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Research Article

EVALUATION OF ANTIHYPERLIPIDEMIC ACTIVITY OF LEAVES OF *PORTULACA OLERACEA LINN* AGAINST DEXAMETHASONE INDUCED HYPERLIPIDEMIA IN RATS

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ABSTRACT

Medicinal plants play pivotal role in preventing various disease. Hyper lipidemia is a major contributor to pathogenesis of cardiovascular diseases and diabetes mellitus. *Portulaca oleracea* Linn belonging to the family Portulacaceae. *P oleracea* is an herbaceous weed with many pharmacological activities like anti inflammatory, antibacterial, antiplasmodial activity. Objective: The present study is an attempt to investigate its antihyperlipidemic activity as mentioned in siddha literature.

Methods: Administration of dexamethasone was given (10 mg/kg .S.C) to the adult wister rats for 8 days induces hyperlipidimea characterized by marked increase in serum cholesterol and triglyceride levels along with increase in atherogenic index.

Result: The Ethanolic extract of leaves of *Portulaca oleracea* Linn. (200 and 400 mg/kg) treatment has showed significant inhibition against dexamethasone induced hyperlipidemia in rats by maintaining the serum levels of cholesterol, triglycerides and near to the normal levels.

Keywords: *Portulaca Oleracea linn*, Hyperlipidemia, Dexamethasone, Diabetes mellitus

INTRODUCTION

Atherosclerosis, referred to as a "silent killer" is one of the leading causes of death in the developed countries and is on the rise in developing countries like India[1]. The American heart association has identified the primary risk factor associated with atherosclerosis as elevated levels of cholesterol and triglycerides in the blood. Therefore therapists consider the treatment of hyperlipidema to be one of the major approaches towards decelerating the atherogenic process[2]. Allopathic hypolipidemic drugs are available at large in the market but the side effects and contraindications of these drugs have masked their popularity. Recently herbal hypolipidemics have gained importance to fill the lacunae created by the allopathic drugs[3].

Major pharmaceutical companies are currently conducting extensive research on plant materials gathered from the rain forests and other places for their potential medicinal value[4]. The world health organization (WHO) estimates that 4 billion people, 80 percent of the world population presently use herbal medicine for some aspect of primary health care. Who notes that of 119 plant-derived pharmaceutical medicines, about 74 percent are used in modern medicine in ways that correlated directly with their traditional uses as plant medicines by native cultures *Portulaca oleracea* L. (Portulacaceae) is listed in the World Health Organization as one of the most used medicinal plants and it has been given the term 'Global Panacea'[5].

In folk medicine, it is utilized as an antipyretic, anti-scorbutic, antiseptic, antispasmodic, diuretic, antihelmentic and for treatment of urinary disorders. The aerial parts of the plant are used medicinally for reducing pain and swelling[6]. Recent pharmacological studies have shown muscle relaxant activity, reduction in locomotor activity, increased in the onset time of pentylenetetrazole-induced convulsion, analgesic, anti-inflammatory effects and antioxidant properties. It is reported that extracts of *P. oleracea* has inhibitory effect on lipopolysaccharide (LPS) and interferon-γ (IFN- γ) induced NO production[7]. It was shown that *P. oleracea* is a rich source of omega-3 fatty acids, gallotannins, kaempferol, quercetin, apigenin and glutathione aqueous extract of *P. oleracea* does not have any cytotoxicity or genotoxicity effect and it is safe for daily use[8]. The antihyperlipidemic effects of ethanolic extract of *Portulaca oleracea* Linn (EELPO) against Dexamethasone induced hyperlipidemia in rats have not been reported so far scientifically. Hence the present study has been carried out to evaluate the antihyperlipidemic effect of ethanolic extract of *Portulaca oleracea* Linn (EELPO) against Dexamethasone induced hyperlipidemia in rats.

MATERIALS AND METHODS

Chemicals

Petroleum ether and Ethanol were obtained from Merck India Ltd. Gemfibrozil was purchased from Cadila, Dexamethasone and all other chemicals used in present study were of analytical grade and purchased from Sigma (St Louis, MO).

Plant materials

Portulaca oleracea Linn. Plant leaves were collected in the month of November 2010 from Karripatti, Salem, Tamilnadu, India. The plant was then taxonomically identified and authenticated by the botanist Mr.A.Balasubramanium, consultant, and central siddha research.

