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HYPOLIPIDEMIC ACTIVITY OF *LUFFA AEGIPTIACA* FRUITS IN CHOLESTEROL FED HYPERCHOLESTEROLEMIC RABBITS

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ABSTRACT:

The methanolic extract of *Luffa aegiptiaca* fruits were investigated for their possible hypolipidemic effect on hypercholesterolemia induced Newzealand white rabbits by feeding the animals with normal diet supplemented with 1% cholesterol and 10% ground nut oil for 8 weeks. Rabbits with normal diet and hypercholesterolemic diets through out the experiment were used as negative and positive control respectively. There was a significant increase in the weight of hypercholesterolemic rabbits when compared to normal control. Methanolic extract of *Luffa aegiptiaca* fruits significantly reduced serum total cholesterol of hypercholesterolemic rabbits by 29%, Triglycerides by 52%, LDL Cholesterol by 22% and it also increased serum HDL by 38%. This observation demonstrated that *Luffa aegiptiaca* fruits have strong hypolipedemic effect which compared with improved HDL / LDL ratio is an indication of the possible use of this fruits in the treatment of the diseases associated with hyperlipidemia such as Ishcaemic heart diseases and arteriosclerosis.

Key words: Hypolipidaemic, Luffa aegiptiaca fruits and hyper cholesterolemic Rabbit.

1. NTRODUCTION

Hyperlipidemia, is a major risk factor in the initiation and progression of atherosclerotic lesions, conditions such as coronary heart disease, ischemic cerebrovascular disease and peripheral vascular disease. This leads to high mortality and morbidity rate in developed countries. This is mainly due to altered lipoprotein metabolism. Hyperlipidemia also has an indirect role by stimulating the production of oxygen free radicals from polymorphonuclear leukocytes and monocytes .It is considered as one of the five leading causes of the death in the world.[1, 2] Standard treatment of dyslipidemia with statins and with the other available agents having adverse effects.[3] No currently available therapy is directed towards prevention of endogenous oxidation of LDL-Cholesterol which is widely considered as an essential modification in the pathogenesis of atherosclerosis. Thus, there is a need for development of newer pharmacological agents which are more efficient in lowering LDL-Cholesterol and/or preventing formation ox-LDL which are devoid of side effects.[4] Nowadays there is an increasing interest toward the potential health benefits of medicinal plants. Many indigenous Indian

medicinal plants have been found to be useful to manage the hyperlipidemia. Such as *Allium Sativum* (Garlic) [5], *Curcuma longa* (Termeric)[6], *Glycyrrhiza glabra* [7]. Apple [8]. *Aloe barbadensis* (Aloe vera) [9], *Ocimum sanctum*,(Tulasi) [10].

Luffa aegiptiaca Mill fruts commonly known as Luffa, is a climbing herb from the botanical family Cucurbitaceae . The main uses of Luffa are Dyslipidemic, Anti-Diabetic, Hepatoprotective, Anti-Hypertensive and Diuretic. . As a food habit of British people Luffa aegiptiaca Mill fruits are extensively using along with egg preparations, which is possibly reduces the cholesterol from the egg. *Luffa aegyptiaca* tender fruit is taken as vegetable, the course sponge of mature fruit is used as a bath scrub and juice of leaves cures conjunctivitis. *Luffa aegiptiaca* fruts has over 100 different chemical components, including mucilage, reducing sugars, resins, alkaloids, organic acids, tannins, saponins, and proteins. It also contains Mono unsatuarated fatty acids, satuarated fatty acids, fiber, flavonoids, Niacin and Ascorbic acid which helps to reduce hypercholesterolemia, [11&12]. In this study the prolonged effect (8 weeks) of the methanolic effect of *Luffa aegiptiaca* fruits in total cholesterol (TC), Low density lipoprotein cholesterol (LDL), High density lipoprotein cholesterol (HDL) and Triglycerides are studied in high cholesterol fed hypercholesterolemic rabbits. Hence on the above facts no study has been carried out on methonolic extract of *Luffa aegiptiaca* fruit in high cholesterol fed hypercholesterolemic rabbits. Thus the present study is an attempt to test the hypolipidemic activity of *Luffa aegiptiaca* fruit.

