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A randomized controlled clinical trial and preclinical efficacy of an Ayurvedic formulation *Arjuna Ksheerapaka Churna* for dyslipidemia

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Arjuna Ksheerapaka is an Ayurvedic formulation prepared as a milk decoction of the powdered bark of *Terminalia arjuna* (Roxb.) Wight & Arn. It is clinically used for a variety of cardiovascular conditions. The current study investigated the efficacy of *Arjuna Ksheerapaka Churna* (AKC) against dyslipidemia using a preclinical model and a randomized, active-controlled clinical trial. Different groups of Sprague Dawley rats were fed with a high-fat diet (HFD) for 7 weeks, followed by 4 weeks of AKC treatment. After that, biochemical and histopathological parameters were studied with rosuvastatin as a reference standard. In the clinical study, 30 patients were randomized in 2 groups (n = 15/group) and provided with AKC 6 g/day or rosuvastatin 10 mg/day orally for 8 weeks. The body mass index, serum biochemical, and haematological parameters were studied at the baseline (start of the treatment) and endpoint (end of the treatment). In the preclinical experiment, a marked decrease in the serum total cholesterol, low-density lipoprotein (LDL), triglycerides, fasting blood glucose, and elevated high-density lipoprotein (HDL) was recorded in the AKC-treated groups compared to the vehicle control. AKC administration also decreased the serum aminotransferases level in contrast to the rosuvastatin treatment. The clinical study showed a marked reduction in triglycerides, total cholesterol, and LDL at the end of AKC treatment compared to the baseline. The effectiveness of AKC on triglycerides, total cholesterol, and LDL reduction at the endpoint was found to be equipotent to that of rosuvastatin. However, insignificant change was observed in the haematological and other biochemical parameters in both groups at the endpoint compared with the baseline. The preclinical results concluded AKC to be safer in a HFD rat model compared to rosuvastatin as it reduced the elevated serum aminotransferases level. The clinical effectiveness of AKC was found to be equipotent to rosuvastatin. Both, clinical and preclinical studies supported the therapeutic efficacy of AKC in dyslipidemia.

Keywords: Ayurveda, Cardiovascular, Dyslipidemia, High-fat diet, Rosuvastatin, *Terminalia arjuna*

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Dyslipidemia is a lipid metabolism disorder characterized by increased circulating cholesterol, low-density lipoprotein (LDL), triglycerides, and reduced high-density lipoprotein (HDL). There has been substantial evidence that dyslipidemia, especially elevated plasma LDL levels, increases the risk of cardiovascular diseases. Hypercholesterolemia is the most prevalent among the different forms of dyslipidemias[1,2]. The World Health Organization (WHO) estimates that dyslipidemia, particularly elevated total cholesterol, accounts for around 2.6 million annual deaths and about 29.7 million total DALYs (Disability-Adjusted Life Years) globally[3]. The prevalence of dyslipidemia varies according to geographical location. Several factors like socioeconomic status, dietary fat intake, obesity, and gender significantly affect the prevalence of dyslipidemia[4,5]. Elevated blood levels of certain lipids also increases the risk of atherosclerosis, which is considered the primary risk factor for potentially life-threatening conditions like stroke, peripheral vascular diseases, and coronary heart diseases[6].

Correcting the deranged lipid levels with suitable therapeutic interventions, along with dietary and lifestyle modifications, helps in dyslipidemia management to a significant extent. Several

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pharmacotherapeutic agents, such as statins, fibrates, niacin, and resins, and their combinations have been used to manage altered lipid levels. However, the use of these agents has been associated with some mild to severe adverse effects like myalgia, myopathy, new-onset type II diabetes mellitus, and rhabdomyolysis[7,8]. In addition to safety and efficacy concerns, patients' adherence is equally challenging with these agents, as they generally have to be continued for an extended period. Therefore, it is crucial to look for alternative therapies with more safety and compliance. A lot of research is in progress to scientifically validate the therapeutic efficacy of traditional knowledge-based leads in experimental studies[9]. *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn is an important medicinal plant described in Ayurveda as *Hridya* (cardioprotective property). In Ayurveda, it has been mentioned that *Arjuna* has *Laghu* (light in nature; easy to digest), *Ruksha Guna* (dryness), *Kashaya Rasa* (Astringent), *Katu Vipaka* (post digestion effect with acid predominance), *Shita Virya* (cold in potency), and *Hridya Prabhava* (cardiac health) properties. Owing to all these properties, *Arjuna* seems to possess good *Lekhana Karma* (scraping action) and *Medhara* (fat reduction) effects. *Ksheerapaka* (milk decoction) is one of the unique classical formulations described in Ayurveda. The formulation is prepared by mixing coarsely powdered *T. arjuna* bark with milk and water in specified ratios. The mixture is heated on a low flame, concentrated, kept at room temperature, and consumed orally. As per the text, *Rukshata* (dryness) of *T. arjuna* bark and *Kaphakaritva* (*Kapha* aggravation property) of milk is reduced by preparing the drug as *Ksheerapaka*[10].

