



Acute toxicity and anti-dyslipidemic activity of *Arogyavardhini* compound in fructose-induced dyslipidemia in albino rats

Bharatkumar C Padhar¹, Alankruta R Dave² and Mukeshkumar Nariya^{3*}

¹PG Department of Kayachikitsa, National Institute of Ayurveda, Jaipur 302002, Rajasthan, India

²Department of Kayachikitsa, 3Pharmacology Laboratory, Institute of Teaching and Research in Ayurveda, Jamnagar 361008, Gujarat, India

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Metabolic syndrome is a co-occurrence of obesity, insulin resistance, hypertension, and dyslipidemia caused by improper diet and lifestyle. *Arogyavardhini* compound (AVC) contains an equal quantity of *Arogyavardhini rasa* and *Lasuna* powder. In clinical practice, *Arogyavardhini Rasa* is well known for its antidyslipidemic and weight lowering effect. Therefore, the present experimental study was designed to evaluate the safety of AVC on acute administration and anti-dyslipidemic activity in albino rats. An acute oral toxicity study for AVC was carried out by following OECD 425 guidelines. The anti-dyslipidemic activity was carried out against fructose-induced dyslipidemia in albino rats. No mortality and toxicity were observed and gross behaviours of all the albino rats were found normal during the experimental period of 14 days in the acute toxicity study. Fructose significantly increased blood sugar, triglycerides, SGPT, and alkaline phosphatase levels in albino rats in comparison to the control group. AVC treated group produced a decrease in serum triglyceride, transaminases, and alkaline phosphatase, which suggest that the drug has potential as anti-dyslipidemic and may be protective for degenerative changes produced by fructose in the liver, kidney, and heart of albino rats. From the present study it is concluded that AVC is safe up to an oral dose of 2000 mg/kg in albino rats and has exhibited a protective role in fructose-induced dyslipidemia in albino rats, hence may be useful in metabolic syndrome.

Keywords: Acute toxicity, Antidyslipidemic activity, *Arogyavardhini* compound, Fructose, Metabolic syndrome.

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Introduction

Metabolic syndrome, a combined occurrence of three or more metabolic disorders including obesity, insulin resistance, hypertension and dyslipidemia which are caused by improper diet and lifestyle and is recognized as one of the major public health burdens worldwide especially in the Indian sub-continent^{1,2}. It is estimated that approximately 25% of the world's population has metabolic syndrome and it will increase up to 38% by 2023³. The prevalence of this syndrome is higher in the adult population throughout the world⁴. In the etiopathogenesis of metabolic syndrome, reactive oxygen species (ROS) play an essential role in multiple systems and contribute to cellular metabolic dysfunction. Oxidative stress plays a major role in the pathogenesis of a variety of human diseases, including diabetes, hypertension, atherosclerosis, ageing, Alzheimer's disease, kidney disease, and malignancies⁵.

Arogyavardhini compound (AVC) is a herbo-mineral formulation that contains an equal quantity of *Arogyavardhini rasa* and *Lasuna* (*Allium ascalonicum* L.) powder. It is developed for the treatment of metabolic disorders. *Arogyavardhini Rasa* is having *Deepana* (appetizer), *Pachana* (digestive) properties and being indicated for *Medodosha* (fat metabolism disturbance) as well as *Lasuna* (Garlic) being indicated for *Avaran* (obstruction) of *Vata*. So the combination of both is expected to remove *Medodosha* correcting *Agni* and the function of *Vata* by removing *Avaran*, which are the main culprits in the pathogenesis of disease^{6,7}. Recent researches on *Arogyavardhini Rasa* have proved its antidyslipidemic and weight lowering effect⁸. Antihypertensive, antihyperglycemic, antihyperlipidemic, anticancer and antioxidant effects of *Lasuna* are also proven by various researches^{9,10}. So, AVC is expected to establish its role, especially on dyslipidemia conditions with the presence of other components of metabolic syndrome together. The current experimental study is designed to evaluate the

*Correspondent author
Email: mukeshnariya@gmail.com
Mob.: +91-9429334081

safety of AVC in acute toxicity study and anti-dyslipidemic activity in albino rats for documentation of scientific data on the safety and efficacy of AVC.