2. MATERIALS AND METHODS

2.1. Plant material.

Fruits of *Luffa aegyptiaca* used for the investigation was obtained from the local market of Chidambaram, Tamilnadu, India. The fruits was identified and authenticated by department of Botany,Annamali University, Tamilnadu, India.

2.2. Alcoholic extraction.

The authenticated fruits were cleaned, shadow dried and subjected to pulverization to get coarse powder. The coarsely powdered (1kg) fruit of Luffa aegyptiaca was used for the methanolic extraction in Soxhlet apparatus. The extract was evaporated to dryness under vacuum and dried in vacuum desiccators (15.5%w/w). The residue stored in a refrigerator at $2-8^0$ C for use in the subsequent work.

2.3. Animals.

24 male, age matched New Zealand white rabbits weighing (1-1.5Kg) were procured form central animal house, Annamalai nagar, Annamalai University, Tamil nadu, India. Rabbits were acclimatized for a period of 14 days in their cages under standard environmental conditions of temperature, relative humidity and dark / light cycle. The rabbits were kept on standard pellet diet and water *ad libitum*. The study was implemented by the approval from the Institutional Animal Ethical Committee of Annamali University. Proposal Number 389.

2.4. Acute oral toxicity studies. *Luffa aegiptiaca* fruit extracts at the dose range of 100mg – 2000mg were administered orally to different groups of rats, comprised of ten rats in each group. Mortality was observed after 72 hours .Acute toxicity was determined according to Litchfield and wilcoxon method.

2.5. Experimental design. Four groups of rabbits, six in each received the following treatment schedule, [13].

Group I: Normal diet and water

Group II: Cholesterol enriched diet and water

Group III: Cholesterol enriched diet, water and standard drug (Atorvastatin 3mg/kg Bw/day)

Group IV: Cholesterol enriched diet, water and methanolic extract of *Luffa aegyptiaca* fruits (300mg/kg Bw/day)

After a 2-weeks period of adaptation, the animals were randomly divided into four dietary groups of 6 in each and marked to permit individual identification The group I was fed a standard laboratory rabbit diet (100 g/day).The group II was fed the same amount of standard diet containing 1% cholesterol and 10% ground nut oil (cholesterol enriched diet). The group III was fed with cholesterol enriched diet and the standard drug Atorvastatin 3mg / kg body weight / day by oral catheter and the group IV received the cholesterol enriched diet and methanolic extract of *Luffa aegyptiaca* fruits 300mg/kg Bw/day by oral catheter. To all four groups received water *ad libitum.* Study period was for 8 weeks.

2.6. Induction of hypercholesterolemia in rabbits. Rabbits were made hypercholesterolemia by feeding a high cholesterol fat diet. Deoxycholic acid was mixed thoroughly with powdered standard rabbit diet (100g/day/rabbit). Simultaneously 1% cholesterol was dissolved in10% warmed ground nut oil and this oil solution was added slowly in to powdered mixture to obtain homogeneous soft cake. This cholesterol rich (HFD) preparation was molded in the shape of pellets of about 3g each and fed for 8 weeks by 100g/day/rabbit for the induction of hypercholesterolemia.[14]

2.7. Collection of blood samples and Liver for Lipid profile determination.

Body weights were measured at base line, 4 th week and 8 th week. Over night fasting blood samples were collected in EDTA coated tubes (3mg/ml blood) at base line, on 4th week and 8th week from the marginal ear vein, then anesthetizing with Thiopentone injection (50mg/kg ip) through the marginal ear vein. There after, Animals were sacrificed and liver was excised, immediately washed with cold 0.15M Kcl and kept it at -40 0C till analysis. The collected blood samples were centrifuged for 10 minutes, the serum samples were collected.

2.8. Biochemical analysis of plasma and liver. The concentration of total cholesterol (T C)[15] , High density Lipoprotein Cholesterol (HDL-C)[16] and Triglycerides (T G) [17]**.** were measured by enzymatic colorimetric methods with standard enzymatic kits supplied by Merck India Ltd, Mumbai, India. Serum LDL-C was calculated according to the Friede Wald equation. Liver was homogenized (10%w/v) in cold Phosphate buffer and extracted with CHCl3 and CH3OH (2:1,v/v).This lipid extract was used for the estimation of lipid parameters. Since the TC/HDL-C and LDL-C/HDL-C ratio determine the relative risk of coronary artery disease.

