ANTI-HYPERLIPIDEMIC ACTIVITY OF *Hibiscus sabdariffa* FLOWER BUDS IN TRITON-X100 INDUCED HYPERLIPIDEMIA IN RATS

Kasapogu Ashok Kumar[1] and B.Naga Sudha [2*]

1)Department of Pharmacology,

2)Department of Pharmaceutical Chemistry.

Creative Educational society's College of Pharmacology, JNTU, Ananthapur, Kurnool- 518218, A.P, India.

Address of corresponding Author
B.Naga sudha,
Department of Pharmaceutical chemistry,
CES College of Pharmacy,
Kurnool- 518218, A.P, India
Email.Id: drbnsudha@gmail.com

Mobile no:8247679047

ABSRACT

The purpose :
The current study was planned to assess Anti-hyperlipidemic action of *Hibiscus sabdariffa* ehanolic flower buds extract in Triton X 100 induced Hyperlipidemia in Rats.
Mehod: Here Triton X-100 (100 mg/kg,i.p)induced Hyperlipidemia model is used.In this model the animals were divided into 5groups, in which 6 rats were used in each group. First group considered as , normal, 2nd control, 3rd as standard, 4th and 5th are considered as tested groups.In this method, Triton x-100 (100mg/kg)is induced to 4 groups except normal group. Rats in 4th and 5th groups were treated with an ethanolic flower bud extract of *Hibiscus sabdariffa* (EEHS) 200 and 400(mg/kg/p.o) for 7 days. On 8th day blood was collected by retro orbital sinus puncture,under mild ether anaesthesia.The collected blood samples were centrifuged for 10mins at 2000 rpm and serum samples collected were used for various lipid profile tests.

Results:
It is found that treatment with ethanolic flower buds extract of *Hibisus sabdariffa* (EEHS)essentially diminished the hyperlipidemia i.e diminished degrees of serum Total Cholesterol, Triglycerides, Low Density Lipoprotein Cholesterol (LDL-C), Very Low Density Lipoprotein Cholesterol (VLDL-C) , and increment of serum High Density Lipoprotein Cholesterol (HDL-C) when contrasted with vehicle control and standard medication Atorvastatin (10 mg/kg).

Conclusion
The outcomes exhibited that ethanolic extract of *Hibiscus sabdariffa* buds had critical antihyperlipidemic action.

Key words: Triton X 100, hyperlipidemia, *Hibiscus sabdariffa*, Atrovastatin

INTRODUCTION

Cardiovascular infections are one of the leading causes of death in both developed and emerging countries. Impaired lipid digestion due to oxidative stress is a major risk factor for the development and spread of these diseases[1]. Coronary artery disease, stroke, atherosclerosis, and hyperlipidemia are the leading causes of death[2]. .Hyperlipidemia is manifested by elevated serum absolute cholesterol and decreased levels of low, low and high lipoproteins. Lipid problems associated with hyperlipidemia are believed to be the cause of atherosclerotic cardiovascular disease[3]. Hyperlipidemia is a condition in which there are abnormally high levels of lipids, such as fatty substances, in the blood. This condition is likewise calledhypercholesteromia/hyperlipop-proteinemia.Lipids are fats in the circulation system, generally isolated into cholesterol and triglycerides. Cholesterol courses in the circulatory system and is engaged with the structure and capacity of cells. Triglycerides (TG) are best seen as vitality that is either utilized promptly or put away in fat cells.TG are fabricated in the liver from the nourishments or by being retained from the intestine[2]. .It has been demonstrated that raised plasma levels of cholesterol and of LDL are liable for atherosclerosis in man, and epidemiological information recommends that raised plasma levels of HDL have a defensive effect[3]. Numerous effectual lipid-bringing down engineered drugs exist, none is compelling for all lipoprotein issue, and every single such operator are related with some antagonistic impacts. Along these lines it is a need of the day to look through different materials from normal sources that are less poisonous, more affordable, which can give better wellbeing and adequacy on a long haul use. Natural products from plants are a rich source utilized for quite a long time to cure different diseases.

