

# Hypolipidemic Activity of *Saaranai Chooranam* Against Atherogenic Diet Induced Hyperlipidemia in Experimental Rats in the Management of *Raththa Kothippu* (Systemic Hypertension)

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## ABSTRACT

In worldwide Siddha system of medicine nowadays become most important medicine, it has fewer side effects in chronic usage. Therefore, in medicines of Siddha system should emphasis their Indications, Contraindications and cautions. The trial drug Saaranai chooranam has been mentioned in siddha literature of “Agasthiyar erandayiram – Part-III” for the management of Raththa kothippu (Systemic hypertension). Primary objective of study to evaluate the Hypolipidemic activity of saaranai chooranam against atherogenic diet induced hyperlipidemia in experimental rats. This Observational in vivo study carried out Arulmigu Kalasalingam College of Pharmacy, Tamil Nadu, India. The trial drug contains equal ratio of Saaranai Root and Inthuppu, with adjuvant Ghee or Jagarry. All animals starved for 18 hours and provided water ad libitum before the experiment. The animals were divided into five groups of six rats each. Group I served as normal control administered with 2% Carboxy Methyl Cellulose only, Group II served as hyperlipidemic control rats received atherogenic diet, Group III and IV served as test groups received Saaranai Chooranam 200mg/kg and Saaranai Chooranam 400mg/kg respectively. Group V served as Atorvastatin (10mg/kg/day) considered as standard. All the groups except the normal

control group administered received atherogenic diet After inducing the hyperlipidemia, the respective treatment was continued for 7 days. Animals were given standard pellet diet and water ad libitum. The next day after the completion of experimental study, the blood was taken from the rats, Liver lipid extraction, Biochemical analysis, Histopathology. The data were statistically analyzed by one-way ANOVA followed by Dennett’s t-test, and value  $P < 0.05$  was considered to be significant. The results obtained from the pharmacological screening have led to the conclusions that, Saaranai chooranam has significant antihyperlipidemic activity. Hence it can be exploited as antihyperlipidemic therapeutic agent or adjuvant in existing therapy for the treatment of hyperlipidemia.

**Key words:** Saaranai chooranam, Raththa kothippu, Hyperlipidemia, Akasthiyar erandayiram.

## 1. INTRODUCTION

The Siddha system of medicine is the ancient system of medicine, which has been presented by the ‘siddhars’. The unique nature of this system is its continuous service to humanity and in maintaining the physical, mental and moral health, while many of these contemporaries had completed their forces long ago. According to the Siddhar ‘Aagasthir’ the diseases are widely classified

into 4448 types. Raththa kothippu (Systemic Hypertension) is one of the diseases in the above classification. In this research medicine Saaranai chooranam from the evidence of Agasthiyar erandajirum Part-III, to indicated Raththa kothippu (Systemic Hypertension). The Saaranai (Family: Aizoacea, Botanical name: *Trianthema portulacastrum*) traditionally used as Diuretic, Analgesic, Stomachic, Laxative and blood disorders.

Hypolipidemic refers to the ability to reduce lipid levels in the body, which is important in preventing diseases such as coronary heart disease and cerebral thrombosis. Hyperlipidemia, a condition characterized by elevated levels of lipids in the blood, poses a significant risk factor for various health disorders, notably cardiovascular diseases. Phytochemical compounds are promising alternatives to the current lipid-lowering drugs, which cause many undesirable effects. Hyperlipidemia is a metabolic disorder characterized by elevated levels of one or more lipids and/or lipoproteins in the blood. Depending on the lipid group altered, in this this research Saaranai chooranam analyzed to its Hypolipidemic effects.

## 2. MATERIALS AND METHODS

### 2.1 Trial drug selection, Collection & Authentication of raw materials:

The details about the Saaranai chooranam is selected from Siddha text Dr. S. Venkattarajan, Akasthiyar-2000, Part – III, Page: 102. The required raw materials for preparation of Saaranai chooranam is collected from in and around of Tirunelveli and authenticated by Botanist, department of medicinal botany, Govt. Siddha Medical College, Palayamkottai. The raw materials were purified and the medicine was prepared in the Govt. Siddha Medical College, Palayamkottai.