In Ayurveda, *T. arjuna* is also prescribed topically for treating wounds, haemorrhages, and ulcers[11]. Previous studies revealed that several secondary metabolites like flavonoids, tannins, alkaloids, and saponins might contribute to lipid-lowering effect. The bark of *T. arjuna* contains many of the mentioned bioactive metabolites that may account for its hypolipidemic activity. Similar compounds from other plants have also shown lipid-lowering effects[12-14]. Evidences from various experimental studies have demonstrated the antiatherogenic, hypotensive, inotropic, anti-inflammatory, antithrombotic, and antioxidant activities of *T. arjuna*[15]. Based on the literature, the current study was designed to investigate the preclinical efficacy of *Arjuna Ksheerapaka Churna* (AKC) in a high-fat diet (HFD) rat dyslipidemia model. Further, the study also investigated clinical safety and effectiveness of AKC in a randomized, active-controlled trial. The conventional *Ksheerapaka* formulation was prepared, lyophilized, and converted into powder for better compliance.

Material and Methods

Drugs and chemicals

T. arjuna bark was procured from the Herbal Garden of Research Institute in Indian System of Medicine (RIISM), Joginder Nagar, Himachal Pradesh, India. Rosuvastatin was procured from Sun Pharma Industries Ltd, East-Sikkim, India.

Plant material and AKC preparation

A standard *Arjuna Ksheerapaka* was prepared as described in Sharangadhar Samhita[16]. The dried *T. arjuna* bark was coarsely powdered and mixed with milk 8 times its quantity by weight. After that, water was added to 4 times the amount of milk. The mixture was heated on a mild flame until all the quantity equivalent to the added water evaporated. The concentrate was then converted to powder using a lyophilizer (Operon; FDTE 5012, South Korea). The lyophilization was done under a pre-freezing condition of -25°C for 4 h and sublimation at a constant rate (5°C/h). The powder obtained was packed immediately and stored in a cool and dry place for further use. The analysis of the final preparation was performed as per the ASU Pharmacopoeia, India standards at the Drug Testing Laboratory, RIISM, Joginder Nagar, Himachal Pradesh, India (Ref No. DTL/PP/15/19-352).

Preclinical study

Animals and ethics

Sprague Dawley male rats of 2-3 months' age were used in the present study. The animals were acclimatized 1 week before initiation of the experiments. The rats were kept in the experimental room and housed in the standard polycarbonate cages in groups as specified by CCSEA (Committee for Control and Supervision of Experiments on Animals), Governments of India. The room was maintained at relative humidity of 50-60%, temperature 25±2°C, and 12 h light/dark cycle. All the rats were provided with standard chow diet and RO water *ad libitum* during the acclimatization period. The animal experimental protocol for the study was duly

approved by the IAEC (Institutional Animal Ethics Committee) of the CSIR-Institute of Himalayan Bioresource Technology (CSIR-IHBT), Palampur, Himachal Pradesh, India (Approval No.: IAEC/IHBT P-8/Dec 2019; dated 15th December 2019).

Preclinical experimental protocol

After acclimatization, a group of rats (*veh*; $n = 6$) was fed with standard chow diet, while other animals ($n = 24$) were provided with a HFD. RO water was provided to all the animals' *ad libitum*. The HFD was prepared in-house, as described by Srinivasan *et al.*[17], with minor modifications. Each kilogram (kg) of the HFD contained 365 g powdered standard chow diet, 305 g lard, 250 g casein, 30 g standard vitamin mix, 30 g standard mineral mix, 10 g cholesterol, 5 g soyabean oil, 3 g DL-methionine, 1 g sodium chloride and 1 g yeast powder. After 7 weeks of HFD intake, rats were randomly separated into 4 groups ($n = 6$). A group served as vehicle control (*hfd/veh*) and was daily provided with 0.5% of carboxymethyl cellulose sodium (5 mL/kg; *p.o.*). A standard reference group (*hfd/rst*) was treated daily with rosuvastatin (10 mg/kg; *p.o.*). AKC was daily provided to the remaining 2 groups at 620 mg/kg; *p.o.* (*hfd/akcLD*) and 1240 mg/kg; *p.o.* (*hfd/akcHD*) as a suspension in 0.5% of carboxymethyl cellulose. The treatment was provided for the subsequent 4 weeks along with HFD. The animal dose of AKC was calculated based on the equivalent human dose (described below in the clinical study section), assuming a human weight of 60 kg[18]. At the end of 4 weeks' treatment, the change in body weight of each animal over the mean weight of the *veh* group was calculated. After that, blood was collected from each rat for biochemical estimation *via.,* retro-orbital plexus. All the animals were sacrificed after blood sampling to isolate the liver and the adipose tissue for histopathology.

