

Evaluation of Antihyperlipidemic Effect of Ethanol Extract of *Balanites Aegyptiaca* Fruit in Triton X-100 Induce Hyperlipidemic Rats Animal Models

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Abstract- The major risk factor of cardio vascular disease is related to disturbances in lipids profile result in hyperlipidemia. Our present study is to evaluate antihyperlipidemic effect of ethanol extract of *Balanites aegyptiaca* fruit (EEBAF) in Triton X-100 induce hyperlipidemic rats in animal models. The Triton X-100 solution 100 mg/kg it is used to induced hyperlipidemia by elevating serum triglyceride in animals models. Treatment with ethanol extract of *Balanites aegyptiaca* 200 mg and 400 mg dose significantly decreased in Tc, TG, & LDL-c serum levels and increased in HDL-c serum levels when compared to hyperlipidemic treated rats. The reduction in HDL-c is one major factors of pathogenesis of atherosclerosis. After administration of EEBAF result increased in HDL-c levels. We could conclude that the EEBAF having good cardio-protective agent this effect due Phenolics and Flavonoids present in EEBAF extract.

Index Terms- Hyperlipidemia, Triton X-100, Simvastatin, EEBAF.

I. INTRODUCTION

Hyperlipidemia, or hypercholesterolemia, it is disorders indicate to elevated levels of lipid profiles in the blood³. Thus result in risk of cardio vascular disease consequently lead to atherosclerosis⁴. Some people may not usually experience symptoms but having heart disease and increase the risk of stroke⁵. Two types of lipoproteins, that transport cholesterol to the cells which include Low-density lipoproteins (LDL), it is also termed as bad cholesterol, and high-density lipoproteins (HDL), or good cholesterol. High LDL level leads to a buildup of cholesterol in arteries result in atherosclerosis⁶.

Balanites aegyptiaca it has been used in a variety of folk medicines⁷. Treatment with EEBAF at 400 and 200 mg/kg dose significantly reduced the serum Tc, TG, & LDL-c levels & increased the serum HDL-c levels when compared to hyperlipidemic treated rats (control).

II. MATERIAL & METHODS

a. Plants materials and extraction

The mesocarp fruits of *Balanites aegyptiaca* were purchased from Randhawa Farm-Punjab and authenticated letter was received. The mesocarp fruits of *Balanites aegyptiaca* were

subjected to extraction by maceration using ethanol 95%, the mesocarp fruits (500g) were immersed in the solvent ethanol (1000 ml) for 6 days with frequent shaking⁸. Then it was filtered, and the filtrate was evaporated. The residue obtained was dried and percentage yield was calculated by the formula⁹-

$$\% \text{ Yield} = \frac{\text{Actual Yield}}{\text{theoretical Yield}} \times 100\%$$

Qualitative test ;

Qualitative phytochemical tests for the ethanol extract of *Balanites aegyptiaca* fruit was subjected to qualitative tests for tannins, saponin, flavonoids, terpenoids, phenolic compounds, alkaloids and anthraquinones⁹.

Quantitative test;

Total phenolic content

Total phenolic content (TPC) was determined by Folin-Ciocalteu method¹⁰.

To 10 µl of EEBA, add 50 µl of Folin Ciocalteu reagent and shake for 5 min. To the above mixture, add 150 µl of 20% Na₂CO₃ and mixed properly and made 3 ml with distilled water. After 90 min, the absorbance was measured at 760 nm by using UV spectrophotometer (1600)¹¹.

Gallic acid was used as a standard for calibration. The phenolic content was expressed as milligram of gallic acid equivalent per gram of dry sample (mg GAE/g) using the linear equation based on the calibration curve¹².

Total flavonoids content

One gram of EEBAF was added to 15 ml of ethanol (50%) and extracted for three times by maceration for 2h. Then filtered and make up the volume with ethanol (50%) in volumetric flask up to 100 ml.