### 2.2 Method of preparation:

The adulterants and dust were removed. Saaranai root thoroughly washed in water and soaked in cow's milk. After that it steamed in milk. Dried and grind into the fine

powder sieved and add same quantity of Inthuppu. The drug will be labelled as Saaranai Chooranam.

### 2.3 Compositions of Trial drug:

#### 2.3.1 Saaranai:

Tamil name- Saaranai

English Name - Black pigweed/ giant pigweed

Botanical name - *Trianthema portulacastrum*

Family – Aizoaceae

Part Used – Root

#### 2.3.2 Inthuppu:

Tamil name- Inthuppu / Sainthalavanam

English Name - Rock salt

Botanical name - *Sodium chloridum impura*

Part Used - Salt

### 2.4 Study Design and Controls:

#### 2.4.1 Animals

Wistar albino adult male rats weighing 150-200gm from animal housing facility were housed in polypropylene cages maintained with temperature  $27^{\circ}\text{C} \pm 1^{\circ}\text{C}$  and 12 hours light and dark cycle. The animals were allowed to adapt to the environment for seven days and supplied with a standard pellet diet and water ad libitum. The experimental protocol has got the approval IAEC bearing no: AKCP/IAEC/23/22-23.

**Chemicals:** Atorvastatin obtained from local pharmacy. Diagnostic kits for estimation, anesthetic ether, ethyl acetate, and ethanol were purchased from Merck Diagnostics India Ltd.

**Atherogenic diet:** Experimental hyperlipidemic diet: Experimental diet consists of well-pulverized mixture of cholesterol – 400 mg/kg, cholic acid – 50 mg/kg, and coconut oil. This mixture is made into paste-like molds and is fed to the rats.

## 3 Pharmacological evaluation

### 3.1 Treatment with atherogenic diet:

The prepared atherogenic diet was used in place of normal pellet diet to all the groups except control. Rats were exposed to atherogenic diet and water ad libitum for 20 days and were used to study the effect of Saaranai Chooranam against experimental hyperlipidemia. All animals starved for 18

hours and provided water ad libitum before the experiment. The animals were divided into five groups of six rats each. Group I served as normal control administered with 2% CMC only. Group II served as hyperlipidemic control rats received atherogenic diet. Group III and IV served as test groups received Saaranai Chooranam 200mg/kg and Saaranai Chooranam 400mg/kg respectively. Group V served as Atorvastatin (10mg/kg/day) considered as standard. All the groups except the normal control group administered received atherogenic diet After inducing the hyperlipidemia, the respective treatment was continued for 7 days. Animals were given standard pellet diet and water ad libitum.

### 3.2 Collection of blood

The next day after the completion of experimental study, the blood was taken from the rats under mild anesthetic state by retro orbital sinus puncture. The collected blood samples were centrifuged (2500 rpm) for 10 minutes. Then serum samples were separated and it was used for various biochemical

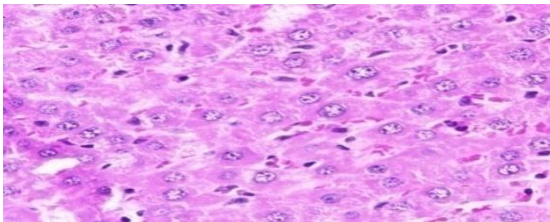
analyses. Then animals were sacrificed and the liver, heart and kidney were taken for histopathological study.

### 3.3 Liver lipid extraction

The liver was homogenized in cold 0.15M KCl and extracted with CHCl<sub>3</sub>: CH<sub>3</sub>OH (2% v/v). This lipid extract was used for the estimation of lipid parameters. The serum and liver were analyzed for serum total cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) by standard enzymatic calorimetric methods. All rats were sacrificed after the collection of blood sample. Liver was excised from the rats to visually detect gross lesions, and weighed to determine weight variation and preserved in 10% neutral formalin for histopathological assessment. The tissue was embedded in paraffin, and then sectioned, stained with haematoxylin and eosin and were examined microscopically.