Serum biochemistry

The collected blood samples were allowed to clot and subjected to centrifugation for the serum separation. The levels of total cholesterol, LDL, triglycerides, HDL, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and glucose were assayed on an automated Biochemical Analyzer (ERBA-EM 200) using standard biochemical kits (ERBA, Transasia, India) as per manufacturer's instruction.

Histopathological examinations

The isolated adipose and liver tissues were kept in 10% neutral buffered formalin. The tissues were washed under running water for overnight, after which they were dehydrated with 50%, 70%, and 90% of alcohol. The clearing was performed with xylene, and embedding was done in paraffin. After that, tissue sections were taken and stained with the help of hematoxylin, and eosin staining by a method described earlier[19]. The stained sections were examined under a microscope (Bright field; Olympus BX53). Images were taken at 10X magnification. Adipocyte size was quantified by measuring the diameter (major axis) of 10 cells per rat section in each group using cellSens imaging software.

Clinical study

Participants

A total of 35 patients were screened based on diagnostic criteria, out of which 30 patients satisfying the inclusion criteria were enrolled for the trial. A written informed consent from each patient was taken prior to inclusion in the study in a language the patient was well versed.

Diagnostic criteria

Patients with one or more of the pathological findings as total cholesterol > 200 mg/dL, LDL > 100 mg/dL, triglycerides > 150 mg/dL, and HDL < 40 mg/dL were considered dyslipidemic.

Inclusion criteria

Both males and females with age 20 to 70 years, ready to participate in the study by providing the written informed consent, and satisfying the diagnostic criteria were included in the trial.

Exclusion criteria

Poorly controlled hypertensive patients with ≥ 160 mm of Hg systolic or ≥ 100 mm of Hg diastolic blood pressure. Patients with concomitant conditions like acute or chronic renal failure, congestive heart failure, ischemic heart disease, other systemic ailments needing prolonged treatment (like tuberculosis, autoimmune disorders, malignancies, *etc.*). Patient who had participated in another study during the past 6 months.

Protocol for clinical study

The trial was a randomized, active-controlled study involving 2 parallel groups (Fig. 1). The trial was performed at the Rajiv Gandhi Government Post-Graduate Ayurvedic College and Hospital, Paprola, Himachal Pradesh, India. It was initiated with the recruitment of patients from 1st July 2020 to 16th April 2021. The outcomes of the trial were reported as per

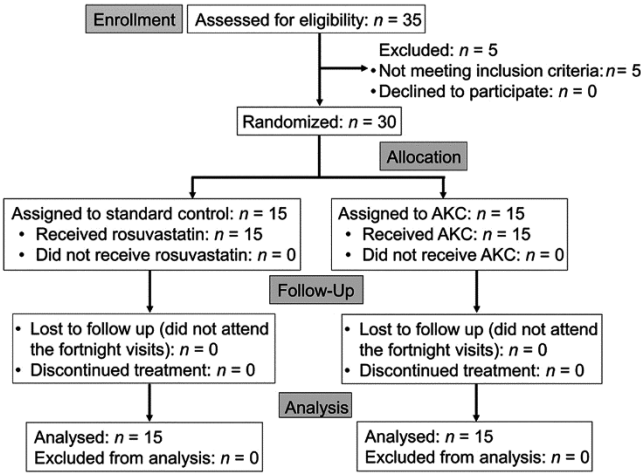

Fig. 1 — CONSORT flowchart of the clinical trial. AKC: *Arjuna Ksheerapaka Churna*