Experimental Animals: Wister rats (150-180gm) of either sex approximately the same age, procured from listed suppliers of Venkataswara Enterprises, Bangalore, India were used for the study. They were housed in polypropylene cages and fed with standard rodent pellet diet (Hindustan Lever Limited, Banglore) and water *ad libitum.* The animals were exposed to alternate cycle of 12hrs of darkness and 12hrs of light. Before each test, the animals were fasted for atleast 12hrs and experimental protocols were subjected to the scrutinization of the Institutional Animal Ethic Committee (P.Col./58/2010/IAEC/VMCP) and were cleared by the same. All experiments were performed in the morning according to current guidelines for care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals. The standard oral gastric canula and syringe were used for drug administration in experimental animals.

Methodology

Preparation of crude drug for extraction

The leaves were dried under shade then coarsely powdered with a mechanical grinder. The powder was passed through sieve no. 40 and stored in an airtight container for the extraction.

Preparation of extracts[9, 10]

Petroleum ether extract of Portulaca *oleracea* Linn.

The shade dried leaves of *Portulaca oleracea* Linn**.** (1 kg) was extracted with petroleum ether (60-80^{o}C), up to 72 hours. After completion of extraction, the solvent was removed by distillation. Dark green colors residue was obtained. The residue was concentrated and then stored in desiccators.

Ethanol Extract of *Portulaca oleracea* Linn.

The marc left after petroleum ether extraction was dried and then extracted with Ethanol 95% (75-78^{o}C), up to 72 hours. After completion of extraction, the solvent was removed by distillation. Dark brown colour residue was obtained. The residue was concentrated and then stored in desiccators.

Aqueous Extract of *Portulaca oleracea* Linn.

The marc left after Ethanol extraction was dried and then macerated with distilled water, up to 72 hours. After completion of extraction, it was filtered and the solvent was removed by evaporation to dryness on a water bath. Brown color residue was obtained and it was stored in a desiccators. The percentage yields of the above extract were shown in table no: 1

Phytochemical Evaluation

The extracts were subjected to phytochemical investigation for plant secondary metabolites like alkaloids, tannins, flavanoids, glycosides, saponins, carbohydrates, phenolic compounds, fixed oils, terpinoids and steroids by utilizing standard methods[11, 12, 13].

The phytochemical evaluation showed the presence of carbohydrate, glycoside, alkaloids, tannins, saponins, phenolic compounds, phytosterols and flavonoids.

Table 1: The percentage yields of the *Portulaca oleracea* Linn. Leaves extracts

Plant name	Part used	Method of extraction	Yield in percentage %		
Portulaca oleracea linn	Leaves	Continuous hot Percolation	Petroleum ether extract 3.8	Ethanol extract 5.2	Aqueous extract 16.2

Table 2: Phytochemical investigation of *Portulaca oleracea* Linn. Leaves extracts

Phytochemicals	Tests	Petroleum ether extract	Ethanol extract	Aqueous extract
Carbohydrates	Fehling's test	+	+	+
Phenolics	Ferric chloride test	-	+	+
Terpenes	Cupper acetate test	-	+	-
Alkaloids	Mayer's test	+	+	-
Flavonoids	Alkaline reagent test	-	+	+
Glycosides	Borntrager's test	-	-	-
Steroids	Liebermann test	+	+	-
Saponins	Foam test	+	-	+

Ethanol Extract of *Portulaca oleracea* Linn. Contain the main phytoconstituents like aristolochic acid (Alkaloid), flavonoids, phenolics, terpenes, phytosterols and carbohydrates. Hence it was taken for pharmacological evaluation.

Pharmacological Evaluation

Dexamethasone induced Hyperlipidemia model

Dexamethasone is a synthetic member of the gluco-corticoid class of hormones. It is used to treat inflammation in different parts of the body and autoimmune disorders. It is also given to cancer patients underlying Chemotherapy to correlate the certain side effects of the antitumour activity. If dexamethasone if given orally or by injection over a period of more than a few days, side effects common to gluco-corticoid may occur : these may include stomach upset, increased appetite leading to significant weight gain, immunosuppressant action, psychiatric disturbances, elevated liver enzymes, fatty liver degeneration, Cushing's syndrome, hypertension, glaucoma, cataract, dermatitis and allergic reaction[14].