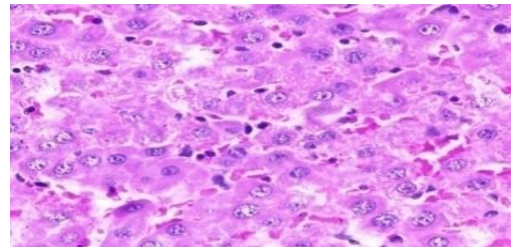
### 3.4 Histopathology study:

Figure No: 1 Normal Control Rat



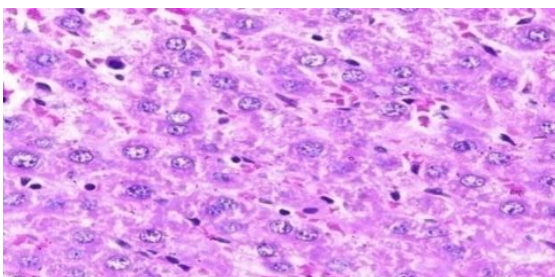
Section of liver parenchyma with hepatocyte, which appear normal & central vein & portal tract appear normal,

Figure No: 2 Hyperlipidemic Control



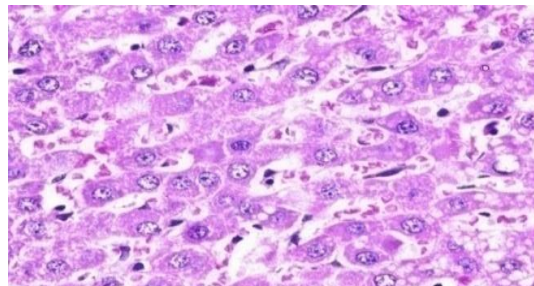
Section of liver parenchyma with scattered focal area of necrosis of hepatocyte.

Figure No: 3 Positive Control



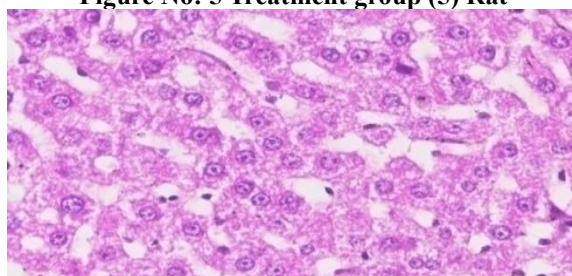
Section of liver parenchyma shows Normal.

Figure No: 4 Treatment group (4) Rat



Section of liver parenchyma with minimal necrosis, and minimal inflammation.

Figure No: 5 Treatment group (5) Rat



Section of liver parenchyma with hepatocyte, which appear normal, central vein & portal tract are normal.

**Statistical evaluation:** All the values were expressed as mean  $\pm$  standard error of mean. The data were statistically analyzed by one-way ANOVA followed by Dennett's t-test,

and value  $P < 0.05$  was considered to be significant.

#### 4. RESULTS

Table 4.1: Effect of Saaranai Chooranam on body weight of atherogenic induced hyperlipidemic rats.

s.no	Groups	Body weight
1	Normal control	146.14 $\pm$ 0.99
2	Hyper lipidemic Control	250.80 $\pm$ 0.46
3	Saaranai Chooranam (LOW)	173.69 $\pm$ 0.77*
4	Saaranai Chooranam (HIGH)	161.33 $\pm$ 0.66*
5	Atorovastatin (10mg/kg/day)	162.55 $\pm$ 0.93

All the values were represented as mean  $\pm$  SEM. All the data were statistically analyzed by one-way ANOVA followed by Dunnett's

test and values  $p < 0.5$  were considered to the significant. \* $p < 0.001$ ; \*\* $p < 0.01$  vs control.