MATERIALS AND METHODS

Collection of plant material

The floral buds of *Hibiscus sabdariffa* was collected from the forms present in Lakshmipuram, Kurnool district, Andhra Pradesh in the month of April and was identified and authenticated.

Preparation of plant extracts

The ethanolic extract of *Hibiscus sabdariffa* floral buds was prepared by using ethanol, by maceration method *Hibiscus sabdariffa* floral buds dissolved in ethanol and place it on heating manrtel for 30 min with stirring at 10 min interval. Filter the solution by using muslin cloth and filterate can be collected.Pour it into china dish or petri plate and allow it to drying.

Preliminary phytochemical analysis [4]

The ethanol extract of *Hibiscus sabdariffa*was subjected to preliminary phytochemical analysis to assess the presence of various phytoconstituents; it revealed the presence of glycosides, flavanoids and tannins.

Acute toxicity test

The acute toxicity tests were performed according to the Organization of Economic Cooperation and Development 423 guidelines [5] .

Antihyperlipidemic studies

Induction of Hyperlipidemia [6-7]

Hyperlipidemia was induced in Wistar albino rats by single dose intraperitoneal infusion of Triton-X (100 mg/kg) in physiological saline solution after overnight fasting for 18 hrs [8]. Animals were divided into five groups of six rats in each

Group 1: Normal group given standard pellet diet, water orally administered for 7 days

Group 2: Triton control group given a single dose of triton at a dose of 100mg/kg, i.p.After 72 hours of triton infusion, received a daily dose (p.o) for 7 days.

Group 3: Single dose of Triton (100 mg/kg, i.p).After 72 hours of triton infusion, received a daily dose of Atrovastatin 10 mg/kg, p.o. for 7 days.

Group 4: Single dose of Triton (200 mg/kg, i.p) after 72 hours of triton infusion, received *Hibiscus sabdariffa* 200mg/kg for 7 days.

Group 5: Single dose of Triton (400 mg/kg, i.p) after 72 hours of triton infusion, received *Hibiscus sabdariffa* 400mg/kg for 7 days.

Collection of blood

On the 8th day, blood was collected by retro orbital sinus, under slight ether anesthesia. The collected blood samples were centrifuged for 10 min, and afterward serum samples were collected and utilized for different biochemical parameters investigation.

Biochemical investigation

The serum was measured for TC [9], TG [10-,11], HDL [12-13], LDL, VLDL by using standard AGAPPE kits.

The part of LDL-C in the serum was determined by utilizing Friedewald'ss equation as follows [14]

LDL-C = Total cholesterol − (HDL-C + VLDL-C)

VLDL-C = Triglyceride/5

Atherogenic index: TC/HDL [15]

Statistical analysis

Results were introduced as mean ± standard error of the mean. The significance of difference among the groups was surveyed utilizing one-way analysis of variance followed by Dunnett's test.

RESULTS AND DISCUSSION:

Table: 1 Phytochemical screening analysis:

S.NO	PHYTOCHEMICAL TEST	RESULTS
1.	Flavanoids	Positive
2	Alkaloids	Negative
3	Tannins	Positive
4	Saponins	Positive
5	Antracene glycosides	Positive
6	Phenols	Positive

Acute toxicity studies:

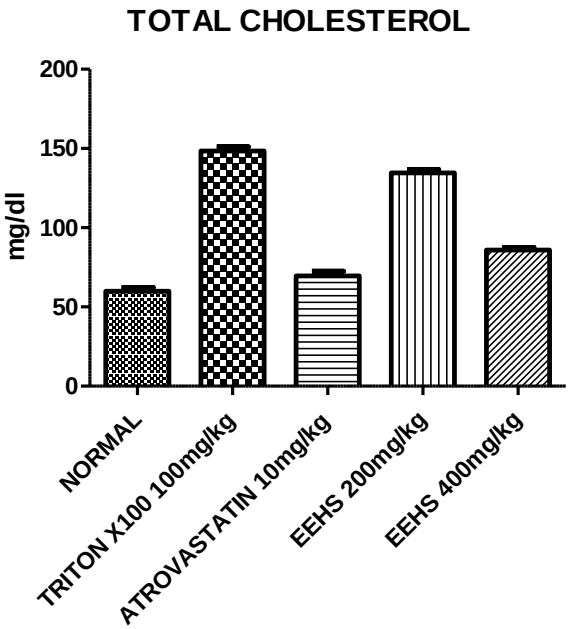
The extract of plant *Hibiscus sabdariffa* was found to be safe up to 2000mg/kg body wt. by oral route. After 72 hr animals were found well tolerated. There was no mortality and signs of toxicity observed. So two dose levels i.e. 200mg/kg, and 400mg/kg body weight were chosen for the present study.(IAEC/CESCOP/AUG-2016-05).

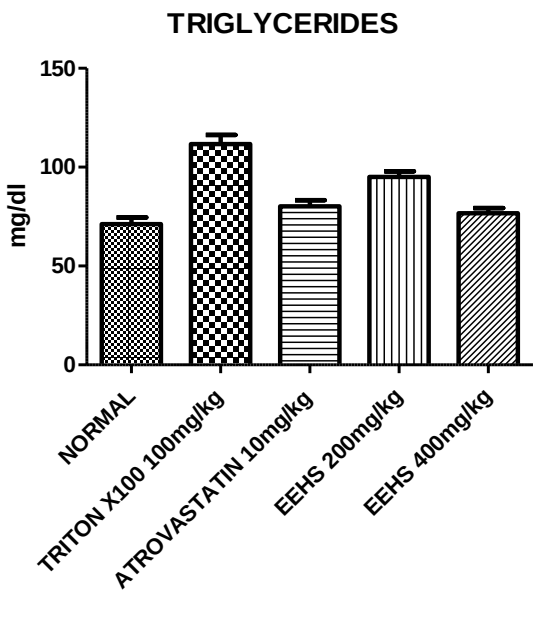
GROUPS	TOTAL CHOLESTEROL (mg/dL)	TRIGLYCERIDES (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)	A.I
Group –I (Normal)	59.92±2.262***	71.17 ± 3.336***	50.17±3.036***	24.1±3.530***	14.22 ±0.93***	1.19 ±0.032***
Group –II (Triton X-100 100 mg/kg i.p)	148.4±2.712***	111.7 ± 4.594***	26.00±3.090***	144.71±3.610***	22.354 ± 1.56***	5.70 ± 0.353***
Group –III (Atorvastatin-10mg/kg P.O)	69.5 ±3.097***	80.17 ± 3.146***	48.00±2.929***	37. ±4.32***	16.021 ±1.48 ***	1.44 ± 0.1546***
Group –IV (200mg/kg of EEHS P.O)	134.5 ±2.277**	95.00 ± 2.989**	41.25±3.029**	111.7±1.89**	18.011 ± 0.68**	3.43 ± 0.382**
Group –V (400mg/kg of EEHS P.O)	85.83±1.768***	76.67 ± 2.624***	45.00±2.580***	59.5±2.63***	15.32 ± 0.62 ***	2.08 ± 0.05***

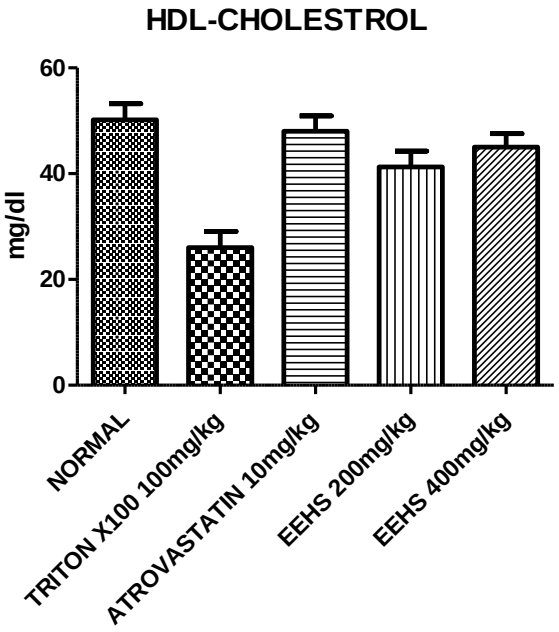
Table: 2 Effect of *EEHS* on biochemical estimation against Triton-X induced hyperlipidemia in rats