the CONSORT guidelines. The subject randomization was done by using a random number table. The patients were allocated to standard control and trial groups in 1:1 ratio. Group I (n = 15) patients were provided with 3 g of AKC with water before or after meal at every 12 h (twice a day) for 8 weeks *p.o.*, whereas Group II (n = 15) patients were given 10 mg rosuvastatin tablet before bedtime for 8 weeks. Furthermore, the patients in both the groups were also asked to adhere a similar activity level and dietary schedule as it was before the study. All the patients recruited for the study completed the trial. The serum total cholesterol, triglycerides, HDL, LDL, AST, ALT, creatinine and blood urea levels of each patient were assayed on a Biochemical Analyzer (ERBA-EM 200). The differential and total leucocyte counts, and haemoglobin level of patients were recorded using a Hematology Analyzer (H360 ERBA, Erba, Transasia, India). Whereas, the standard Westergren method was used to calculate erythrocyte sedimentation rate (ESR). The body mass index (BMI) was determined using Quetelet's index, *i.e.,* BMI = weight (kg) / height (m2)[20]. Body weight was measured using Omron HBF 212 full body composition monitor (Kyoto, Japan). The primary outcome variables of the study included the levels of total cholesterol, triglycerides, HDL, and LDL in the serum. Whereas, the secondary outcome variables comprised total and differential leucocyte counts, BMI, ESR, haemoglobin level, blood urea and serum creatinine. The patients were followed up once in every fortnight for clinical evaluation and adherence assessment. The haematological and biochemical investigations were done before the commencement (baseline observations) and at the trial's end (endpoint observations). The compliance of each patient to the respective treatment was ensured by recording the number of strips/packets returned during the follow-ups. The clinical study protocol was duly approved by the IEC (Institutional Ethics Committee) of the Rajiv Gandhi Government Post-Graduate Ayurvedic College and Hospital, Paprola, Himachal Pradesh, India (Approval No.: Ayu/IEC/2019/1176; dated: 27th September 2019). The trial was strictly conducted according to the revised Declaration of Helsinki[21]. The written informed consent was obtained from the patients prior to enrolling them for the trial. A detailed proforma was designed to record the particulars of the patients related to disease, detailed history, demographic profile, information of general physical and systemic examinations, blood investigations and anthropometric measurements. The experimental trial was registered with the Clinical Trial Registry of India (https://ctri.icmr.org.in/; Registration No.: CTRI/2020/06/026173).

Statistical analysis

Each value was represented as mean ± standard error of the mean. The inter-group variation in the preclinical study parameters was analysed by one-way analysis of variance followed by Tukey's *post hoc* test. The statistical comparison of the clinical parameters among both the groups and within each group (baseline and endpoint) was performed by the Student's t-test. The statistical difference among the parameters was considered significant at $p<0.05$. The statistical analysis was performed by SigmaStat® version 4.0 statistical software.

Results

Effect of AKC on body weight of rats

The mean body weight of vehicle control *hfd/veh* group was increased significantly ($p<0.001$) compared to the naïve *veh* group. In contrast to *hfd/veh* group, there was a marked decrease in body weight of AKC treated *hfd/akcLD* ($p=0.015$) and *hfd/akcHD* ($p=0.003$) groups. However, insignificant ($p=0.08$) change in body weight was observed among rosuvastatin treated *hfd/rst* group and *hfd/veh* group. Interestingly, the body weight in AKC treated *hfd/akcLD* ($p<0.001$) and *hfd/akcHD* ($p=0.005$) groups remained significantly increased compared to *veh* group (Fig. 2a).

Effect of AKC on serum biochemistry of rats

The serum triglycerides and total cholesterol levels were significantly (p<0.001) increased in vehicle control *hfd/veh* group compared to normal *veh* group. AKC at both the doses and rosuvastatin treatment showed a marked (p<0.001) reduction in the levels of cholesterol and triglycerides compared to *hfd/veh* group. The total cholesterol level remained significantly higher in *hfd/akcLD* (p=0.002), *hfd/akcHD* (p=0.004) and *hfd/rst* groups (p=0.011) compared to naive *veh* group (Fig. 2b). Similarly, *hfd/akcLD*, *hfd/akcHD* and *hfd/rst* groups showed a significant (p<0.001) rise in triglycerides level in comparison to *veh* group (Fig. 2c). However, insignificant changes in the cholesterol and triglycerides was observed among AKC and rosuvastatin treated groups.