Gluco- corticoid excess is known to evoke plasma lipid elevation but the pattern of changes appears to vary in several species. Few synthesis of triacyl glycerol in liver is stimulated by the injection of gluco-corticoid in rats and consequently may lead to the accumulation of fatty liver. The stimulation of the triglyceride (TG) could leads to increased secretion of VLDL. Increasing VLDL secretion has been reported when Dexamethasone is injected for several days in rats. Increase in TG level induces imbalance in lipid metabolism leads to hyperlipidemia. Similarly Dexamethasone treatment in newborn rats for 4 days showed widespread increase in serum lipids[15].

Dexamethasone-induced Hyperlipidemia in Rats[16]

Induction of Hyperlipidemia

In order to induce hyperlipidemia by using Dexamethasone, a glucocorticoids excess is known to evoke plasma lipid elevation. Dexamethasone (10mg/kg/day, S.C) was administered to rats for 8 days to induce hyperlipidemia. The animals were divided in to five groups each group contains six rats.

Grouping & Treatment Protocol

Group I: **Normal control**

: No. of animals 6

: Animals were fed with basal diet and water.

: Animal received normal saline.

Group II: **Hyperlipidemic control**

: No. of animals- 6

: Animals were fed with basal diet and water.

: Dexamethasone (10mg/kg/day, S.C) were given to rats for 8 days.

Group III : **Standard group**

: No. of animals 6

: Animals were fed with basal diet and water.

: Rats were treated with gemfibrozil (10mg/kg/day, I.P) along with Dexamethasone treatment.

Group IV: **Test Group I**

: No. of animals 6

: Animals were fed with basal diet and water.

: Rats were treated with *Portulaca oleracea* (200mg/kg/day, P.O) With Dexamethasone treatment

Group V: **Test Group II**

: No. of animals 6

: Animals were fed with basal diet and water.

: Rats were treated with *Portulaca oleracea* (400mg/kg/day, P.O) with dexamethasone treatment.

Procedure

Hyperlipidemia was induced in group II, III, IV, V by subcutaneous injection of dexamethasone (10mg/kg/day, S.C) for 8 days. The rats in normal and hyperlipidemic control group received normal saline while III group received gemfibrozil (10 mg/kg/day, I.P) [Suspend in gum acacia in water] and IV & V group, received extract by oral route in doses of 200mg/kg/day and 400 mg/kg/day respectively throughout the 8 days experiment.

After the experimental period, the overnight fasted experimental rats were sacrificed by decapitation under light ether anesthesia and blood was collected.

Serum from blood was separated and analyzed for biochemical parameters (lipid profiles). The lipid profiles of Dexamethasone induced hyperlipidemia model and the results of antihyperlipidemic effect of extract treated groups of dexamethasone treated groups were shown in table no: 1

Histopathological studies

A portion of liver tissue(liver slices) of normal control, Dexamethasone control and co-treated groups of rats with gemfibrozil and *Portulaca oleracea* (200mg/kg) and *Portulaca oleracea* (400mg/kg) were stored in containers for 12 hours in 10% formalin solution and subjected to histopathological studies by processing with paraffin embedding following standard micro technique. 5 micron section of the livers stained with alum haematoxylin and eosin, were observed microscopically for histopathological changes i.e., normal liver, damaged and recovered liver was studied and compared. The results were shown in fig no: 1.

Statistical evaluation[17, 18]

All the values were expressed as mean ±SEM. The data's were statistically analyzed by one way ANOVA followed by Dunnett's t-test and values $p< 0.05$ was considered to be significant.

RESULT

The present investigation was carried out to evaluate the antihyperlipidemic effect of plant drug EELPO against Dexamethasone induced hyperlipidemia in Wister rats.

When EELPO was evaluated for its antihyperlipidemic activity against Dexamethasone induced hyperlipidemia model, it showed a statistically significant activity in dose of 200mg/kg and 400mg/kg by oral administration.