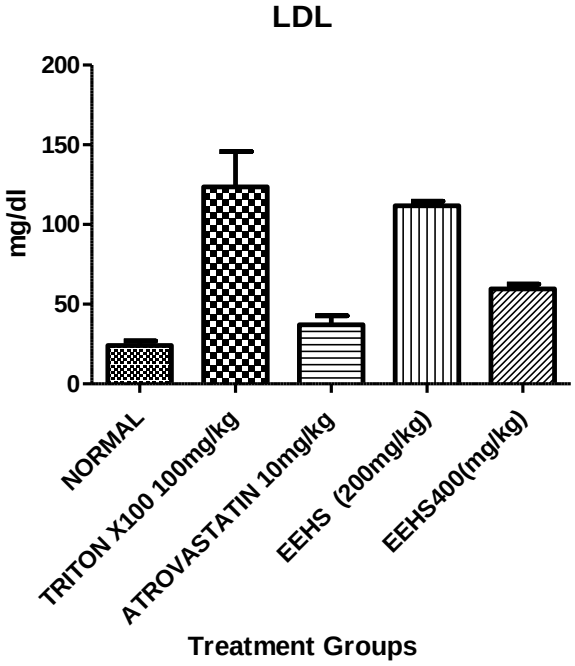
The values expressed as mean ± SEM, where n=6, All the data were analyzed by using one way ANOVA followed by dunnet's test. ***P<0.001, **P<0.01 as compared with control, ###P<0.001, #P<0.05 as compared with the triton-x group.

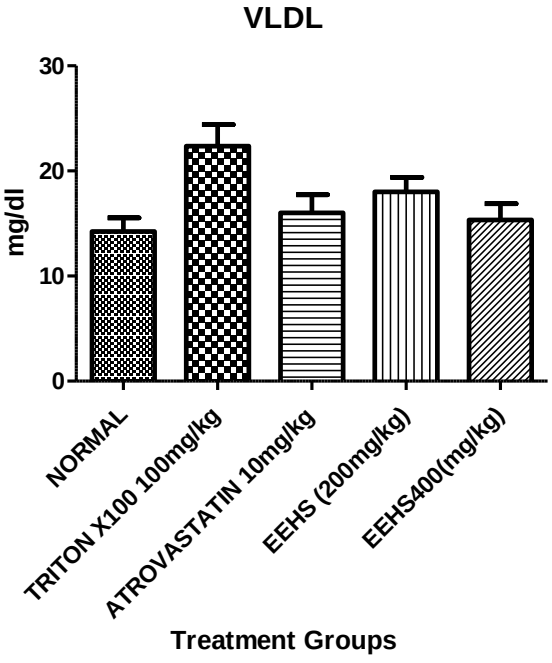
Graphs: Effect of *EEHS* on biochemical estimation against Triton-X induced hyperlipidemia in rats