Materials and Methods

Animals

Charles's foster strain albino rats of either sex weighing between 200±20 g were used for experiments. The animals were obtained from the Animal house attached to the Pharmacology laboratory. They were exposed to 12 hours of light and dark cycles, with ideal husbandry conditions in terms of ambient temperature and humidity. The temperature during the experiment was between 23±3 °C and humidity around 50-60%. Animals were fed ad libitum with Amrut brand rat pellet feed supplied by Pranav Agro Industries and drinking water. The experiment as per the guideline of CPCSEA, India was carried out after obtaining the permission from Institutional Animal Ethics Committee (IAEC/17/2015/07).

Drugs and Chemicals

AVC is a herbo-mineral non-classical Ayurvedic formulation. It was developed by authors consists of *Arogyavardhini rasa* with *Lasuna* for the treatment of metabolic disorders. Table 1 depicts the ingredients of AVC¹¹. *Lasuna* was procured from the garlic and onion market of Ahmedabad, Gujarat. Raw drugs of *Arogyavardhini Rasa* were collected from the Pharmacy, Gujarat Ayurveda University, Jamnagar, Gujarat, India. Raw drugs were identified and authenticated in the Pharmacognosy Laboratory of the Institute for Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurveda, University, Jamnagar. The identification was carried out based on

organoleptic characters and microscopic characters of *Churna* and later on pharmacognostical evaluation of the AVC was carried out. The microphotographs were also taken under the microscope. The formulation was prepared and pharmaceutical analysis of the finished product was carried out as per API parameters for quality compliance¹². All other chemicals used were of analytical grade.

Dose fixation

The therapeutic clinical dose of AVC is considered as 1 g twice a day for the treatment of metabolic disorders¹³. Based on that, the dose of animals was calculated by extrapolating the human therapeutic dose to rat dose (conversion factor 0.018 for 200 g rat) based on body surface area ratio by referring to table of Paget and Barnes (1964)¹⁴. Considering that, rat therapeutic dose was calculated as 90 mg/kg body weight twice a day.

Route of drug administration

The suspension of the test drug was prepared with distilled water to suitable concentration and in uniform volume was administered by oral route with the help of oral feeding cannula. The test drug was administered daily between 9:30 to 10:30 am and 4:30 to 5:30 pm.

Acute oral toxicity study

Acute oral toxicity study of AVC was carried out by following OECD 425 guideline¹⁵ (modified, adopted on 23rd March 2006) with 2000 mg/kg as a limit test. Five female albino rats in each group were treated with 2000 mg/kg sequentially and rats were observed for 14 days. AVC (limit dose) was administered once orally to overnight fasted rats. Gross behaviour and mortality, if any was observed

Table 1 — The ingredients of AVC

Name of drug	Latin name	Part used	Ratio	Form
<i>Suddha Parada</i>	-	-	1 part	Powder
<i>Suddha Gandhaka</i>	-	-	1 part	Powder
<i>Loha bhasma</i>	-	-	1 part	<i>Bhasma</i>
<i>Abharaka bhasma</i>	-	-	1 part	<i>Bhasma</i>
<i>Tamra bhasma</i>	-	-	1part	<i>Bhasma</i>
<i>Haritaki</i>	<i>Terminalia chebula</i> L.	Fruit	2 part	Powder
<i>Amalaki</i>	<i>Emblica officinalis</i> L.	Fruit	2 part	Powder
<i>Bibhitaki</i>	<i>Terminalia bellirica</i> R.	Fruit	2 part	Powder
<i>Suddha Shilajatu</i>	-	Exudates	3 part	Powder
<i>Suddha Guggulu</i>	<i>Commiphora mukul</i> H.	Gum	4 part	Powder
<i>Eranda moola</i>	<i>Ricinus communis</i> L.	Root	4 part	Powder
<i>Katuki</i>	<i>Picrorhiza kurrora</i> R.	Root/Rhizome	22 part	Powder
<i>Nimba patra svarasa</i>	<i>Azadirachta indica</i> A.	Leaves Juice	<i>Mardana</i> for 2 days	
Single bulb <i>Lasuna</i>	<i>Allium ascalonicum</i> L.	Bulb	44 part	<i>Churna</i>

throughout the study period for 14 days. Food was withheld overnight before the experiment and further 2 hours after administration of the test drug.

