by an expert botanist of Department of Life Sciences, Manipur University, Imphal. The medicinal plants of were procured from local farms and shade dried and powdered in a mixer grinder. The powdered of 1000 gm plants were defatted with petroleum ether in soxhlet apparatus for a period of 10 hours. The defatted material was spread out to evaporate the adhering petroleum ether and extracted again with 95% ethanol till the eluent was colourless. Ethanol was removed under reduced pressure to obtain a dark green solid and was stored in a glazed porcelain jar for use. The yield of the TGF and ATR extract were 12% and 14% respectively.

Chemicals

Ethanol, petroleum ether, rosuvastatin, cholesterol and other chemicals were procured from Merck Life Science, India. The kits of BeneSphera™ were purchased for the estimation of total cholesterol (TC), triglycerides (TG) and high density lipoproteins (HDL).

Acute toxicity testing of ATR

The acute toxicity of ATR and TFG were done according to OECD guidelines.

Treatment groups

The albino rabbits of either sex were divided into 7 groups (n=6). Group 1 was vehicle group given 2 % of gem acacia at a dose of 1ml/100 g of body weight. Group 2 was disease control. Group 3 & 4 were treated with ATR 200 and 400 mg/kg respectively. However, Group 5 and 6 were administered TFG at the dose of 200 and 400 mg/kg respectively. Group 7 was treated with rosuvastatin at the dose of 2 mg/kg.

Experimental procedure

On day 0(zero)	→ Fasting basal serum lipid estimation were done on each rabbit.
On day 1 to 30	→ Induction of hyperlipidemia was done by feeding the rabbits with high cholesterol diet (HCD) i.e., 400 mg/kg. body wt. of cholesterol A.R. powder dissolved in 5ml of coconut oil as a cocktail except in group 1which was treated with distilled water (DW).
On day 31 to 60	→The animals were treated with ATR, TFG and rosuvastatin in their respected groups.

Overnight fasting blood samples from rabbits were collected on day 31 and day 61 and serums were analysed for total cholesterol (TC), triglycerides (TG) and high density lipoproteins (HDL) and by the enzymatic method as described previously using suitable commercial kits.[26,27]

On day 61, all animals were sacrificed for the histopathological examination of thoracic aorta and were performed according to previously described method.[28,29]

Coagulation profile study

Along with the lipid profile estimation, the effect of two plant extracts on coagulation profile such as bleeding time, clotting time and prothrombin time was also estimated and compared to day 0, 31 and 61 in the same 42 rabbits which were experimentally induced with HCD for the antihyperlipidemic studies.

Histopathology of aorta

At the end of experiment, rabbits were sacrificed with high dose phenobaritone. The aorta was isolated after incising the chest wall. The isolated tissue was rinsed with chilled normal saline and preserved in 10% formalin. The slices of aortic tissue specimens were stained by Hematoxyline-Eosin method. All histopathological evaluations were performed by a pathologist of Department of Pathology, RIMS, Imphal.

Statistical analysis

The data were analysed by IBM SPSS version 21.0. The results of the studies were expressed as MEAN ± SEM. Analysis was done by using one way ANOVA followed by bonferroni test post hoc. A probability level of ($p<0.05$) was considered significant.

RESULTS

Acute toxicity testing of ATR & TFG

No adverse effect, organ toxicity or mortality was detected in albino rats upto 2g/kg of body wt.

Antihyperlipidemic Study

Serum lipid changes after induction of hyperlipidemia from day 0 to day 31:-

Changes in serum total cholesterol

No significant difference was found in the mean fasting basal of total cholesterol values among the groups. After HCD for 30 days, the cholesterol was raised significantly ($p<0.001$) in the group 2, 3, 4, 5, 6 & 7 as compared to day 0 and total cholesterol value found to be 370.66 ± 9.37, 377.66 ± 10.31, 391.50 ± 8.95,389.00 ± 8.74, 376.16 ± 16.09 and 336.16 ± 8.46 mg/dl respectively. After the 30 days treatment of ATR, TFG and rosuvastatin on the day 61, ATR 200 and 400 significantly reduced the cholesterol level to 338.33 ± 8.96 ($p<0.01$) and 228.66 ± 11.21 ($p<0.001$) mg/dl respectively. Moreover, TFG (200 and 400 mg/kg) also significantly ($p<0.01$) decreased the total cholesterol level as compared to day 30 (Table-1).