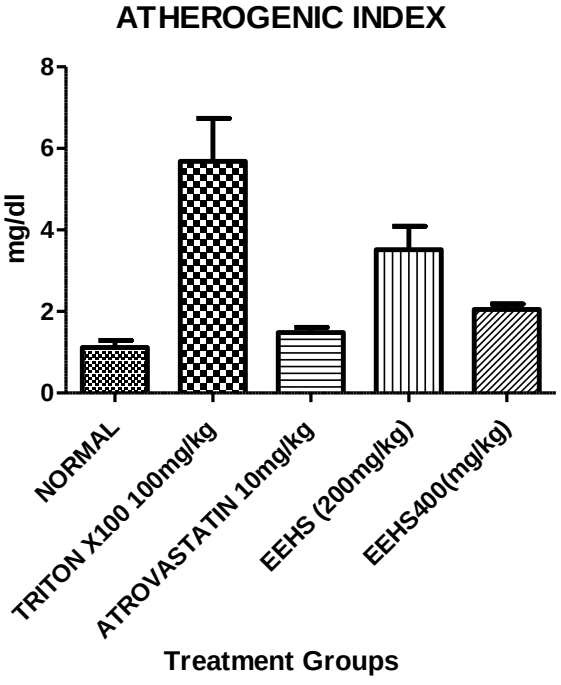





HDL-CHOLESTROL
mg/dl
60
40
20
0
NORMAL
TRITON X100 100mg/kg
ATROVASTATIN 10mg/kg
EEHS 200mg/kg
EEHS 400mg/kg


LDL
mg/dl
200
150
100
50
0
NORMAL
TRITON X100 100mg/kg
ATROVASTATIN 10mg/kg
EEHS (200mg/kg)
EEHS400(mg/kg)
Treatment Groups


VLDL
mg/dl
30
20
10
0
NORMAL
TRITON X100 100mg/kg
ATROVASTATIN 10mg/kg
EEHS (200mg/kg)
EEHS400(mg/kg)
Treatment Groups


ATHEROGENIC INDEX
mg/dl
8
6
4
2
0
NORMAL
TRITON X100 100mg/kg
ATROVASTATIN 10mg/kg
EEHS (200mg/kg)
EEHS400(mg/kg)
Treatment Groups

DISCUSSION

A major cause of cardiovascular disease is a disorder of lipid metabolism. Many classes of lipid-lowering drugs are used for treatment, but none of the available drugs are completely effective, completely safe, or free of side effects. Efforts are therefore being made to find safe and reliable specialists who may help improve lipid digestion and prevent cardiovascular infections. Traditional medical practitioners have used this plant to treat hyperlipidemia, so it has been considered advantageous to study cases of interim onset hyperlipidemia. Triton release by the liver was accompanied by a marked reduction in VLDL and LDL catabolism [17] . In this study, his EEHS was chosen to measure the antihyperlipidemic behavior of the therapeutically used triton atorvastatin drug. EEHS (Hibiscus Sabdariffa Ethanol Extract) clearly shows that the 200 and 400 mg/kg doses reduced overall TG and cholesterol levels. The reduction of TCs by EEHS extracts was associated with a reduction of the LDL division, a target of some lipid-lowering drugs. This result suggests that the cholesterol-lowering effects of natural concentrates may be the result of rapid catabolism of LDL-C by liver receptors, ultimately to bile acids. It is generally accepted that decreased plasma HDL is a risk factor for the development of atherosclerosis. HDL facilitates the movement of cholesterol from peripheral tissues such as the vascular septum to the liver for catabolism. Increased HDL may interfere with surgery for atherosclerosis. Increased HDL (cardioprotective lipid) levels may be due to increased movement of lecithin-cholesterol acetyltransferase. Lecithin-cholesterol acetyltransferase plays a key role in binding free cholesterol to HDL and returning it to VLDL, a medium-thickness lipoprotein recovered from the heart. liver cells. The increase in HDL cholesterol and the decrease in cholesterol associated with its LDL portion in the results may be due to increased cholesterol excretion and decreased cholesterol retention in the gastrointestinal tract. Several studies have shown that increased HDL-C is associated with decreased coronary artery risk. Elevated TC and LDL-C levels are important coronary risk factors. Saponins are known to have lytic activity on erythrocyte membranes, and this property has been exploited for saponin localization. The hemolytic activity of saponins is thought to be a result of the aglycon moiety's preference for layered sterols, particularly cholesterol, to form insoluble complexes[17]. This result clearly suggests that this lipid-lowering movement of the regenerating plant may be due to the proximity of key saponins in the concentrate. EEHS (200 and 400 mg/kg) and Triton antihyperlipidemic activity were part of

the subgroup treated with contrast agent and standard atorvastatin. However, further studies are needed to gain further insight into possible mechanisms.