One ml aliquot of the sample was taken in a test tube and diluted with 10 ml of distilled water. Add 1.5 ml Folin Ciocalteu's reagent and allowed to incubate at room temperature for 5 min. 4 ml of 20% (w/w) Na₂CO₃ was added, adjusted to 25 ml with distilled water, agitated and left to stand for 30 min at room temperature. The absorbance of the sample was measured at 765 nm against blank¹³

III. CHEMICALS

All the chemicals and reagents used in the study were of analytical grade and molecular biology grade. Triton X-100 was purchased from Sigma Aldrich chemical private limited-Bangalore-India. Reagents for estimation of SGOT, SGPT, lipid profile Purchased from Unitron Bio Medicals, Bangalore- India. Standard drugs Simvastatin were purchased from MedPlus Pharmacy, Bangalore India

IV. ANIMALS & EXPERIMENTAL DESIGN

Male Albino Wistar rats weighing 180g-200g¹⁴ were procured from Adita Biosys Pvt Ltd, Bangalore. Animals were housed in polypropylene cages with paddy husk as bedding material. They were provided with standard pellet rodent diet (Amrut Laboratory animal feed, Sangli) and free access to water. We had taken approval from Institution Animals Ethics Committee, Nargund College of Pharmacy, Bangalore. Has approved the experimental protocol (IAEC/NCP/109/2020). All the procedures were performed in accordance with the Committee for the Purpose of Control and supervision of Experiments on Animals (CPCSEA).¹⁵The biomedical disposal was sent to Maridi Bio Industries Pvt, Ltd, Bangalore .

Anti-hyperlipidemic studies

The rats were divided into five groups of six rats in each group (n=6). From group II to V hyperlipidemia was induced by single dose of Triton X-100 (100 mg/kg intraperitoneal injection) over 18 hours fasting rats¹⁶.

Group- I: Normal control .

Group -II: hyperlipidemic control .

Group -III: standard group (simvastatin10 mg /kg) p.o.

Group -IV: EEBAF (200 mg/ kg) p.o.

Group -V: EEBAF (400 mg / kg) p.o.

After 3 days of hyperlipidemia induced, the rats where treated with EEBAF for 14 days. The high dose of EEBAF where administered

at 7 pm . (200 mg of EEBAF and 10 mg of simvastatin) p.o was administered at morning .

Collection of blood:

After 14 days of treatment over night fasting for 18 hours blood samples were collected in blood collection tubes from all fasted group animals by retro orbital under ether anesthesia. Serum was collected by centrifuging tubes at 4000 rpm for 20 min in cold centrifuge (Remi C-854/6). After blood collection, all the animals were sacrificed by overdose of anesthesia pentobarbitone (100 mg/kg IP). Isolate liver, carotid artery washed with ice-cold saline and kept in 10 % formalin solution and sent to histopathology lab **Dr. Vamshi's, Biological sciences & Research Centre-Bangalore.**

Biochemical parameters analysis:

The serum was stored at -20°C and used further for measured the biochemical parameters such as HDL-cholesterol (HDL-c), Total cholesterol (TC), Triglyceride (TG), Aspartate amino transferase (AST) and Alanine amino transferase (ALT) levels.

Statistical analysis

All the data were subjected to column statistical analysis so as to obtain the Mean \pm S.E.M values for the group. These values were used to assess the treatments are significant using one way analysis of variance (ANOVA) followed by Dennett's test (Graph Pad Prism 9 for Windows, Version 9.1.2(226). P value (P<0.05) and (P<0.01).

V. RESULT:

The mesocarp fruit of EEBAF were found to have percentage yield 34.75%.

Qualitative test

Table Qualitative phytoconstituents of EEBAF.

S.No	Test	State
1	Reducing sugar	+
2	Tannins	-
3	Steroid	+
4	Terpenoids	-
5	Flavonids	+
6	Saponin	+
7	Glycoside	+

(+)indicate present of constituent , (-)indicate absent of constituent.

Quantitative studies (Total Phenolic and Flavonoid content).

Formulations	Total Phenolic Content mg/g	Total Flavonoid Content mg/g
EEBAF	1.04±0.4	0.58±0.1

Effect on lipid profiles

Ethanol extract of *Balanites aegyptiaca* fruit (400 mg/kg) in Triton X-100 induce hyperlipidemia resulted significantly

(P<0.001) decreased in Tc, LDL-c, and TG , and increased in HDL-c levels when compared with hyperlipidemic treated rats.