The animals were observed continuously for 6 hours after the dosing. The careful cage side observation was done without disturbing the animal attention and at the end of every hour, animals were individually exposed to an open arena for recording the behavioural changes like increased or decreased motor activity, convulsions, Straub's reactions, muscle spasm, catonia, spasticity, opisthotonus, hyperesthesia, muscle relaxation, anaesthesia, arching and rolling, lacrimation, salivation, diarrhoea, writhing, mode of respiration, changes in skin colour, exitus, C.N.S. depression- hypoactivity, passivity, relaxation, ataxia, and narcosis etc. All the animals were observed at ½, 1, 2, 3, 4, 5, 6, 24 hours after administration of test drug and daily once for any mortality during the experimental period (14 days). The body weight of each animal was recorded just before treatment on days 1, 7 and 14.

Anti-dyslipidemic activity

Charles's foster strain albino rats of either sex weighing 200 ± 20 g were randomly divided into four groups, each consisting of six rats ($n=6$). Group I received Normal control rats received distilled water (10 mL/kg, po) (NC), Group II Fructose control rats, received distilled water (10 mL/kg, po) + 10% fructose in drinking water (FRC), Group III, treated group received AVC (90 mg/kg/day, po) + 10% fructose in drinking water (AVC), and Group IV Pioglitazone (10 mg/kg/day, po) + 10% fructose in drinking water.

Test drugs and reference standard drugs were administered to respective groups for 30 days. Pioglitazone was used as a standard drug for anti-dyslipidemic activity and the dose was fixed at 10 mg/kg/day¹⁶. The 10% w/v fructose solution was given in drinking water *ad libitum* for 24 hours during the study period of 30 days in all the groups to induce dyslipidemia except in the normal control (Group I). Chronic fructose feeding is associated with insulin resistance and metabolic abnormalities¹⁶.

On the 31st day, after overnight fasting, the rats were weighed again and blood was collected from retro-orbital puncture under light ether anaesthesia. Serum was separated and used for estimation of different serum biochemical parameters like total cholesterol, triglyceride, HDL-cholesterol, fasting blood glucose, SGPT, and alkaline phosphatase, using

a fully automated biochemical random-access analyzer (BS-200, Lilac Medicare Pvt. Ltd., Mumbai). In the end, the rats were sacrificed and important organs were dissected such as the liver, kidney, and heart. The organs were cleaned of extraneous tissue, weighed immediately and then transferred to a 10% buffered formalin solution for fixing. They were processed later for histopathological studies.

Statistical analysis

The data are expressed as Mean \pm SEM for six rats in each group. Statistical comparisons were performed by Student's Paired and Unpaired 't' test and one-way ANOVA followed by Dunnett's multiple 't' test by using Sigma stat software (version 3.1) for all the treated groups with the level of significance set at $P < 0.05$.

Results and Discussion

Acute oral toxicity study

OECD 420, 423, and 425 guidelines were employed using a single-sex (preferably females) to reduce variability and as a means of minimizing the number of animals used. This is because literature surveys of conventional LD₅₀ tests show that usually there is little difference in sensitivity between the sexes but, in those cases where differences were observed, females were generally slightly more sensitive¹⁷. Hence, in the present study, acute toxicity was evaluated in female albino rats. AVC at a dose level of 2000 mg/kg orally did not produce any mortality in any of the treated rats which suggest that LD₅₀ value may be higher than 2000 mg/kg by oral route. In the present study, normal progressive weight gain was observed in both normal control and treated groups in comparison to the initial weight. As per UN classification, any substance which has oral LD₅₀ of more than 2000 mg/kg is considered as low hazard potential. Thus, as per the above criterion, the AVC can be categorized as a substance with low health hazard potential (Class 4 of Globally Harmonized System and United Nations 6.1 Packing group III)¹⁸.

Anti-dyslipidemic activity

Lipid pathology in human beings is quite similar to fructose-induced dyslipidemia in animals, so the fructose-induced dyslipidemia model is considered a suitable model to evaluate anti-dyslipidemic activity in experimental models. Fructose results in obesity and weight gain through several mechanisms¹⁹. In a clinical study, Havel's *et al.* observed that fructose

does not cause a level of satiety equivalent to that of a glucose-based meal²⁰. Specifically, the differences in the effect of fructose and glucose consumption (consumed as beverages with three meals) on *ad libitum* food intake and hunger rating were observed on the day after the exposure to the sweetened beverages. Fructose is not able to stimulate insulin and leptin and inhibit the ghrelin which may affect the satiety centre in the central nervous system. In the present study, administration of 10% fructose solution led to a significant increase in body weight of fructose control group albino rats when compared to normal control rats. AVC attenuated the fructose-induced weight gain in albino rats (Table 2). This indicates that the test drug has antagonizing effect against fructose-induced changes in body weight. Fructose consumption leads to a non-significant increase in liver weight and a significant decrease in kidney weight in comparison to the normal control group. AVC did not produce any effect on the relative weight of liver, kidney, and heart of albino rats (Table 3).