Table 1: Effect of ATR & TFG treatment on total cholesterol level in HCD induced hyperlipidemia in rabbits.

S. No.	Treatment Groups	TC level (mg/dl) Day 0	TC level (mg/dl) Day 31	TC level (mg/dl) Day 61
1	Vehicle control	54.33 ± 1.33	55.00 ± 1.80	59.83 ± 3.19
2	HCD	57.16 ± 2.74	370.66 ± 9.37^{a***}	360.00 ± 9.76
3	ATR200 + HCD	56.83 ± 1.77	377.66 ± 10.31^{a***}	338.33 ± 8.96^{b**}
4	ATR400+ HCD	54.16 ± 4.02	391.50 ± 8.95^{a***}	228.66 ± 11.21^{b***}
5	TFG200 + HCD	57.16 ± 2.42	389.00 ± 8.74^{a***}	346.33 ± 9.74^{b**}
6	TFG400 + HCD	54.83 ± 2.67	376.16 ± 16.09^{a***}	302.50 ± 9.01^{b**}
7	Rosuvastatin + HCD	57.00 ± 2.25	336.16 ± 8.46 ^{a***}	159.50 ± 11.26^{b***}

Data were represented in Mean ± SEM, (n=6),** $p<0.01$ &*** $p<0.001$. 'a' compared with Day 0. 'b' compared with Day 31.TC - Total cholesterol, ATR - *Allium tuberosum* Rottler ex Spreng, TFG -*Trigonella foenum graecum.,* HCD - High cholesterol Diet.

Changes in the triglycerides

The base line serum TG (mg/dl) levels in the group 1,2,3,4,5,6 & 7 were found to be 41.83 ± 2.5, 43.00 ± 2.00, 41.50 ± 1.64, 38.00 ± 2.76, 37.33 ± 1.60, 37.33 ± 1.85 and 42.00 ± 2.32 mg/dl respectively and have no significant difference among the groups. The HCD significantly ($p<0.001$) increased the TG levels on days 31 in all the groups. However, one month treatment with ATR 400, TFG 400 and rosuvastatin significantly ($p<0.001$) ameliorated the risen TG levels as compared day 31. (Table- 2)

Table 2: Effect of ATR & TFG treatment on triglycerides level in HCD induced hyperlipidemia in rabbits.

S. No.	Treatment Groups	TG level (mg/dl) Day 0	TG level (mg/dl) Day 31	TG level (mg/dl) Day 61
1	Vehicle control	41.83 ± 2.5	49.83 ± 3.19	55.66 ± 3.96
2	HCD	43.00 ± 2.00	173.50 ± 9.11^{a***}	165.33 ± 5.46
3	ATR200 + HCD	41.50 ± 1.64	175.83 ± 7.16^{a***}	153.50 ± 5.40^{b*}
4	ATR400+ HCD	38.00 ± 2.76	177.00 ± 8.22 ^{a***}	88.66 ± 4.83^{b***}
5	TFG200 + HCD	37.33 ± 1.60	176.66 ± 4.95^{a***}	148.00 ± 3.26^{b**}
6	TFG400 + HCD	37.33 ± 1.85	171.33 ± 6.26^{a***}	142.33 ± 4.70^{b***}
7	Rosuvastatin + HCD	42.00 ± 2.32	176.16 ± 2.65 ^{a***}	72.66 ± 8.85^{b***}

Data were represented in Mean ± SEM, (n=6),* $p<0.05$&*** $p<0.001$. 'a' compared with Day 0. 'b' compared with Day 31.TG–triglycerides, ATR - *Allium tuberosum* Rottler ex Spreng, TFG -*Trigonella foenum graecum.*HCD - High cholesterol diet.