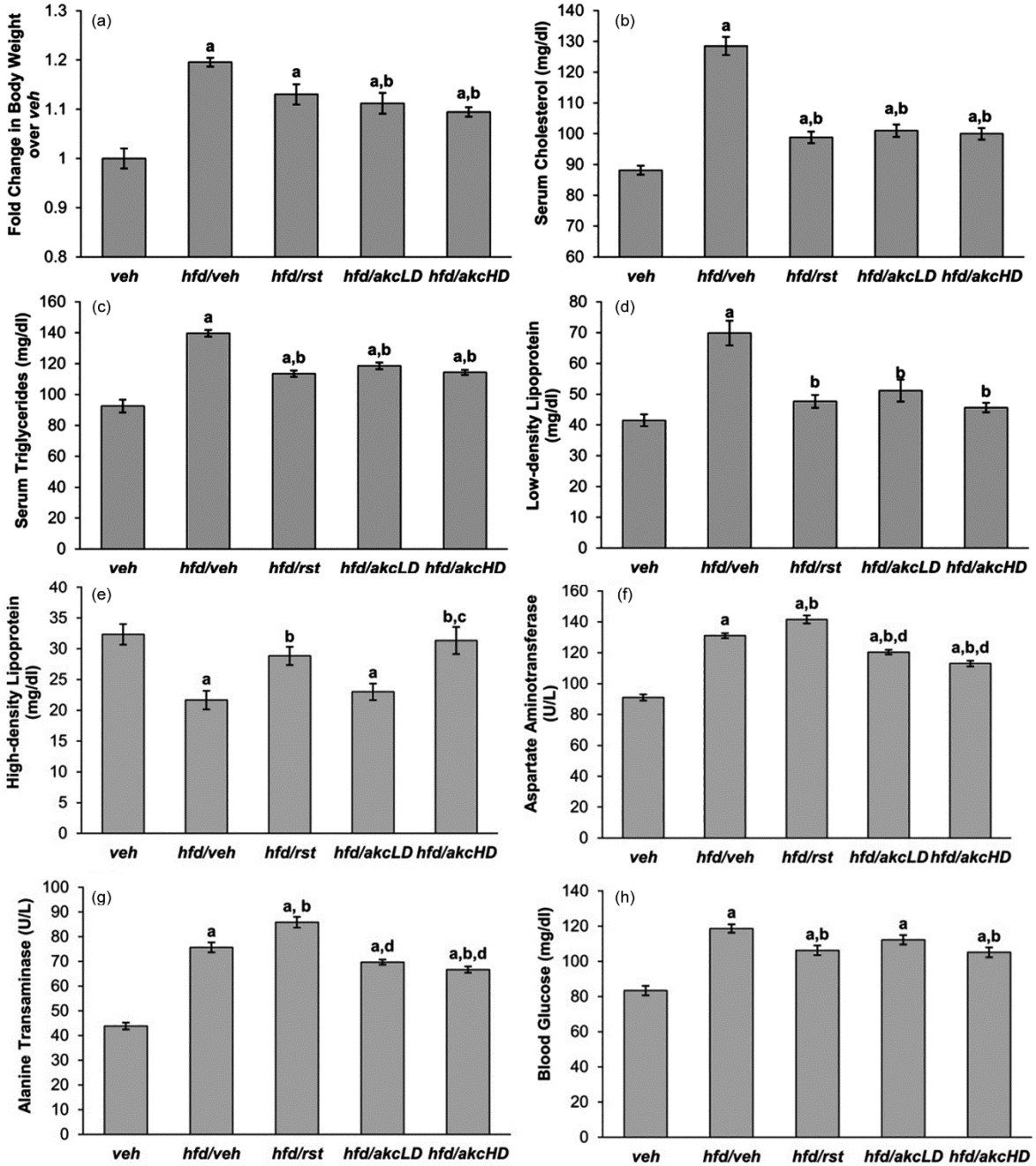

Fig. 2 — Effect of AKC on rats (a) body weight, serum (b) cholesterol, (c) triglycerides, (d) low-density lipoprotein, (e) high-density lipoprotein, (f) aspartate aminotransferase, (g) alanine transaminase and, (h) fasting blood glucose with rosuvastatin as a standard. [a]p<0.05 compared to *veh*; [b]p<0.05 compared to *hfd/veh*; [c]p<0.05 compared to *hfd/akcLD* and; [d]p<0.05 compared to *hfd/rst*

The result showed a significantly ($p<0.001$) increased the serum LDL level of HFD fed *hfd/veh* group in comparison to *veh* group. A marked ($p<0.001$) reduction in LDL level was noted in the groups treated with low and high dose of AKC and standard rosuvastatin compared to *hfd/veh* group. The level of LDL in all the 3 treated groups remained normal as insignificant changes was observed compared to *veh* group (Fig. 2d).

There was a marked increase in HDL level of *hfd/akcHD* ($p=0.003$) and *hfd/rst* ($p=0.041$) groups compared to normal *veh* group. The HDL level remained significantly ($p=0.001$) reduced in *hfd/veh* group as that of *veh* group. AKC treatment at low dose in *hfd/akcLD* group remained significantly ($p=0.005$) reduced compared to *veh* group, and showed insignificant changes ($p=0.979$) in HDL level in comparison to *hfd/veh* group. AKC treatment in *hfd/akcHD* also showed a significant ($p=0.013$) increase in HDL level in comparison to *hfd/akcLD* group (Fig. 2e).