After 8 days treatment of Dexamethasone, a significant rise in lipid and lipoprotein levels were observed in serum in Dexamethasone induced group, when compared to normal group. The results were depicted in (Table No.3)

Effect of EELPO in Biochemical Parameters in Serum

Effect on Total Cholesterol and total triglycerides

Total cholesterol levels in Dexamethasone induced group have significantly increased compared to normal rats. The values have risen to 117.83 ± 1.687mg/dl compared to normal rat group, in which values lie in the range 65±1.352 mg/dl. This indicates hypercholesteremia. In the treatment group treated with EELPO (200mg/kg) & EELPO (400mg/kg), the values are reduced to 83±2.307 ($p<0.001$) and 79.0±2.387mg/dl ($p<0.01$) respectively. There is a significant reduction in total cholesterol values in EELPO treatment group. On the other hand Gemfibrozil also have significantly reduced serum total cholesterol levels to 71.50±1.352mg/dl ($p<0.001$) (Table no: 3).

The triglyceride levels have reached as 151.83±1.667mg/dl in Dexamethasone induced group compared to normal rats where the values are 63.83±1.777mg/dl. This indicates triglyceridemia. In the group treated with EELPO **(**200mg/kg) and EELPO (400mg/kg) the values are significantly reduced to 78.16±1.687mg/dl ($p<0.01$) and 74.16±1.687mg/dl ($p<0.01$) respectively. In the Gemfibrozil treated group, the values are reduced to 67.33±0.764mg/dl ($p<0.001$) (Table No: 3).

Effect on Phospholipids

Phospholipids are amphatic lipid constituents of a membrane. They play an essential role in the synthesis of plasma lipoproteins. They function in transduction of messages from cell-surface receptors to certain messengers that control cellular processes and as surfactants[19].

Phospholipid levels in Dexamethasone induced group have significantly increased compared to normal rats. The values have risen to 132.1±2.983mg/dl compared to normal rat group, in which values lie in the range 92.73±1.166mg/dl. In the treatment group treated with EELPO (200mg/kg) and EELPO (400mg/kg), the values are reduced to 104.65±1.777 mg/dl ($p<0.01$) and 99.32±1.721mg/dl ($p<0.01$) respectively. There is a significant reduction in phospholipid values in EELPO treatment group. On the other hand Gemfibrozil also have significantly reduced serum phospholipid levels to 95.37±1.515mg/dl ($p<0.001$) (Table No: 3).

Effect on Free fatty acid

Free fatty acid levels in Dexamethasone induced group have significantly increased compared to normal rats. The values have risen to 35.1±0.152mg/dl compared to normal rat group, in values lie in the range 20.37±0.396mg/dl. In the treatment group treated with EELPO (200mg/kg) & EELPO (400mg/kg), the values are reduced to 27.73±0.307 ($p<0.001$) and 26.37±0.258mg/dl ($p<0.001$) respectively. There is a significant reduction in free fatty acid values in EELPO treatment group. On the other hand, Gemfibrozil also have significantly reduced serum free fatty acid levels to 22.62±0.223mg/dl ($p<0.001$) (Table No: 3).

Effect on High density lipoprotein (HDL) cholesterol

HDL-Cholesterol in Dexamethasone induced group have significantly decreased compared to normal rats. The values have reduces to 26.16±0.307mg/dl compared to normal rat group, 38.66±1.687mg/dl. In the group treated with EELPO (200mg/kg) and EELPO (400mg/kg) the values were 24.33±0.3 ($p<0.01$) and 28.33±0.333 mg/dl ($p<0.01$) respectively. In Gemfibrozil treated group, the values were 34.33±0.421 mg/dl ($p<0.001$) (Table No.3)

Effect on Low density lipoprotein (LDL) Cholesterol & Very low density lipoprotein (VLDL) Cholesterol

LDL-Cholesterol in Dexamethasone induced group have significantly increased to 54.33±1.687 mg/dl compared to normal rat group, 13.66±0.333mg/dl. In the group treated with EELPO (200mg/kg) and EELPO (400mg/kg), the values were reduced to 31.5±0.223mg/dl ($p<0.001$) & 27.5±0.223 mg/dl ($p<0.001$) respectively. There is significant reduction in LDL-Cholesterol values in EELPO treatment group. Gemfibrozil has significantly reduced LDL-Cholesterol level to 21.66±0.33 mg/dl ($p<0.001$) (Table No.3).

VLDL-Cholesterol in Dexamethasone induced group have significantly increased to 38.33±1.542 mg/dl compared to normal rat group, 13.16±0.307 mg/dl. In the group treated with EELPO (200mg/kg) and EELPO (400mg/kg), the values are reduced to 29.33±0.333 ($p<0.01$) and 25.33±0.333 mg/dl ($p<0.01$) respectively. There is significant reduction in EELPO treatment group. Gemfibrozil has significantly reduced VLDL-Cholesterol level to 18.16±0.307 mg/dl ($p<0.001$) (Table No.3 & 4).