Fructose consumption in high amounts leads to postprandial hypertriglyceridemia and visceral adipose deposition contributing to hepatic triglyceride accumulation and protein kinase C activation. Fructose initiates hepatic insulin resistance independently of visceral adiposity and free fatty acid delivery²¹. It has been found that hepatic production of triglycerides is much greater with fructose consumption as compared with equimolar concentrations of glucose consumption²². Fructose is metabolized in the liver and having less ability of expression of the fructose transporter GLUT5 in pancreatic β -cells does not stimulate insulin secretion²³. Consumption of fructose-sweetened beverages with meals produced a rapid and prolonged elevation of plasma triglycerides and Apolipoprotein B compared with glucose-sweetened beverages²⁴. Fructose increases the incidence of hypertension, non-alcoholic fatty liver diseases and diabetes²⁵.

A significant increase in serum triglyceride and an insignificant increase in serum cholesterol were

observed in the fructose control group in comparison to the normal control group. Treatment with AVC attenuated the increased serum level of triglyceride and cholesterol level but did not affect HDL-cholesterol level when compared to the fructose control group (Table 4) which may suggest anti-hyperlipidemic activity in rats. *Tamra* (Copper) is one of the important components of AVC. Recent research works on copper indicates that copper deficiency is associated with specific effects on systemic lipid metabolism²⁶. Early work showed feeding rats with a copper-deficient diet revealed hypercholesterolemia, cardiac hypertrophy, haemorrhage, inflammation, and focal necrosis²⁷. The other contents of AVC like *Guggulu*²⁸, *Lasuna*²⁹, and *Triphala*³⁰ possess antihyperlipidemic activity which is believed to play a significant role in the decrease of serum cholesterol and triglycerides in AVC treated group.

Table 2 — Effect of test drugs on body weight of albino rats during anti dyslipidemic study

Treatments	Body weight (g)		
	Initial	Final	% change
NC	176.67±9.72	202.50±11.80*	14.62↑
FRC	170.00±8.94	228.33±12.76**	34.31↑
AVC	182.00±14.63	232.00±17.72**	27.47 ↑
Pioglitazone	179.00±4.28	238.33±10.05**	33.14 ↑

Data: Mean±SEM; ↑-Increase; * $P < 0.01$; ** $P < 0.001$ when compared to initial body weight (Paired 't' test)

Table 3 — Effect of test drugs on the relative weight of organs of albino rats during anti dyslipidemic study

Treatments	Relative weight		
	Liver (g/100 g BW)	Heart (mg/100 g BW)	Kidney (mg/100 g BW)
NC	3.122±0.127	311.49±14.36	705.81±17.06
FRC	3.374±0.115	308.83±6.55	626.06±26.22*
AVC	3.077±0.057	293.92±8.49	652.91±41.74
Pioglitazone	3.521±0.201	301.59±16.45	625.03±37.83

Data: Mean±SEM; ↑- Increase; ↓- Decrease; * $P < 0.05$ when compared to normal control group (Unpaired 't' test)

Table 4 — Effect of test drugs on blood sugar, cholesterol and triglyceride levels in albino rats during anti-dyslipidemic study

Treatments	Blood sugar	%	Cholesterol	%	Triglyceride	%
	(mg/dL)	change	(mg/dL)	change	(mg/dL)	change
Control	69.833±5.32	--	43.33±4.31	--	65.33±12.16	--
FRC	118.67±13.59 [®]	69.93↑	49.67±4.99	14.63↑	148.17±27.12 [®]	126.80↑
AVC	115.75±11.56	2.46 ↓	45.00±3.66	9.40↓	102.50±34.03	30.82↓
Pioglitazone	108.33±7.07	8.71 ↓	50.67±3.49	16.93↑	77.83±11.48 [#]	47.47↓

Data: Mean±SEM; ↑-Increase; ↓ - Decrease; [®] $P < 0.05$, when compared to the normal control group (Anova followed by Dunnett's multiple 't' test); [#] $P < 0.05$, when compared to the fructose control group (Unpaired 't' test).