Changes in the HDL

The fasting mean basal values for serum HDL levels were not changed significantly among the groups and 20.83 ± 1.62, 19.00 ± 1.23, 20.50 ± 1.80, 18.16 ± 2.05, 19.00 ± 1.96, 18.83 ± 0.98 and 21.00 ± 1.57 mg/dl were found in the group 1,2,3,4,5,6 & 7 respectively. After feeding for one month with HCD to group 2,3,4,5, 6 & 7, the HDL values raised significantly ($p<0.001$) up to35.00 ± 4.02, 73.83 ± 6.26, 77.00 ± 1.91, 74.50 ± 4.57, 56.16 ± 2.5 and 34.33 ± 4.30mg/dl respectively as compared to baseline of day 0.Only TFG 400 mg/kg significantly ($p<0.05$) increased the HDL levels after one month of treatment on day 61 as compared to day 31(Table-3).

Table 3: Effect of ATR & TFG treatment on HDL level in HCD induced hyperlipidemia in rabbits.

S. No.	Treatment Groups	HDL level (mg/dl) Day 0	HDL level (mg/dl) Day 31	HDL level (mg/dl) Day 61
1	Vehicle control	20.83 ± 1.62	22.33 ± 1.81	23.33 ± 1.94
2	HCD	19.00 ± 1.23	35.00 ± 4.02^{a*}	36.66 ± 3.98
3	ATR200 + HCD	20.50 ± 1.80	73.83 ± 6.26^{a***}	74.66 ± 6.01
4	ATR400+ HCD	18.16 ± 2.05	77.00 ± 1.91 ^{a***}	79.00 ± 1.84
5	TFG200 + HCD	19.00 ± 1.96	74.50 ± 4.57^{a***}	75.51 ± 4.45
6	TFG400 + HCD	18.83 ± 0.98	56.16 ± 2.5^{a***}	74.66 ± 6.01^{b*}
7	Rosuvastatin + HCD	21.00 ± 1.57	34.33 ± 4.30 ^{a**}	29.66 ± 1.14

Data were represented in Mean ± SEM, (n=6),* p<0.05, ** p<0.01 &*** p<0.001. 'a' compared with Day 0. 'b' compared with Day 31.HDL=High density lipoproteins, ATR - *Allium tuberosum* Rottler ex Spreng, TFG -*Trigonella foenum graecum.*HCD - High cholesterol diet.

Changes in the LDL

No significant changes were found in basal serum LDL levels among the groups on day 0. One-month HCD feed significantly (p<0.001) increased the LDL levels in all the groups as compared to day 0. One month treatment of ATR (400 mg/kg), TFG (200 & 400 mg/kg) and rosuvastatin (2 mg/kg) significantly ameliorated the increased LDL levels (Table-4).

Table 4: Effect of ATR &TFG treatment on LDL level in HCD induced hyperlipidemia in rabbits.

S. No.	Treatment Groups	LDL level (mg/dl) Day 0	LDL level (mg/dl) Day 31	LDL level (mg/dl) Day 61
1	Vehicle control	25.13 ± 1.50	22.70 ± 2.06	25.36 ± 3.32
2	HCD	29.56 ± 3.32	300.96 ± 12.59 ^{a***}	290.26 ± 12.40
3	ATR200 + HCD	28.03 ± 3.35	268.66 ± 14.34^{a***}	232.96 ± 9.95
4	ATR400+ HCD	28.40 ± 3.92	279.10 ± 9.09 ^{a***}	131.93 ± 12.22 ^{b***}
5	TFG200 + HCD	30.70 ± 1.92	279.16 ± 6.52^{a***}	242.35 ± 5.86^{b***}
6	TFG400 + HCD	28.53 ± 2.21	285.73 ± 14.09^{a***}	198.23 ± 10.69^{b***}
7	Rosuvastatin + HCD	27.60 ± 2.68	266.60 ± 5.07 ^{a***}	114.80 ± 10.75^{b***}

Data were represented in Mean ± SEM, (n=6),*** p<0.001. 'a' compared with Day 0. 'b' compared with Day 31. LDL- Low density lipoproteins, ATR - *Allium tuberosum* Rottler ex Spreng, TFG -*Trigonella foenum graecum* and HCD - High cholesterol diet.