HFD fed rats of *hfd/veh* group showed a marked increase ($p<0.001$) in the AST (Fig. 2f) and ALT (Fig. 2g) compared to normal *veh* group animals. AKC treated *hfd/akcLD* ($p=0.006$) and *hfd/akcHD* ($p<0.001$) groups showed a marked decrease in AST levels compared to *hfd/veh* group. However, a marked ($p=0.005$) decrease in the ALT level was only noted at a higher dose of AKC in *hfd/akcHD* group compared to *hfd/veh* group. In contrast, the level of both AST ($p=0.007$) and ALT ($p=0.002$) was significantly elevated following treatment with rosuvastatin in *hfd/rst* group, compared to vehicle control *hfd/veh* group. Furthermore, both the AKC treated groups showed a marked ($p<0.001$) reduction in the levels of AST and ALT compared to *hfd/rst* group. All the HFD fed animals of *hfd/akcLD*, *hfd/akcHD* and *hfd/rst* groups showed a marked ($p<0.001$) increase in AST and ALT levels compared to normal diet fed animals of *veh* group.

The fasting blood glucose level was observed to be significantly ($p<0.001$) elevated in all the HFD fed groups (*hfd/veh*, *hfd/akcLD*, *hfd/akcHD* and *hfd/rst*) compared to the naive *veh* group (Fig. 2h). The glucose level was significantly reduced in *hfd/akcHD* ($p=0.011$) and *hfd/rst* ($p=0.022$) groups in comparison to the vehicle control *hfd/veh* group. AKC treatment at lower dose in *hfd/akcLD* group showed insignificant ($p=0.45$) change on the blood glucose level in comparison to *hfd/veh* group.

Effect on histological changes in rat adipose tissue and liver

There was a marked ($p<0.001$) increase in the adipocyte size in the vehicle control *hfd/veh* group in comparison to naïve *veh* group. However, treatment with rosuvastatin, AKC 620 mg/kg and AKC 1240 mg/kg showed a marked ($p<0.001$) reduction in the adipocyte size compared to *hfd/veh* group. However, the adipocytes size in *hfd/rst* ($p<0.001$), *hfd/akcLD* ($p<0.001$) and *hfd/akcHD* ($p=0.003$) remained significantly increased compared to normal *veh* group. Insignificant change in the adipocyte size was observed among rosuvastatin and AKC treated groups (Fig. 3a). Feeding of HFD resulted in the deposition of fat vacuoles in the hepatocytes and sinusoidal congestion (Fig. 3b). The severity of fatty changes was less in *hfd/akcLD* and *hfd/akcHD* groups in contrast to *hfd/veh* group. In *hfd/rst* group the severity of fatty changes was less compared to *hfd/veh* group; however, mild sinusoidal dilatational and occasional inflammatory foci were the additional features.

Effect on clinical parameters

The patients in both Group I and II completed the entire allocated treatment of AKC and rosuvastatin respectively during the trial. The mean age of the subjects in both groups was 48.87 (± 7.65; standard deviation) years. Among 30 randomized patients, 16 (53.33%) were females, and the remaining were males. The findings of all clinical parameter in Group I and II at baseline and endpoint of respective treatment are shown in Table 1. A significant decrease in total cholesterol ($p=0.005$), LDL ($p=0.012$), and triglycerides ($p<0.001$) was observed following 8 weeks of treatment with AKC compared to the baseline value. Similarly, daily treatment with rosuvastatin significantly reduced total cholesterol ($p<0.001$), triglycerides ($p=0.002$), and LDL ($p<0.001$) compared to the baseline. The mean HDL level of the recruited patients in both groups was normal. Interestingly, insignificant change in the level of HDL was observed when compared within the groups among baseline and endpoint and between AKC and rosuvastatin-treated groups at the treatment endpoint. Notably, body mass index and fasting blood glucose remained unchanged in both the groups in comparison to each other at the end of the treatment and within the group between the start and end of the treatment. Similarly, an insignificant change was noted in both Group I and II on the remaining studied biochemical parameters, including ALT, AST, blood urea, serum creatinine, total leucocyte and differential