Table 5 — Effect of test drugs on HDL-cholesterol, SGPT and Alkaline phosphatase levels in albino rats during anti-dyslipidemic study

Treatments	HDL-cholesterol (mg/dL)	% change	SGPT (IU/L)	% change	ALP (IU/L)	% change
Control	31.16±1.72	--	43.16±1.42	--	106.66±9.71	--
FRC	37.83±5.06	19.67↑	65.83±5.75 [@]	52.52↑	241.33±37.59 [@]	126.26↑
AVC	41.60±9.15	9.96↑	60.40±2.84	8.24↓	196.80±11.91	18.45↓
Pioglitazone	36.33±2.39	3.96↓	75.50±2.77	14.68↑	220.83±28.22	8.49↓

Data: Mean±SEM; ↑-Increase; ↓ - Decrease; [@]P <0.05, when compared to the normal control group (Annova followed by Dunnett's multiple 't' test)

Hypolipidemic activity of guggulsterone present in *Guggulu* can be explained through several mechanisms, like removal of excess cholesterol from the body through conversion of cholesterol to bile acids and subsequent excretion through the entero-hepatic circulation pathway³¹. Inhibition of HMG-CoA reductase and 14-alpha-demethylase reduction by garlic powder and its oil substances reduces cholesterol biosynthesis significantly³².

AVC did not affect the BSL in comparison to the fructose control group (Table 4). However, *Katuki* is also an ingredient of AVC, which is in larger quantity in *Arogyavardhini rasa*, which possesses anti-hyperglycemic activity. Aqueous extract of *Picrorhiza kurroa* increases the insulin-mediated translocation of GLUT-4 from the cytosol to the plasma membrane and increase glucose uptake by skeletal muscles and improves glycaemic control³³. Other content of AVC like *Triphala*³⁴, *Nimba*³⁵ leaves juice and *Shilajatu*³⁶ also possess antihyperglycemic activities.

There was a significant elevation of SGPT and alkaline phosphatase levels in the fructose control group (Table 5). The liver contains the highest amount of SGPT in comparison to other tissues. Elevation of SGPT is indicative of liver injury due to leakage of this enzyme from the tissue into the serum. An increased level of alkaline phosphatase suggests obstructive liver damage. Treatment with AVC attenuated the increased levels of SGPT and alkaline phosphatase in albino rats, which suggest the hepatoprotective role of the drug in fructose-induced dyslipidemia in rats.

Overall, the AVC reduced triglyceride, transaminase, and alkaline phosphatase, which suggest that the drug has potential as anti-dyslipidemic and may be protective for degenerative changes produced by 10% fructose in the liver, kidney, and heart of albino rats. The results of biochemical parameters corroborate with the histopathological study. The histopathological study also clearly indicates the presence of pathological

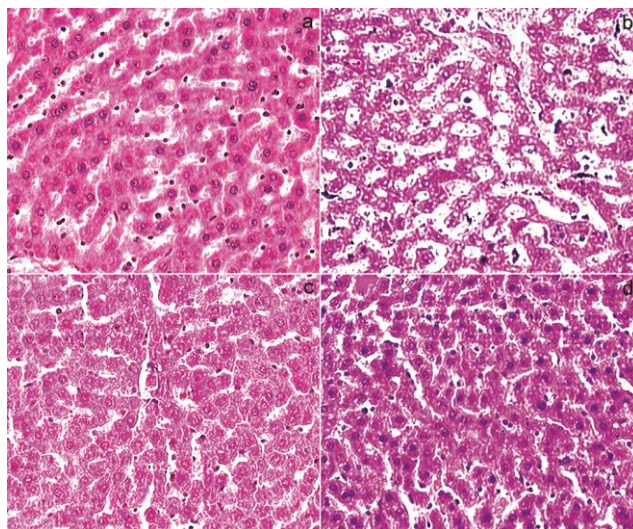


Fig. 1 — Photomicrographs of representative sections of Liver taken at x 400 magnification. a) Normal cytoarchitecture (Normal control group), b) Sinusoidal inflammation, fatty changes and necrosis (fructose control group), c) Mild fatty changes (AVC treated group), and d) Almost normal cytoarchitecture (Pioglitazone treated group).

changes in liver, kidney, and heart of fructose treated rats whereas the AVC prevented dyslipidemia-induced pathological changes in these organs (Fig. 1-3).