CONCLUSION

This study aimed to investigate the antihyperlipidemic effect of Hibiscus sabdariffa extract in Triton X-100-induced hyperlipidemic rats. Administration of Triton-X-100 (100 mg/kg) to rats increased total cholesterol, total triglycerides, VLDL and LDL, and decreased HDL levels. Hibiscus sabdariffa was administered to rats with Triton-induced hyperlipidemia at various doses including 200 and 400 mg/kg daily (orally). Atorvastatin 10 mg/kg was used as a reference standard. Treatment with 400 mg/kg plant extract significantly decreased levels of TC, TG, VLDL and LDL ($p<0.05$). Additionally, the extract was found to cause a significant ($p<0.05$) increase in HDL levels. The arteriosclerosis index was also decreased in a dose-dependent manner. Therefore, it can be concluded that Hibiscus sabdariffa extract 400 mg/kg can effectively suppress Triton-X 100-induced hyperlipidemia in rats, suggesting a potential protective function in coronary artery disease.

ACKNOWLEDGEMENT

This work was carried out by Creative Educational Society's College of Pharmacy, Kurnool, and Andhra Pradesh

Conflicts of Interest

We declare that we have no conflict of interest.

REFERENCES

1.Amit G, Vandana S, et al, Hyperlipidemia: An Updated Review. International Journal of Biopharma Toxicology Research 2011; 1: 81-89.

2..Ankurrohilla, NidhiDagar, et al., Hyperlipidemia- a deadly pathological condition. International Journal of Current Pharmaceutical Research 2012; 4:15-18.

3. Robbins and Cotran: Pathological Basics of disease. Published by Elsevier ,7th ed.2004:158.

4.Kokate CK, Purohit AR, Gokhale SB: Pharmacognosy. Nirali Prakashan, 38th ed. 2006. Pathway to screen phytochemical nature of natural drugs; p. 607.

5.OECD: Guideline, 423, Acute Oral Toxicity: Environmental Health and Safety Monograph Series on Testing and Assessment No. 24. Environmental directorate. 2000.

6.Keshetty V, Pabba S, Gudipati et al., Antihyperlipidemic activity of methanolic extract of garlic (*Allium sativum* L.) in triton X-100 induced hyperlipidemic rats. Journal of Pharmacy Research 2009; 2 :777–80.

7.Sudha S, Karthic R, Naveen, et al., Anti hyperlipidemic activity of *Spirulina platensis* in Triton x-100 induced hyperlipidemic rats. Hygeia: Journal for Drugs and Medicines 2011; 3:32–37.

8.Harnafi, H, Serghini Caid, H, el Houda Bouanani, N, Aziz, M, & Amrani, S. Hypolipemic activity of polyphenol-rich extracts from Ocimum basilicum in Triton WR-1339-induced hyperlipidemic mice. Food Chemistry 2008: 108 :1: 205–212.

9.Allain, C.C, *et al*.; Clin.Chem 20 ,1974, 470 *ADL*.

10. BucoloG,David M. “Clin. Chem.” 1973, 19.476 .

11.Werner M.,GabrielsonD.G.,EastmanG.,Eastman G. “Clin.Chem.” 1981, 27.268.

12.Burstein M, Scholnic H.R, Morfin R. Journal of Lipid Research1970.

13. Burtis, C.A., Ashwood, E.R., Bruns, D.E: Textbook of Clinical Chemistry and Molecular Diagnostics. WB Saunders Comp, 5th edition, 2012.

14. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry1972; 18:499–502.

15. Abbott RD, Wilsonpw-Atherosclerogenic1988:3: 207-11.

16. Otway S, Robinson DS. The effect of a non-ionic detergent (Triton WR 1339) on the removal of triglyceride fatty acids from the blood of the rat. Journal of Physiology 1967; 190:309–19.