Coagulation Profile Study

Changes in bleeding time (BT)

Fasting mean basal values for BT in the group 1,2,3,4,5,6 & 7 were found to be 90.16 ± 1.51, 88.83 ± 1.51, 89.50 ± 1.66, 88.50 ± 1.54, 91.33 ± 1.14, 88.16 ± 1.19 and 89.16 ± 1.62 seconds respectively. After one month feeding of HCD, no significant changes were found among the groups. However, ATR (200 & 400 mg/kg) treatment significantly (p<0.05) increased BT as compared to day 31. Similarly, the TFG at the dose 400 mg/kg also enhanced the BT significantly (p<0.001). TFG 200 mg/kg and rosuvastatin therapy did not produce any significant change in BT (Table-5).

Table 5: Effect of ATR, TFG &rosuvastatin treatment on bleeding time in hyperlipidemic albino rabbits

S. No.	Treatment Groups	Day 0 BT (sec.)	Day 31 BT (sec.)	Day61 BT (sec.)
1	Vehicle control	90.16 ± 1.51	88.00 ± 1.46	91.83 ± 1.79
2	HCD	88.83 ± 1.51	86.33 ± 1.05	87.83 ± 0.87
3	ATR200 + HCD	89.50 ± 1.66	87.33 ± 1.72	93.50 ± 0.99^{b*}
4	ATR400+ HCD	88.50 ± 1.54	87.83 ± 1.62	94.16 ± 1.24^{b*}
5	TFG200 + HCD	91.33 ± 1.14	88.83 ± 1.51	91.33 ± 1.70
6	TFG400 + HCD	88.16 ± 1.19	88.16 ± 1.49	96.83 ± 1.40^{b**}
7	Rosuvastatin + HCD	89.16 ± 1.62	88.16 ± 1.64	92.16 ± 1.19

Data were represented in Mean ± SEM, (n=6),* p<0.05,*** p<0.001, 'b' as compared to day 31. BT - Bleeding time, ATR- *Allium tuberosum* Rottler ex Spreng, TFG -*Trigonella foenum graecum.*HCD - High cholesterol diet.

Changes in clotting time (CT)

Fasting mean basal values for CT in the group 1,2,3,4,5,6 & 7 were found to be 102.00 ± 0.96, 99.66 ± 0.88, 101.33 ± 1.28, 96.83 ± 2.12, 100.16 ± 1.42, 99.83 ± 1.24 and 101.00 ± 1.34 seconds respectively. One month treatment of HCD did not show any significant change in CT. Only ATR (200 and 400 mg/kg) treatment, significantly ($p<0.001$) increased the CT as compared to CT of day 31 (Table-6).

Table 6: Effect of ATR, TFG &rosuvastatin treatment on clotting time in hyperlipidemic albino rabbits

S. No.	Treatment Groups	Day 0 CT (sec.)	Day 31 CT (sec.)	Day61 CT (sec.)
1	Vehicle control	102.00 ± 0.96	100.50 ± 0.76	103.66 ± 3.96
2	HCD	99.66 ± 0.88	97.50 ± 0.84	100.16 ± 0.98
3	ATR200 + HCD	101.33 ± 1.28	98.00 ± 0.51	111.00 ± 1.93^{b***}
4	ATR400+ HCD	96.83 ± 2.12	98.66 ± 0.88	120.33 ± 1.20^{b***}
5	TFG200 + HCD	100.16 ± 1.42	99.16 ± 0.47	101.33 ± 0.80
6	TFG400 + HCD	99.83 ± 1.24	99.66 ± 0.88	103.16 ± 0.70
7	Rosuvastatin + HCD	101.00 ± 1.34	98.16 ± 0.65	101.33 ± 0.66

Data were represented in Mean ± SEM, (n=6), *** $p<0.001$, 'b' as compared to day 31. CT- Clotting time, ATR - *Allium tuberosum* Rottler ex Spreng, TFG -*Trigonella foenum graecum.* HCD - High cholesterol diet.