Effect on Atherogenic Index

Atherogenic index = Total serum Cholesterol total

Total serum HDL- cholesterol

Atherogenic index in Dexamethasone induced hyperlipidemia control is increased to 4.50 compared to normal rat group, 1.65. In the group treated with EELPO (200mg/kg) and EELPO (400mg/kg), the values are significantly reduces to 3.41 and 2.78 respectively. Gemfibrozil has significantly reduced the values to 2.21. (Table No. 4)

Table 3 & 4: Effect of Ethanolic extract of leaves of *Portulaca oleracea* Linn against dexamethasone induced hyperlipidemiain rats

Group No	Treatment/ Dose	Total Cholesterol (mg/dl)	Total Triglyceride (mg/dl)	HDL Cholesterol (mg/dl)	LDL Cholesterol (mg/dl)
1	Normal Control	65±1.352	63.83±1.777	38.66±1.687	13.66±0.333
2	Dexamethasone (10mg/kg) S.C	117.83±1.687	151.83±1.667	26.16±0.307	54.33±1.687
3	Dexamethasone (10mg/kg) S.C+Gemfibrozil (10mg/kg)P.O	71.50±1.352*	67.33±0.764*	34.33±0.421*	21.66±0.33*
4	Dexamethasone + EELPO-I	83.0±2.307*	78.16±1.687*	24.33±0.33*	31.5±0.223
5	Dexamethasone + EELPO-II	79.0±2.387*	74.16±1.687*	28.33±0.333*	27.5±0.223

Group No	Treatment/ Dose	VLDL Cholesterol (mg/dl)	Atherogenic Index	Phospholipids (mg/dl)	Free Fatty Acid(mg/dl)
1	Normal Control	13.16±0.307	1.65	92.73±1.166	20.37±0.396
2	Dexamethasone (10mg/kg) S.C	38.33±1.542	4.50	132.1±2.983	35.1±0.152
3	Dexamethasone (10mg/kg) S.C+Gemfibrozil (10mg/kg)P.O	18.16±0.307**	2.21	95.37±1.515*	22.62±0.223*
4	Dexamethasone + EELPO-I	29.33±0.333**	3.41	104.65±1.777 **	27.73±0.307*
5	Dexamethasone + EELPO-II	25.33±0.333**	2.78	99.32±1.721 **	26.37±0.258*

All the values were represented as mean ± SEM. All the data were statistically analysed by one way ANOVA followed by Dunnet's test and values P< 0.5 were considered to the significant.

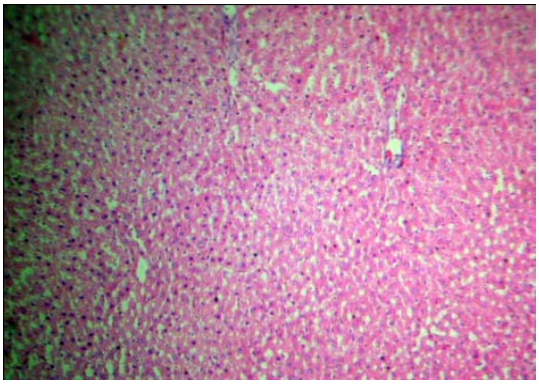
* - P< 0.001; ** - P< 0.01 Vs Control

Histopathology

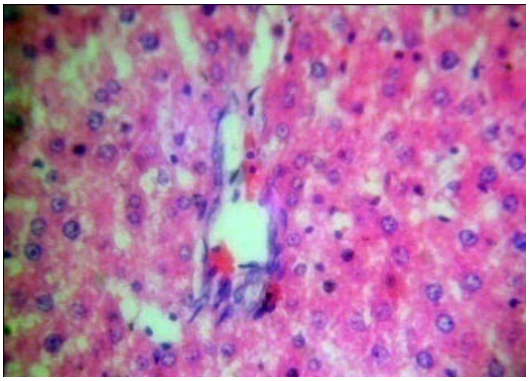
Microscopical examination of liver section of control group [Figure No. 1(a)] showed normal arrangement of hepatocytes with clear broad of central vein at portal layer. Microscopical examination of liver section of dexamethasone treated group (figure No. b) showed various degrees of pathological changes such as centrilobulor fatty degeneration, cloudy swelling and necrosis of hepatic cells.