2.9. Statistical analysis.

All the values of body weight and biochemical estimations were expressed as mean ± standard error of mean (S.E.M) and analyzed for ANOVA and post hoc Dunnet's t-test. Difference between groups were discussed significant at $P<0.01$ levels

Groups	0th week	4th week	8th week
I. Normal	1000 ± 7.3	1023 ± 10.00	1042 ± 6.85
II. Cholesterol group	1100 ± 12.6	1185± 5.7	1376± 4.5
III. *Luffa aegyptiaca* fruits group	1077 ± 5.4	*1111 ± 7.05	*1162 ± 2.24
IV. Atorvastatin group	1050± 8.8	*1078 ± 3.5	*1098 ± 3.4

Table: 1Effect of *Luffa aegyptiaca* fruits on body weight in cholesterol induced hypercholesterolemic rabbit

Values are given as mean ± S E M for groups of six animals each * P<0.01(Dunnet t – test). Hypercholesterolemic group was compared with the normal group and the extract treated group were compared with the Hypercholesterolemic group.

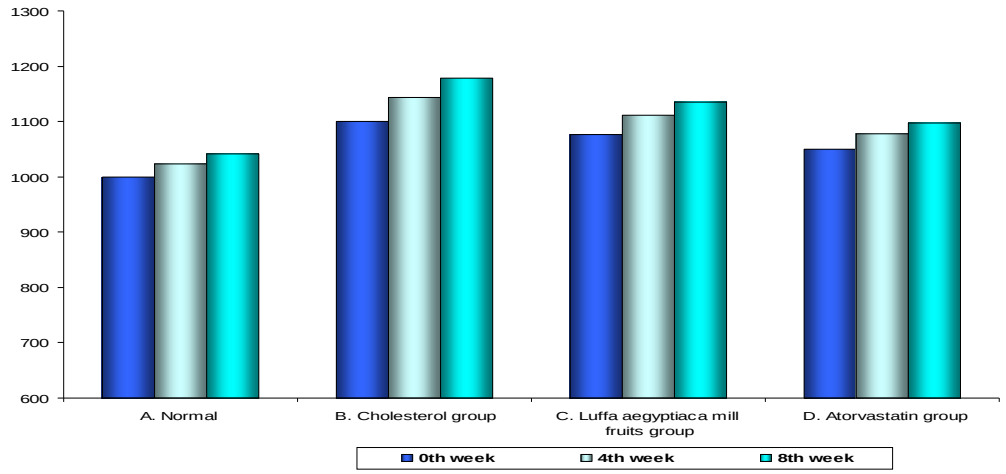
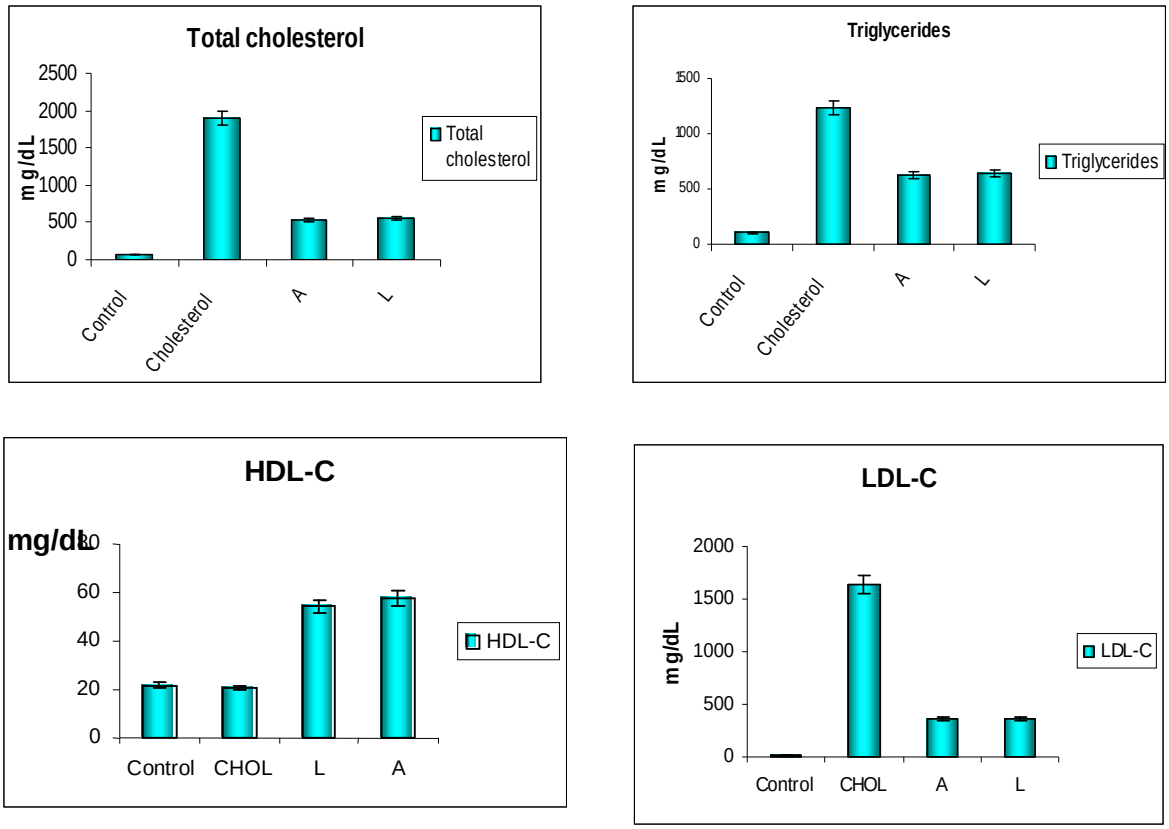