Changes in prothrombin time (PT)

The basal mean fasting prothrombin time were found in the group 1, 2 ,3, 4,5 ,6 & 7 as 7.13 ± 0.08, 7.08 ± 0.14, 7.03 ± 0.23, 7.15 ± 0.11, 7.56 ± 0.22, 7.18 ± 0.17 and 7.00 ± 0.12 seconds respectively. Thirty days HCD treatment did not produce any significant change in PT. However, one month treatment of ATR (200 and 400 mg/kg) significantly ($p<0.001$) increased the PT as compared to PT of day 31 (Table-7).

Table 7: Effect of ATR, TFG & rosuvastatin treatment on prothrombin time in hyperlipidemic albino rabbits

S. No.	Treatment Groups	Day 0 PT (sec.)	Day 31 PT (sec.)	Day61 PT (sec.)
1	Vehicle control	7.13 ± 0.08	7.13 ± 0.84	7.16 ± 0.04
2	HCD	7.08 ± 0.14	7.00 ± 0.13	7.26 ± 0.18
3	ATR200 + HCD	7.03 ± 0.23	6.92 ± 0.10	8.21 ± 0.08 ^{b***}
4	ATR400+ HCD	7.15 ± 0.11	7.08 ± 0.14	8.81 ± 0.09 ^{b***}
5	TFG200 + HCD	7.56 ± 0.22	7.04 ± 0.13	7.17 ± 0.11
6	TFG400 + HCD	7.18 ± 0.17	7.08 ± 0.14	7.51 ± 0.12
7	Rosuvastatin + HCD	7.00 ± 0.12	7.01 ± 0.15	7.32 ± 0.15

Data were represented in Mean ± SEM, (n=6), *** $p<0.001$, 'b' as compared to day 31. PT- Prothrombin time, ATR - *Allium tuberosum* Rottler ex Spreng, TFG -*Trigonella foenum graecum.* HCD - High cholesterol diet.

Histopathology of aorta

Histopathology of Group 1 (Vehicle control)

Aorta showed normal histology. Sections from the aorta showed inner layer tunica lined by single layer of endothelial cells with the subendothelial layer comprising of loose connective tissue containing elastic fibres and few smooth muscle cells. The middle tunica media, the thickest layer is separated from intima by internal elastic lamina. Tunica media consist of numerous elastic laminae in a circular and oblique arrangement and between them; there is a thin layer of connective tissue containing collagen fibres and smooth muscle cells, tunica in time and tunica media. Tunica adventitia is the thin outer layer containing bundles of collagen fibres and few elastic fibres, fibroblasts, mast cells and vasa vasorum (Fig. 1).

Histopathology of Group 2 (High cholesterol diet)

Section from the aorta showed plaque at least half as thick as tunica media with accumulation of intracellular lipid, foamy macrophages and smooth muscle cell consistent with atherosclerotic plaque (Fig. 1).