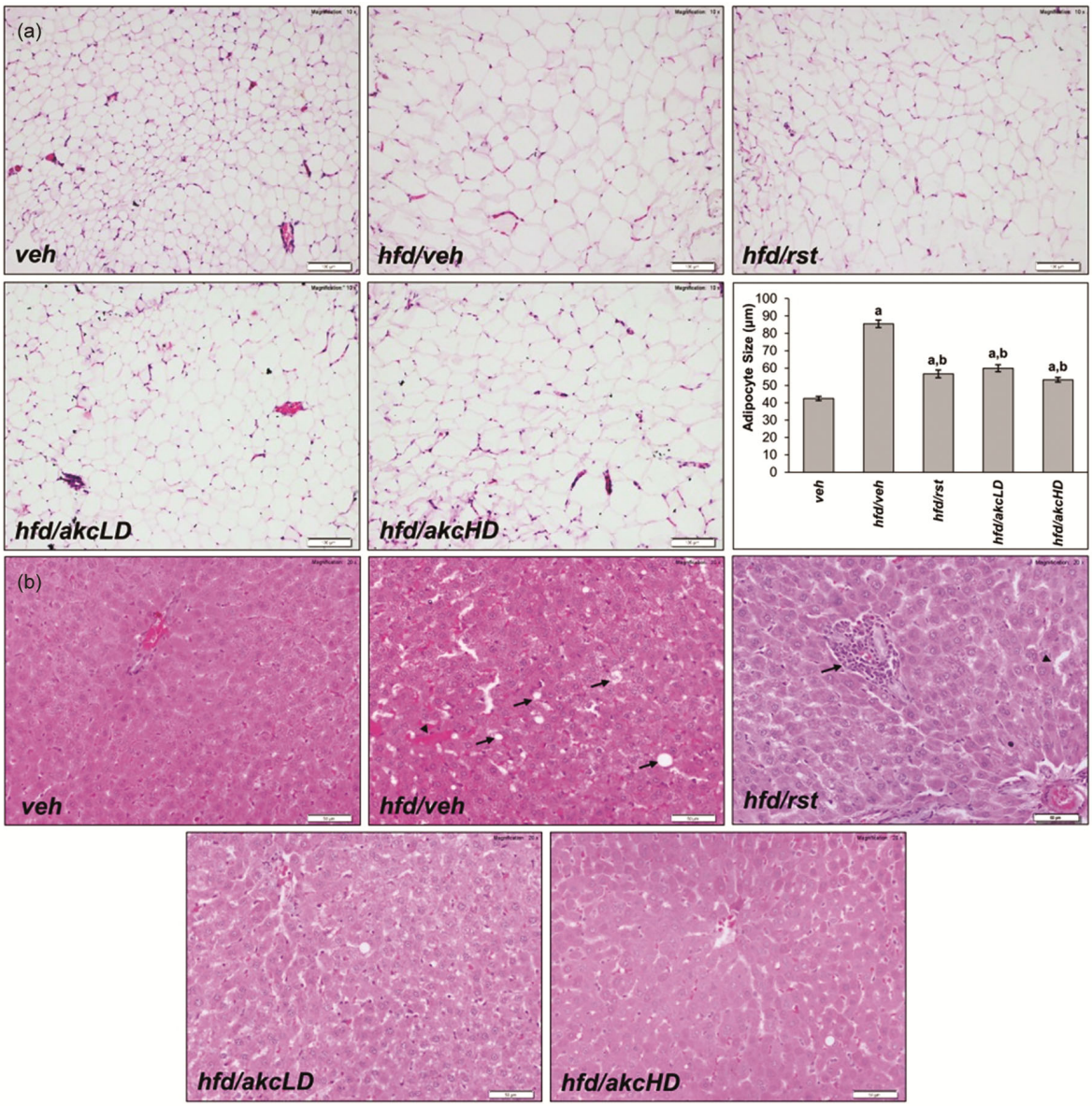

Fig. 3 — Effect of AKC on adipocyte size (a) with rosuvastatin as a standard. The histopathological analysis of the liver sections (b) showed normal structure in *veh* group, fat vacuoles in the hepatocytes (arrow) and sinusoidal congestion (arrowhead) in *hfd/veh* group, mild sinusoidal dilatation (arrowhead) and inflammatory cells (arrow) in *hfd/rst* group, whereas reduced severity of changes in the liver tissues were observed in *hfd/akcLD* and *hfd/akcHD* groups. [a]$p<0.05$ compared to *veh* and; [b]$p<0.05$ compared to *hfd/veh*.

leucocyte counts, ESR and haemoglobin when comparison was made at the baseline and endpoint of the respective treatments.