Figure 1. Effect of *Luffa aegyptiaca* fruits on body weight in cholesterol induced hypercholesterolemic rabbits

Group	Total cholesterol (mg/dl ± SEM)	TG (mg/dl± SEM)	HDL-C (mg/dl ± SEM)	LDL-C (mg/dl ± SEM)
A) Control	64. 83 ± 4.67	103.08 ± 2.45	21.89 ± 2.77	22.33 ± 2.8
B) Cholesterol	1905.83 ± 95.7	1240.17 ± 65	20.71 ± 1.31	1637.83 ± 92.8
C) Atorvastatin	*537.78 ± 13.3	*630.13 ± 23.5	*57.64 ± 1.64	*357.39 ± 10.46
D) Luffa aegyptiaca mill fruits group	*559.53 ± 20.48	*648.43 ± 42.36	*51.37 ± 1.97	*369.61 ± 12.75

Table :2 Effect of *Luffa aegyptiaca* fruits on serum lipid profile in cholesterol induced hypercholesterolemic rabbits after 8th weeks

Values are given as mean ± S E M for groups of six animals each * P<0.01 (Dunnet t – test). Hypercholesterolemic group was compared with the normal group and the extract treated group were compared with the Hypercholesterolemic group.



Group-I. Control, Group-II cholesterol, Grou-III- A- Atorvastatin 3mg/kg Bw/day), Group-IV-L-*Luffa aegyptiaca* fruits300mg/kg Bw/day).

Figure: 2 Effect of *Luffa aegyptiaca* fruits on serum lipid profile in cholesterol induced hypercholesterolemic rabbit after 8th weeks.

Group	Total cholesterol (mg/dl ± SEM)	TG (mg/dl± SEM)	HDL-C (mg/dl ± SEM)	LDL-C (mg/dl ± SEM)
A) Control	9. 03 ± 0.67	8.32 ± 0.45	3.02 ± 0.18	3.35 ± 0.08
B) Cholesterol	22.35 ± 1.62	19.64 ± 1.11	2.32 ± 0.31	3.93 ± .02
C) Atorvastatin	*13.25 ± 1.35	*13.91± 1.4	*2.81 ± 0.12	*3.54± 0.13
D) Luffa aegyptiaca mill fruits group	*14.36 ± 1.62	*14.70 ± 1.06	*2.57 ± 0.17	*3.74 ± .02

Table: 3 Effect of *Luffa aegyptiaca* fruits on liver lipid profile in cholesterol induced hypercholesterolemic rabbits after 8th weeks.

Values are given as mean ± S E M for groups of six animals each * P<0.01(Dunnet t – test). Hypercholesterolemic group was compared with the normal group and the extract treated group were compared with the Hypercholesterolemic group.