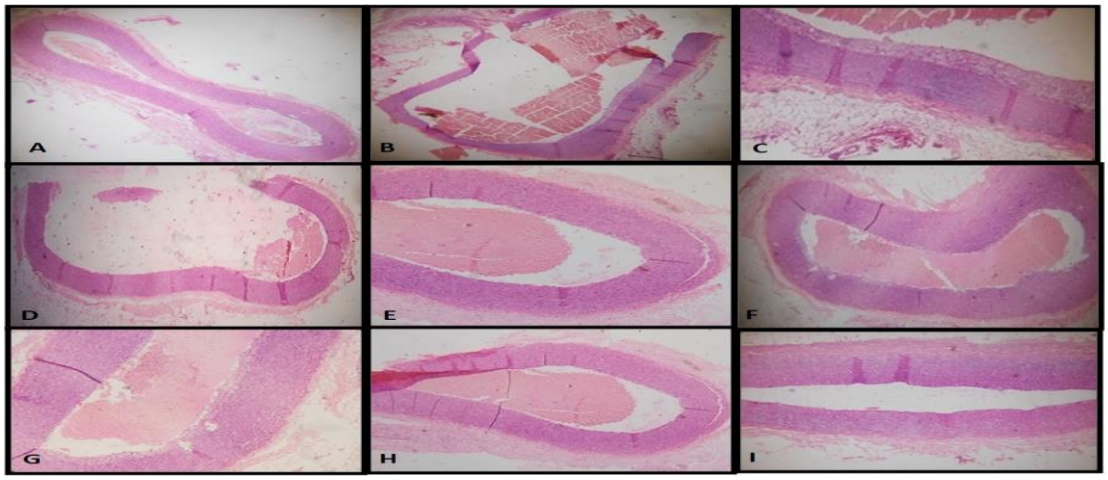

Figure 1: Cross sections of the aorta: (A): Group fed with 2 % of gum acacia, magnification ×10, (B): fed high cholesterol diet, magnification ×10, (C): fed high cholesterol diet, magnification ×40 (D): group fed ATR200 mg/kg with high cholesterol diet, magnification ×10, (E): group fed ATR 400 mg/kg with high cholesterol diet, magnification ×10, (F): Group fed TFG 200 mg/kg with high cholesterol diet, magnification ×10, (G): Group fed TFG 200 mg/kg with high cholesterol diet, magnification ×40, (H): Group fed TFG 400 mg/kg with high cholesterol diet, magnification ×10, and (I): Group fed rosuvastatin with high cholesterol diet, magnification ×10.

Histopathology of Group 3 (ATR 200 + HCD)

Sections from the aorta showed similar findings as in histopathology of Group 1 (Vehicle control). No plaque was seen (Fig. 1).

Histopathology of Group 4 (ATR 400 + HCD)

Sections from the aorta showed similar findings as in histopathology of Group 1 (Vehicle control). No plaque was seen (Fig. 1).

Histopathology of Group 5 (TFG 200 + HCD)

Section showed few foamy macrophages, smooth muscle comprising less than half the media layer consistent with atherosclerotic plaque (Fig. - 1).

Histopathology of Group 6 (TFG 400 + HCD)

Aorta showed normal histology. Sections from the aorta showed similar findings as in histopathology of Group 1 (Vehicle control). No plaque was seen (Fig. 1).

Histopathology of Group 7 (Rosuvastatin 2 mg/kg + HCD)

Aorta showed almost normal histology. Sections from the aorta showed similar findings as in histopathology of Group 1 (Vehicle control). No plaque was seen (Fig. 1).

Comparison of hypolipidemic effect between ATR and TFG on day 61

There were no significant differences in the hypolipidemic effect between ATR and TFG at lower dose 200 mg/kg. However, at higher dose of 400 mg/kg ATR showed significantly ($p<0.001$) more effective than TFG in case of triglyceride, total cholesterol and low-density lipoprotein cholesterol levels (Fig. 2).