Further, the AVC is having *Triphala* as one of the contents, which is a well-known drug having rich antioxidants, antihyperlipidemic, cardioprotective, and many more pharmacological activities. Ingredients of *Triphala* are reported with different pharmacological actions. *Haritaki* is reported to have antioxidant³⁷ and cardioprotective actions³⁸. *Bibhitaki* has been reported to have antioxidant³⁷ and hepatoprotective activities³⁹. *Amalaki* has antioxidant⁴⁰, cardioprotective⁴¹, and hepatoprotective action⁴².

Thus, the observed anti-dyslipidemic activity of the AVC may be attributed to the involvement of one or more mechanisms viz., by interfering with the absorption of the cholesterol from dietary sources, by interfering with the re-esterification or incorporation

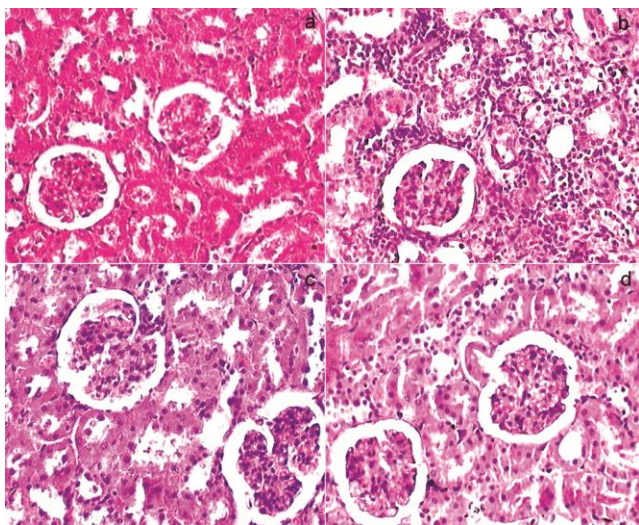


Fig. 2 — Photomicrographs of representative sections of Kidney taken at x 400 magnification. a) Normal cytoarchitecture (Normal control group), b) Degenerative fatty changes and cell infiltration (fructose control group), c) Almost normal cytoarchitecture (AVC treated group), and d) Almost normal cytoarchitecture (Pioglitazone treated group).

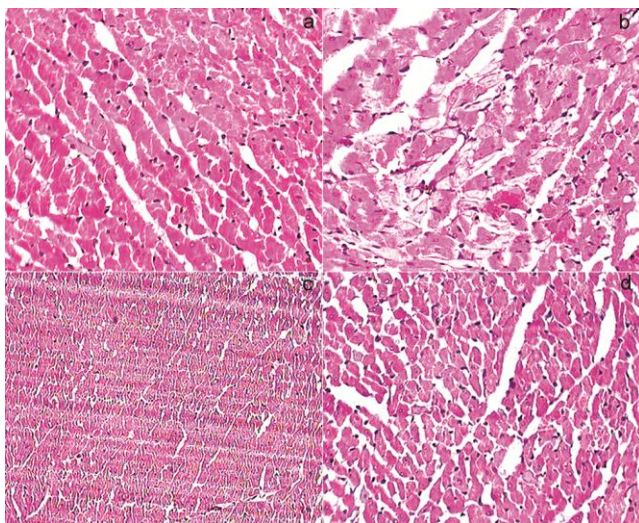


Fig. 3 — Photomicrographs of representative sections of Heart taken at x 200 magnification. a) Normal cytoarchitecture (Normal control group), b) Fatty changes (fructose control group), c) Almost normal cytoarchitecture (AVC treated group), and, d) Almost normal cytoarchitecture (Pioglitazone treated group).

of fatty acids to form chylomicrons in the intestinal epithelial cells, by interfering with the formation of endogenous triglycerides in the tissues by inhibiting the enzyme diacylglycerol transferase, by interfering with the transport of triglycerides from the endoplasmic reticulum to a microsomal site which is by microsomal triglyceride transport protein, by inhibiting the activity of the lipoprotein lipase at different sites, or by inhibiting the activity of the rate-

limiting enzyme in cholesterol bio-synthesis- HMG-CoA (3-hydroxy 3-methyl 3-methylglutaryl CoA).

Conclusion

From the present study, it is concluded that the *Arogyavardhini* compound is safe up to an oral dose of 2000 mg/kg in albino rats. It has a protective role in fructose-induced dyslipidemia in albino rats, hence may be useful in metabolic syndrome.

Conflict of interest

The authors declare no conflict of interest

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