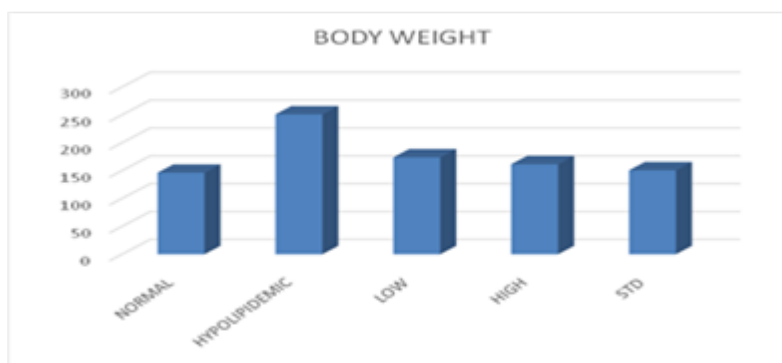


Figure: 4.1 Effect of Saaranai Chooranam on body weight of atherogenic induced hyperlipidemic rats.

Total body weight in the hyperlipidemia-induced group have significantly increased compared to normal rats. The values have risen to 250.80 $\pm$ 0.46mg/dl compared to Group I (normal rat group), in which values lie in the range 146.14 $\pm$ 0.99mg/dl. This indicates hypercholesterolemia. In the treatment group treated with Saaranai

Chooranam (200 mg/kg) and Saaranai Chooranam (400 mg/kg), the values are reduced 173.69 $\pm$ 0.77\* ( $P < 0.001$ ) and 161.33 $\pm$ 0.66\*mg/dl ( $P < 0.01$ ), respectively. There is a significant reduction in total cholesterol values in Saaranai Chooranam treatment group. On the other hand, atorvastatin also has significantly reduced



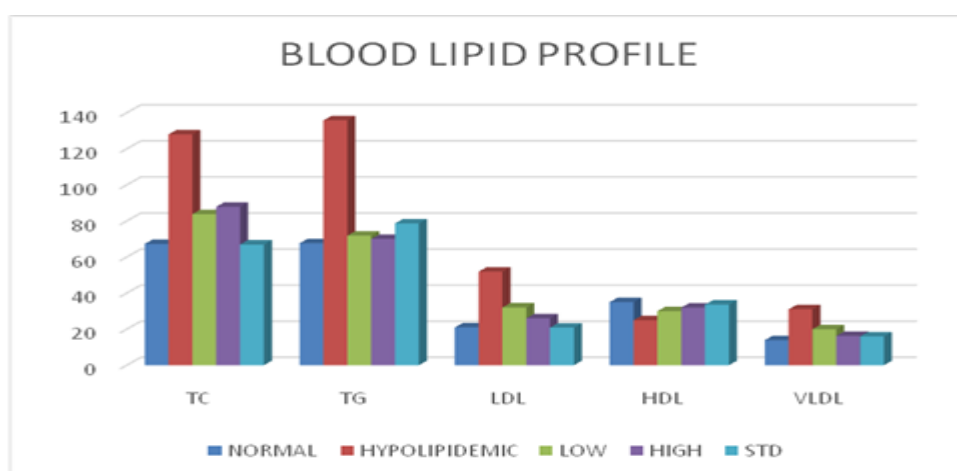
serum total cholesterol levels to 162.55±0.93mg/dl ( $P < 0.001$ ) [Table 1].

**Table 2: Effect of Saaranai Chooranam on Blood lipid profile of atherogenic -induced hyperlipidemic rats.**

Group	Treatment	T.C.	T.G.	LDL	HDL	VLDL
I	Normal Control	67.25±0.47	67.65±0.45	20.93±0.06	34.99±0.98	13.86±0.01
II	Hyperlipidemic Control	127.99±0.83	135.79±0.26	51.86±0.87	24.97±0.98	30.99±0.85
III	Saaranai Chooranam (LOW)	83.76±0.94*	71.85±0.83*	31.99±0.98*	29.94±0.62*	19.94±0.24*
IV	Saaranai Chooranam (HIGH)	87.85±0.144*	69.95±0.76*	25.99±0.99*	31.95±0.68*	16.24±0.49*
V	Atorvastatin 10Mg/kg	66.9±0.73**	78.63±0.15**	20.83±0.39*	33.6±0.90*	15.99±0.91**

All the values were represented as mean ± SEM. All the data were statistically analyzed by one-way ANOVA followed by Dunnett's

test and values  $p < 0.5$  were considered to be significant. \* $p < 0.001$ ; \*\* $p < 0.01$  vs control,