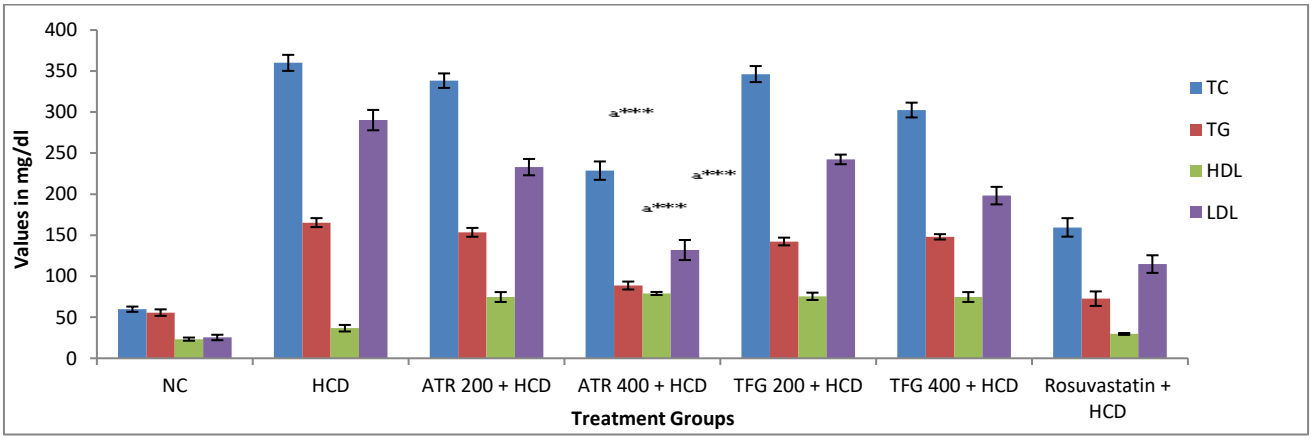

Figure 2: Comparison of hypolipidemic effect between ATR and TFG in HCD induced hyperlipidemic albino rabbits on day 61. Data were represented in Mean ± SEM, (n=6), *** $p<0.001$, 'a' as compared to TFG 400. HDL=High density lipoproteins, LDL=Low density lipoproteins, ATR - *Allium tuberosum* Rottler. ex Spreng, TFG - *Trigonella foenum graecum.* HCD - High cholesterol diet, TC - Total cholesterol, TG - Triglycerides, HDL -High density lipoproteins and LDL - Low density lipoproteins.

Comparison between ATR and TFG effect in coagulation profile on day 61.

The bleeding time did not show any significant change among the groups. However, ATR at both the dose 200 and 400 mg/kg significantly (p<0.001) increased the clotting and prothombin time as compared to TFG 200 and 400 mg/kg on day 61 (Fig. 3).

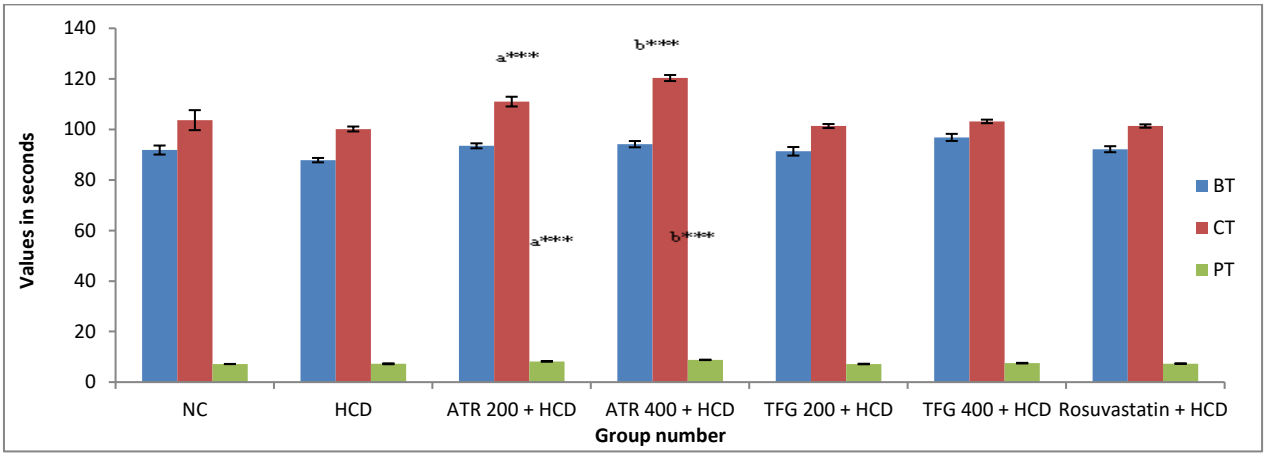

Figure 3: Comparison of coagulation profile between ATR and TFG in HCD induced hyperlipidemic albino rabbits on day 61. Data were represented in Mean ± SEM, (n=6), *** p<0.001,'a' as compared to TFG 200, 'b' as compared to TFG 400. ATR - *Allium tuberosum* Rottler ex Spreng, TFG - *Trigonella foenum graecum.* HCD - High cholesterol diet, BT- Bleeding time, CT- Clotting time and PT- Prothombin time.