3. RESULTS

3.1. Serum lipid profile.

The sequential changes in serum TC, TG, LDL-C, HDL-C, Were summarized **Table 2** and in **Figure 2**. High cholesterol diet significantly increased the level of serum TC, LDL-C and TG with less concentration of HDL-C compared to baseline ($p< 0.01$). There was no statistically significant difference in serum lipid profile between groups at baseline. After 8 weeks of treatment with *Luffa aegyptiaca* fruits the concentrations of serum TC, LDL-C and TG were significantly lower, with an increased HDL-C concentration in treatment group as compared to control group, by 29%, 22%, 32%, and 38% respectively.

3.2. Liver lipid profile.

The sequential changes in liver TC, TG, LDL-C and HDL-C, Were summarized in **Table 3**. High cholesterol diet significantly increased the level of liver TC, LDL-C and TG with less concentration of HDL-C compared to baseline. There was no statistically significant difference in liver lipid profile between groups at baseline. After 8 weeks of treatment with *Luffa aegyptiaca* fruits the concentrations of TC, LDL.C and TG were significantly lower, with an increased HDL-C concentration in treatment group as compared to control group.

3. 3. Body weight.

The body weights were summarized in **Table 1** and Figure **1**. Group II (high Cholesterol fed group animals) were increased their weight from 0^{th} to 8^{th} week significantly when compared to the other groups of animals. There was no statistically significant difference in body weight between groups at baseline. The body weight of group III and IV (animals treated with Atorvastatin and *Luffa aegyptiaca*) significantly less when compared to that of group II hypercholesterolemic group.

4. DISCUSSION

In light of the results, Methanolic extract of *Luffa aegyptiaca fruits* on high fat fed hypercholesterolemic rabbits exhibited significant hypolipidemic activity as indicated by the parameters like body weight , serum and liver lipid profiles. This activity is attributed to the number of chemical ingredients present in *Luffa aegyptiaca* fruit such as Ascorbic acid, Niacin, Fiber, MUFAs(Linoleic acid, Oleanolic acid, Oleic acid) and stearic acid . This suggested that *Luffa aegyptiaca mill* fruits act through multiple mechanisms. Niacin has been proven to reverse atherosclerosis by reducing total cholesterol, triglyceride, very-low-density lipoprotein (VLDL), and low-density lipoprotein (LDL) [18&19], and increasing high-density lipoprotein (HDL) by blocks the breakdown of fats in adipose tissue, it causes a decrease in free fatty acids in the blood and, as a consequence, decreased secretion of VLDL and cholesterol by the liver. By lowering VLDL levels, niacin also *increases* the level of high-density lipoprotein (HDL) or "good" cholesterol in blood, and therefore it is sometimes prescribed for patients with low HDL, who are also at high risk of a heart attack**.** [20]*Luffa aegyptiaca* fruits containing Fiber. Various soluble fibers reduce total and LDL cholesterol**.** [21&22]

Ascorbic acid is an anti-oxidant, since it protects the body against stress. Prevention of endogenous oxidation of Cholesterol will reduce the concentration of LDL-C and their by act as hypolipidemic agent.[23&24] The content of Stearic acid in *Luffa aegyptiaca* fruits also play an important rule in reducing serum lipoprotein lipids, apolipoproteins, lipoprotein(a), and lipid transfer proteins in healthy subjects.[25] MUFAs (monounsaturated fatty acids)like Linoleic acid, Oleanolic acid and Oleic acid reduces low-density lipoprotein (LDL) cholesterol,

while possibly increasing high-density lipoprotein (HDL) cholesterol. However, their true ability to raise HDL is still in debate. In children, consumption of monounsaturated oils is associated with healthier serum lipid profiles. [26&27] Flavonoids in *Luffa aegyptiaca* fruits may act by making liver cells more efficient to remove LDL-C from blood. To do this, flavonoids increase LDL receptor densities in liver and by binding to apolipoprotein. The above evidence strongly support that the *Luffa aegyptiaca* fruits act as hypolipidemic through multiple mechanisms.

High fat fed rabbit is an ideal model to study the hypolipidemic agents.

5. CONCLUSION

The methanolic extract of *Luffa aegyptiaca* fruits at a dose of 300mg/kg exhibited significant hypolipidemic activity in high fat fed hypercholesterolemic rabbits. This showed by the reduction of serum and liver TC, LDL-C and TG, with an increased HDL-C concentration in treatment group. Apart from the suggested actions listed in discussion part, absence of acute toxicity may also offer a new hope to the treatment of hyperlipidemea in future.

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