**Figure:2 Effect of Saaranai Chooranam on Blood lipid profile of atherogenic -induced hyperlipidemic rats.**

Total cholesterol levels in the hyperlipidemia-induced group have significantly increased compared to normal rats. The values have risen to 127.99±0.83mg/dl compared to Group I (normal rat group), in which values lie in the range 67.25±0.47mg/dl. This indicates hypercholesterolemia. In the treatment group treated with Saaranai Chooranam (200 mg/kg) and Saaranai Chooranam (400 mg/kg), the values are reduced to 83.76±0.94 ( $P < 0.001$ ) and 66.9±0.73mg/dl ( $P < 0.01$ ), respectively. There is a significant reduction in total cholesterol values in Saaranai Chooranam treatment group. On the other hand, atorvastatin also has significantly reduced serum total cholesterol levels to 66.9 ± 1.16 mg/dl ( $P < 0.001$ ) [Table 1].

The TG levels have reached as 127.99±0.83±1.53mg/dl hyperlipidemia-

induced group compared to normal rats where the values are 135.79±0.26mg/dl. This indicates triglyceridemia. In the group treated with Saaranai Chooranam (200 mg/kg) and (400 mg/kg), the values are significantly reduced to 71.85±0.83\*mg/dl ( $P < 0.01$ ) and 69.95±0.76mg/dl ( $P < 0.01$ ), respectively. In the atorvastatin treated group, the values are reduced to 78.63±0.15mg/dl ( $P < 0.001$ ) in [Table 1]. LDL-cholesterol in atherogenic-induced group has significantly increased to 51.86±0.87 mg/dl compared to normal rat group, 20.93±0.06mg/dl. In the group treated with Saaranai Chooranam (200 mg/kg) and (400 mg/kg), the values were reduced to 31.99±0.98 and 25.99±0.99mg/dl ( $P < 0.001$ ), respectively. There is a significant reduction in LDL-cholesterol values in Saaranai Chooranam treatment group.

atorvastatin has significantly reduced LDL-cholesterol level to 20.83±0.39mg/dl ( $P < 0.001$ ) [Table 1].

HDL-cholesterol in atherogenic -induced group has significantly decreased compared to normal rats. The values have reduced to 24.97±0.98mg/dl compared to normal rat group, 34.99±0.98mg/dl. In the group treated with Saaranai Chooranam (200 mg/kg) and (400 mg/kg), the values were 29.94±0.62 ( $P < 0.01$ ) and 31.95±0.68mg/dl ( $P < 0.01$ ), respectively. In atorvastatin treated group,

the values were 33.6±0.90mg/dl ( $P < 0.001$ ) [Table 4].

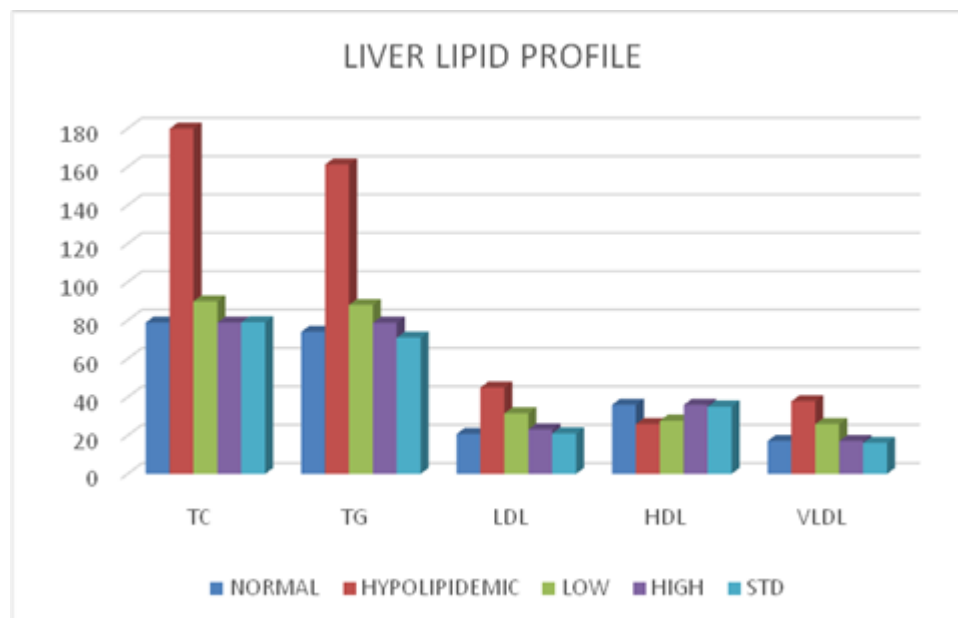
VLDL-cholesterol in atherogenic-induced group has significantly increased to 30.99±0.85mg/dl compared to normal rat group, 13.86±0.01mg/dl. In the group treated with SC (200 mg/kg) (400 mg/kg), the values are reduced to 19.94±0.24 ( $P < 0.01$ ) and 16.24±0.49\*mg/dl ( $P < 0.01$ ), respectively. There is a significant reduction in Saaranai Chooranam treatment group. atorvastatin has significantly reduced VLDL cholesterol level to 15.99±0.91mg/dl ( $P < 0.001$ ) [Tables 1].