DISCUSSION

The trend of use of complementary and alternative medicine (CAM) is increasing fast throughout the world mainly for chronic illness like diabetes, hypertension, arthritis, epilepsy, stroke, hyperlipidemia etc.[30,31] The hypolipidemic potential of various medical plants like *Allium sativum, Boswellia carterii, Citrus limon, Ginkgo biloba, Trigonella foenum, Withania somnifera, Zingiber officinale* etc. have been reported.[15,32] *Allium tuberosum* and *Trigonella foenum* are the most commonly used medicinal plant for the treatment of hyperlipidemia.[33] The hypolipidemic property of *Allium tuberosum* and *Trigonella foenum* were already proven.[15,18] But, there was a lacking of evidence based comparative data on their efficacy and potency. So, the present study was done to compare their antihyperlipidemic effect in HCD induced hyperlipidemia in albino rabbits.

The high cholesterol diet induced hyperlipidemia is one most commonly used experiment model. Previously various studies also used to analyze the hypolipidemic effect of plant extract in the HCD induced hyperlipidemia in rabbits.[34,35] The significant (p<0.001) increase in lipid prolife levels (triglycerides, total cholesterol, high density lipoprotein cholesterol and low-density lipoprotein) were found in the present study after 30 days treatment HCD. Similarly, Cheong *et al.,* also reported the elevation of lipid profile levels with HCD administration for 4 week in the rabbits.[36]

The present study reported that treatment of rosuvastatin (2 mg/kg) significantly (p<0.001) reduced the elevated lipid profile levels. However, rosuvastatin (2.5 mg/kg) therapy also ameliorated the altered lipid in the HCD induced hypercholesterolemia in rabbits.[37] The hypolipidemic potential of ATR and TFG were established by various experiment studies.[15,38] TFG at the doses of 200 and 400 mg/kg significantly (p<0.01 and p<0.001) reduced all the lipid profile parameters (TC, TG and LDL) respectively in the present study. But, in case of ATR only 400 mg/kg significantly (p<0.001) improved the HCD induced hyperlipidemia. Saponin is one of the most important phyto-constituent of TFG and ATR responsible for their hypolipidemic property.[20,39] The ameliorating property of saponin probably due to hastened cholesterol metabolism, inhibited cholesterol synthesis, and improved reverse cholesterol transport.[40] In the comparative study of ATR and TFG at the lower dose 200 mg/kg did not show any significant difference in antihyperlipidemic property. However, at higher dose of400 mg/kg, ATR was found to be more effective in lowering TC, TG and LDL levels than TFG.

In the present study, high cholesterol diet did not produce any change in coagulation profile (BT, CT and PT) of blood. ATR therapy on both the dose at 200 and 400 mg/kg significantly (p<0.001) increased the CT and PT of blood. The fibrinolytic property of allium has been already reported and the organosulphur phytocompounds are mainly responsible for that.[41] TFG 400 mg/kg treatment only significantly (p<0.01) enhanced the bleeding time. Spirostanol is a type of saponin, present in *Trigonella foenum-graecum* hat inhibits the platelet aggregation.[42] However, in the comparison between ATR and TFG on coagulation profile, it was found that ATR are more effective than TFG in case of CT and PT.

The histopathological analysis of the thoracic aorta of HCD group indicate the grade II atherosclerotic changes with half as thick as tunica media with accumulation of intracellular lipid, foamy macrophages and smooth muscle

cell. Which was consistent with atherosclerotic plaque (Chekanov Scale). It leads to the narrowing of the lumen. Sections of thoracic aorta from animals treated with TFG400 and ATR400 showed no atherosclerotic lesions. It proved that ATR and TFG at a dose of 400 mg/kg were effective in the treatment of atherosclerosis in hyperlipidemic albino rabbits.

CONCLUSIONS

The findings of the present study demonstrated that both the medicinal plant ATR and TFG have antihyperlipidemic property in the HCD induced hyperlipidemia in albino rabbits. However, comparative study illustrated that ATR was more effective than TFG.

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