Table 1; effect of EEBAF on lipid profiles

Groups	Control	Hyperlipidemic control	Standard group	EEBAF 200 mg	EEBAF 400 mg
Tc (mg/dl)	33.74±10.5	92.86±16.6**	35.73±11.5**	46.67±8.6 **	36.30±12***
TG (mg/dl)	46.40±12.4	120.43±16.9*	44.9±34.6**	51.6±12.03 ns	63.37±10.9**
HDL (mg/dl)	28.53±3.5	23.83±9.2**	26.7±10.1**	24.73±4.3**	25.1±4.8***
LDL (mg/dl)	16.9±5.9	22.44±5.9**	12.3±9.3***	13.9±7.6**	12.9±5.9**

All value are expressed as mean ±SEM and n=6 for all group. Simvastatin and *EEBAF* (200 mg & 400 mg/kg) were compared with Triton X-100 treated group. Data was analyzed using one way ANOVA followed by Dunnett’s t test. ns-non significant,(*P<0.0014,***P<0.0040,**P<0.007.P<0.0004)

HDL - High density lipoprotein, LDL- Low density lipoprotein, TG- Triglyceride, Tc – Total cholesterol

Effect of Biological rhythms (Chronopharmacological)

400 mg of *EEBAF* dose resulted more significance compared to 200 mg dose at morning times. *EEBAF* showed significance effect increased antioxidants enzymes when compared to Triton X-100 treated rats.

Liver biomarkers effect:

The dose of (*EEBAF* 400 mg) dose significantly (P<0.05) decreased in SGOT and SGPT levels when compared with Triton X-100 treated rats and mild significantly decreased in Simvastatin treated rats.

Table 2: effect of EEBAF in liver biomarker enzyme

Experimental groups/Treatment	SGOT(U/1)	SGPT (U/1)
Normal control	54.27±8.80	51.09± 8.26
Triton X-100,100mg/kg.b.w,I.P	154.64±22.29**	141.1± 10.5***
Triton X-100 + Simvastatin 10mg/kg, p.o	51.90±2.20 **	101.41 ± 12.7*
Triton X-100 + 200mg/kg,b.w.P.o <i>Balanites aegyptiaca</i> extract	72.30±20.88 *	87.21 ± 16.6 **
Triton X-100 + 400mg/kg.b.w.P.o <i>Balanites aegyptiaca</i> extract	45.94±19.12**	64.43± 12.25 ***

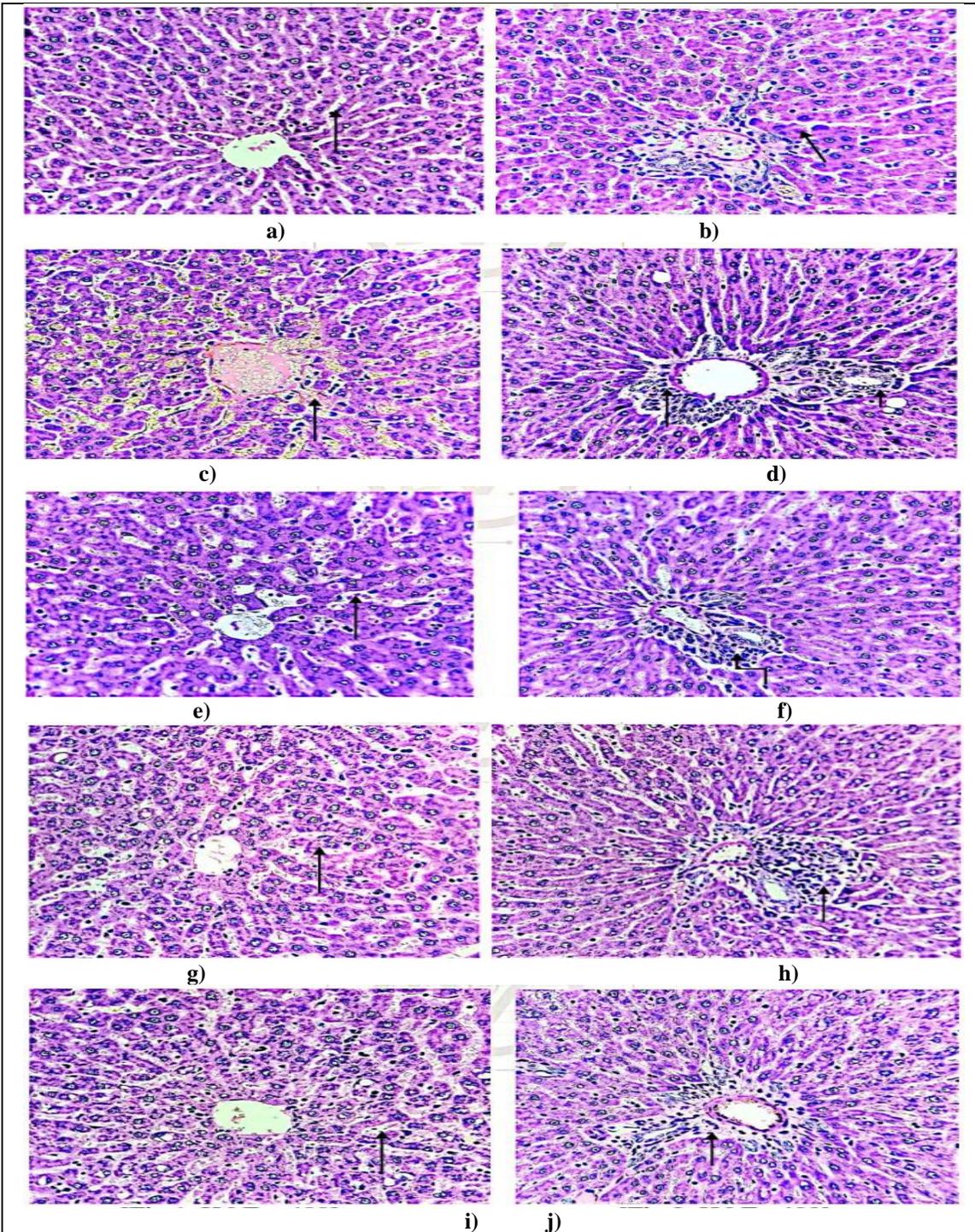
All value are expressed as mean \pm SD and n=6 Data was analyzed using one way ANOVA followed by Dunnett's t test. ns- non significant, *P<0.05, ***P<0.002. **Antioxidants enzyme**