**Table 3: Effect of Saaranai Chooranam on liver lipid profile of atherogenic -induced hyperlipidemic rats.**

Group	Treatment	T.C	T.G.	LDL	HDL	VLDL
I	Normal Control	78.84±0.91	73.95±0.17	20.76±0.04	35.99±0.95	17.09±0.98
II	Hypolipidemic Control	179.86±0.92	161.19±0.99	44.99±0.86	25.95±0.77	37.77±0.77
III	Saaranai Chooranam (LOW)	89.88±0.16*	87.97±0.28*	31.52±0.38*	27.70±0.10*	25.94±0.89*
IV	Saaranai Chooranam(HIGH)	78.8±0.10*	78.72±0.93*	23.1±0.94*	35.90±0.04*	17.07±0.21*
V	Atorvastatin (10mg/kg/day)	78.94±0.33*	70.95±0.90*	21.02±0.05*	35.04±0.10*	16.08±0.05*

All the values were represented as mean ± SEM. All the data were statistically analyzed by one-way ANOVA followed by Dunnett's

test and values  $p < 0.5$  were considered to the significant. \* $p < 0.001$ ; \*\* $p < 0.01$  vs control,



**Figure 3: Effect of Saaranai Chooranam on liver lipid profile of atherogenic -induced hyperlipidemic rats.**

Total cholesterol levels in the hyperlipidemia-induced group have significantly increased compared to normal rats. The values have risen to 179.86±0.92mg/dl compared to Group I (normal rat group), in which values lie in the

range 78.84±0.91mg/dl. This indicates hypercholesterolemia. In the treatment group treated with Saaranai Chooranam (200 mg/kg) and Saaranai Chooranam (400 mg/kg), the values are reduced 89.88±0.16 ( $P < 0.001$ ) and 78.94±0.33mg/dl ( $P < 0.01$ ),

respectively. There is a significant reduction in total cholesterol values in Saaranai Chooranam treatment group. On the other hand, atorvastatin also has significantly reduced serum total cholesterol levels to  $78.94 \pm 0.33$  mg/dl ( $P < 0.001$ ) [Table 1].

The TG levels have reached as  $161.19 \pm 0.99$  mg/dl in hyperlipidemia-induced group compared to normal rats where the values are  $73.95 \pm 0.17$  mg/dl. This indicates triglyceridemia. In the group treated with Saaranai Chooranam (200 mg/kg) and (400 mg/kg), the values are significantly reduced to  $87.97 \pm 0.28$  mg/dl ( $P < 0.01$ ) and  $78.72 \pm 0.93$  mg/dl ( $P < 0.01$ ), respectively. In the atorvastatin treated group, the values are reduced to  $70.95 \pm 0.90$  mg/dl ( $P < 0.001$ ) [Table 1].