Microscopical examination of liver section of EELPO (200mg/kg & 400mg/kg) treated group (Figure No. d & e) clearly showed normal hepatic cells and central vein which are comparable with Gemfibrozil treated group (Figure No.c)

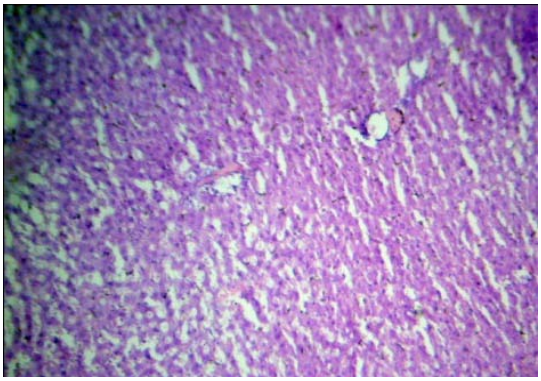
The histopathological study showed recovery of the damaged liver cells in the drug treated group. The reputed cells of the intoxicated liver were reformed. The degree of vascularisation was also reduced as compare to hyperlipidemic group.



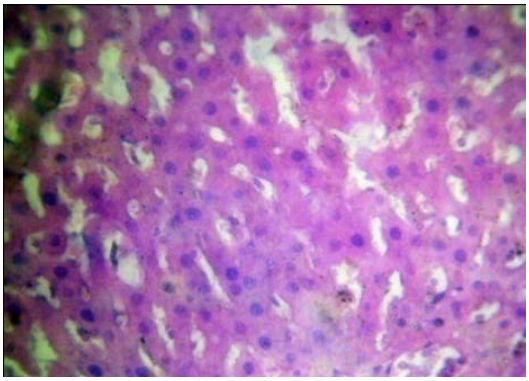
a) NORMAL



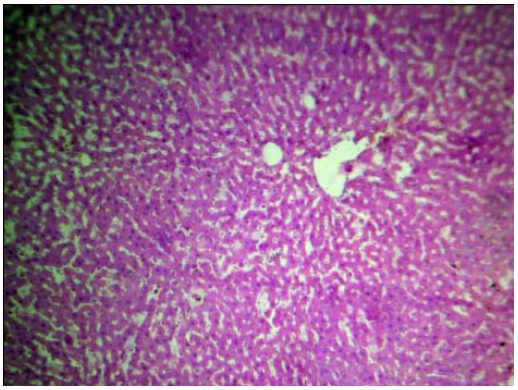
b) DEXAMETHASONE (10mg/kg)



c) DEXAMETHASONE(10mg/kg) + STANDARD DRUG (10mg/kg)



d) DEXAMETHASONE + EELPO (2OOmg/kg)



e) DEXAMETHASONE + EELPO (4OOmg/kg)

Fig. 1: Histopathology of liver of normal and Dexamethasone induced hyper lipidemia model

DISCUSSION

Treatment with Ethanolic extract of *Portulaca oleracea* Linn. (EELPO) produced a significant decrease in the serum level of lipids in the dexamethasone induced hyperlipidemia in rats. Beta sitostrol a phytostirol is reported as useful in the treatment of hyperlipidemia[20]. Hypolipidemic effect of proteins, gums, saponins and beta sitostrol have been reported by several authors. The Ethanolic extract of *Portulaca oleracea* Linn. (EELPO) contains carbohydrates, glycosides, alkaloids, tannins, saponins, phenolic compounds, phytosterols and flavonoids. The high amount of phytostrols and alkaloids may be responsible for the hypolipidemic effect. It was found that EELPO was more effective in higher dose as compared to lower dose as an antihyperlipidemic agent against dexamethasone induced hyperlipidemia model. It also improves HDL- cholesterol levels and lower atherogenic index.

Further experiments are required to prove the mechanism and advantage of ethanolic extract of *Portulaca oleracea* Linn. (EELPO) over other drugs.

CONCLUSION

These results suggested that ethanolic extract of Portulaca oleracea Linn. (EELPO) possess significant anti hyperlipidemic activity.

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