Table 3; effect of EEBAF in antioxidants enzyme

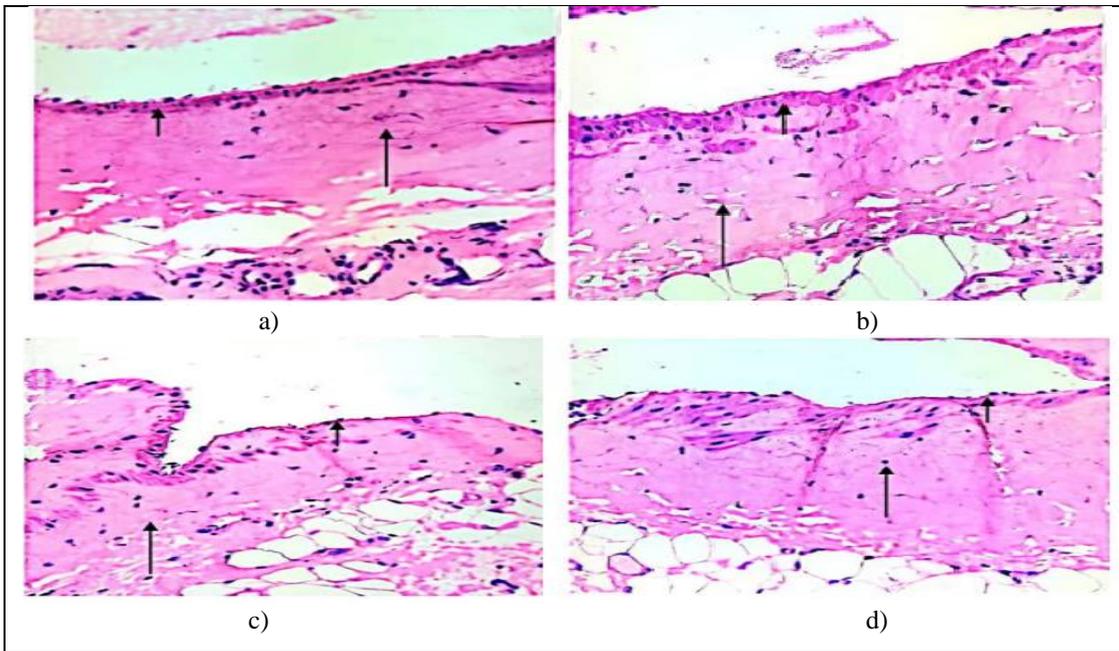
Experimental groups/Treatment	SOD(units/mg of protein)	CAT(units/ mg of protein)	TP (units/ mg of protein)
Normal control	5.1 \pm 1.12	87.67	9.65 \pm 0.66
Triton X-100,100mg/kg.b.w,I,P	2.38.11 \pm 0.4**	31.69 \pm 4.8**	13.43 \pm 3.4*
Triton X-100 + Simvastatin 10mg/kg, p.o	4.1 \pm 2.48 *	83.83.4 \pm 9.4***	8.2 \pm 2.9*
Triton X-100 + 200mg/kg,b.w.P.o Balanites aegyptiaca extract	3.2 \pm 5.5**	61.04 \pm 6.9**	8.1 \pm 2.5*
Triton X-100 + 400mg/kg.b.w.P.o Balanites aegyptiaca extract	4.03 \pm 5.5*	65.62 \pm 4.5***	7.2 \pm 0.7*