LDL-cholesterol in atherogenic-induced group has significantly increased to  $44.99 \pm 0.86$  mg/dl compared to normal rat group,  $20.76 \pm 0.04$  mg/dl. In the group treated with Saaranai Chooranam (200 mg/kg) and (400 mg/kg), the values were reduced to  $31.52 \pm 0.38$  and  $23.1 \pm 0.94$  mg/dl ( $P < 0.001$ ), respectively. There is a significant reduction in LDL-cholesterol values in Saaranai Chooranam treatment group. atorvastatin has significantly reduced LDL-cholesterol level to  $21.02 \pm 0.05$  mg/dl ( $P < 0.001$ ) [Table 1].

HDL-cholesterol in atherogenic -induced group has significantly decreased compared to normal rats. The values have reduced to  $25.95 \pm 0.77$  mg/dl compared to normal rat group,  $35.99 \pm 0.95$  mg/dl. In the group treated with Saaranai Chooranam (200 mg/kg) and (400 mg/kg), the values were  $27.70 \pm 0.10$  ( $P < 0.01$ ) and  $35.90 \pm 0.04$  mg/dl ( $P < 0.01$ ), respectively. In atorvastatin treated group, the values were  $35.04 \pm 0.10$  mg/dl ( $P < 0.001$ ) [Table 4].

VLDL-cholesterol in atherogenic-induced group has significantly increased to  $37.77 \pm 0.77$  mg/dl compared to normal rat group,  $17.09 \pm 0.98$  mg/dl. In the group treated with Saaranai Chooranam (200 mg/kg) and (400 mg/kg), the values are reduced to  $25.94 \pm 0.89$  ( $P < 0.01$ ) and  $17.07 \pm 0.21$  mg/dl ( $P < 0.01$ ), respectively. There is a significant reduction in Saaranai Chooranam treatment

group. atorvastatin has significantly reduced VLDL-cholesterol level to  $18.1 \pm 0.95$  mg/dl ( $P < 0.001$ ) [Tables 1].

## DISCUSSION

The reduction in cholesterol may indicate the increased oxidation of mobilized fatty acids by inhibition or lipolysis. The present investigation showed that all atherogenic induced rats displayed hyperlipidemia as shown by their elevated levels of serum and liver cholesterol, triglyceride, VLDL, LDL and also the reduction in the HDL level. Literature reveals that an increase in HDL cholesterol and decrease in TC, LDL cholesterol and TG is associated with a decrease in the risk of ischemic heart diseases. In general, consumption of more fat may lead to the production of increased VLDL, resulting in the formation of maximum amounts of LDL which may stick to the walls of the blood vessels causing blockages for the normal flow of blood. The strong association between the risk of coronary artery diseases (CAD), high levels of LDL-C and low levels of HDL-C has been well established. Atherogenic has been widely used to block the clearance of triglyceride-rich lipoproteins to induce acute hyperlipidemia particularly, in rats it has been used for screening natural or chemical hypolipidemic drugs. The results showed that Saaranai Chooranam produced a significant reduction in cholesterol level and also it reversed atherogenic induced hypolipidemic in rats. Similarly, Saaranai Chooranam at a dose of 200 and 400 mg/kg significantly lowered both plasma triglycerides and cholesterol levels. The reduction of total cholesterol by the Saaranai Chooranam at the dose level of 200 and 400 mg kg may be associated with a decrease of LDL, which is the ultimate aim of many hypolipidemic agents.

This study suggests that cholesterol-lowering activity of the Saaranai Chooranam may increase the fecal excretion of bile acids and neutral sterols with the consequent reduction of hepatic cholesterol because of its use in the biosynthesis of these bile acids. These

fractions also slow down the rate of diffusion through the intestinal mucosa thereby reducing the absorption of cholesterol and triglycerides.

## CONCLUSION

The results obtained from the pharmacological screening have led to the conclusions that, ac have significant antihyperlipidemic activity. Hence it can be exploited as antihyperlipidemic therapeutic agent or adjuvant in existing therapy for the treatment of hyperlipidemia and it's also used to treat Raththa kothippu (Systemic Hypertension)

### Declaration by Author

**Ethical Approval:** Approved

**Acknowledgement:** None

**Source of Funding:** None

**Conflict of Interest:** The author declares no conflict of interest.

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