All value are expressed as mean \pm SD and n=6 Data was analyzed using one way ANOVA followed by Dunnett's t test. ns-non significant P .value P < 0.05,*P<0.002.

Histopathological result of liver :



Histopathology result of artery:



VI. DISCUSSION

Triton X-100 has been widely used to block the clearance of triglycerides rich lipoproteins to induce hyperlipidemia in experimental animals. The intraperitoneal injection of Triton X-100 (100 mg/kg) results significant increased in plasma cholesterol, TGs and LDL and make hyperlipidemic rats. This may be due to reduction in LDL and VLDL catabolism in liver. The antihyperlipidemic activity of ethanol extract of *Balanites aegyptiaca* fruit (200 and 400 mg/kg) against Triton X-100 shows a significant decrease in TC, HDL, LDL, TG ($P < 0.0014$, $P < 0.0040$, $P < 0.007$, $P < 0.0004$) and significant increase in HDL ($P < 0.001$) in a dose dependent manner comparing with standard simvastatin treated group. However, there is a necessity for further research to work for more insight to the possible mechanisms.

The administration of ethanol extract of *Balanites aegyptiaca* fruit 400 mg/kg at evening 7 pm showed more significantly normalizes the hyperlipidemic conditions by increasing HDL and decreasing triglyceride, total cholesterol, LDL levels and HDL levels when compared to low dose and triton X-100 treated hyperlipidemic rats.

Triton X-100 injected wistar albino rats resulted in increase in SGOT and SGPT levels. The administration of ethanol extract of *Balanites aegyptiaca* fruit 200 and 400 mg/kg, has not shown altered liver biomarkers such as SGOT and SGPT. Therefore, the ethanol extract of *Balanites aegyptiaca* fruit is safe on long term use.

Triton X-100 injected wistar albino rats showed focal necrosis, periportal hepatocytes exhibit moderate chronic inflammation with focal steatosis and central veins and sinusoids were congested. The administration of ethanol extract of *Balanites aegyptiaca* fruit 200 and 400 mg/kg, reverses the structural damages when compared to Triton X-100 treated group. It indicated to reduce the degeneration, inflammation and congestion for high dose.

Triton X-100 injected wistar albino rats showed intact endothelial lining with loss of integrity in the thickness of tunica intima and tunica adventitia. The tunica media appears poorly oriented with loss of elastic fibers. Low dose of ethanol extract of *Balanites aegyptiaca* fruit (200 mg/kg) administered rats showed focal loss of integrity in the thickness of tunica intima and tunica adventitia it indicate that *Balanites aegyptiaca* showed effect in artery wall with reduced in artery thickness. High dose of ethanol extract of *Balanites aegyptiaca* (400 mg/kg) administered rats showed intact endothelial lining with almost intact integrity in the thickness of tunica intima and tunica adventitia, the tunica media appears oriented with intact elastic fibers.

The antihyperlipidemic activity of ethanol extract of *Balanites aegyptiaca* fruit could be due to phenolic and flavonoid present.

VII. CONCLUSION

Based on our results, we concluded that ethanol extract of *Balanites aegyptiaca* fruit possess potent antihyperlipidemic activity and antioxidant activity. It does not have effect on liver markers. Chronotherapy of this reverses lipid profile histological structures more significantly than low dose. Therefore, it has more beneficial in the management of antihyperlipidemia conditions and associated diseases.

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