Discussion

The current study investigated the preclinical and clinical efficacy of AKC. The results showed that AKC administration for 4 weeks in rats kept on HFD for 7 weeks reduced the body weight, total serum cholesterol, triglycerides, LDL, blood glucose, and increased HDL. The treatment also reduced the adipocyte size and prevented pathological changes in the rat liver. In contrast to vehicle control and rosuvastatin-treated groups, AKC treatment reduced the rat serum aminotransferases level. The protective effect of AKC was also observed in the dyslipidemic patients after 8 weeks of the treatment, indicated by decreased total cholesterol, triglycerides, and LDL. The effect of AKC in the patients was equipotent to that of rosuvastatin.

Previous studies in rodent models have shown that HFD-induced dyslipidemia causes a significant

Table 1 — Clinical characteristics at baseline and at endpoint of AKC intervention in the randomized active-controlled clinical study

Variables	Group	Baseline (Mean±SEM)	Endpoint (Mean±SEM)	p value (between baseline and endpoint)	Inter-group p value (at endpoint)
BMI (kg/m^2)	I	26.19±1.00	25.67±0.97	= 0.712	= 0.462
	II	26.71±0.77	26.59±0.76	= 0.912	
Total Cholesterol (mg/dL)	I	236.00±13.22	184.90±10.18	= 0.005	= 0.913
	II	236.93±5.56	186.10±3.73	< 0.001	
Triglycerides (mg/dL)	I	250.67±14.49	184.53±10.18	< 0.001	= 0.306
	II	255.13±12.87	199.73±10.43	= 0.002	
LDL (mg/dL)	I	129.40±11.41	94.73±5.87	= 0.012	= 0.502
	II	127.87±7.10	89.80±4.23	< 0.001	
HDL (mg/dL)	I	49.67±3.99	52.20±2.89	= 0.612	= 0.471
	II	53.13±2.94	55.06±2.64	= 0.629	
FBG (mg/dL)	I	90.80±3.16	88.27±2.55	= 0.539	= 0.719
	II	91.00±2.94	89.60±2.63	= 0.725	
Blood Urea (mg/dL)	I	28.67±1.40	27.20±1.23	= 0.437	= 0.176
	II	25.40±1.41	24.60±1.41	= 0.691	
Creatinine (mg/dL)	I	0.88±0.04	0.83±0.03	= 0.380	= 0.380
	II	0.91±0.02	0.88±0.04	= 0.568	
AST (I.U./L)	I	31.33±1.69	30.06±1.51	= 0.580	= 0.716
	II	30.47±1.88	29.13±2.03	= 0.632	
ALT (I.U./L)	I	32.20±1.47	31.20±1.30	= 0.614	= 0.256
	II	34.53±1.45	33.33±1.30	= 0.543	
Hb (g%)	I	13.22±0.43	13.37±0.42	= 0.805	= 0.235
	II	12.53±0.39	12.69±0.37	= 0.768	
TLC (per cumm)	I	7120±431.07	6900±420.20	= 0.718	= 0.403
	II	7540±387.64	7413±433.57	= 0.829	
Lymphocytes (%)	I	33.86±1.06	32.14±1.32	= 0.318	= 0.558
	II	33.13±1.06	31.08±1.20	= 0.213	
Mixed cells (%)	I	7.98±0.26	7.62±0.26	= 0.336	= 0.812
	II	6.51±0.483	7.50±0.42	= 0.132	
Neutrophils (%)	I	59.63±1.54	58.13±1.34	= 0.469	= 0.067
	II	60.35±0.98	61.41±1.08	= 0.473	
ESR (mm fall infirst hour)	I	16.87±1.54	15.60±0.89	= 0.481	= 0.297
	II	17.87±2.05	14.13±1.06	= 0.116	

The patients of Group I (n = 15) were provided with 3 g of AKC *p.o.* every 12 h. Group II (n = 15) patients were given a rosuvastatin 10 mg *p.o.* tablet before bed time for 8 weeks. The baseline value represents observation at start of the intervention, whereas the endpoint value indicates observation at the end of the respective treatment. AST: Aspartate aminotransferase; ALT: Alanine transaminase; ESR: Erythrocyte sedimentation rate; BMI: Body mass index; FBG: Fasting blood glucose; Hb: Haemoglobin; HDL: High-density lipoprotein; LDL: Low-density lipoprotein and; TLC: Total leukocyte count

increase in the serum triglycerides, LDL, total cholesterol, and decrease in HDL levels due to enhanced absorption and secretion by intestine and decreased metabolism of cholesterol[22,23]. HFD rich in saturated fatty acids lead to elevated 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase activity, thereby, promoting cholesterogenesis. Dietary saturated fatty acids could lead to down regulation of LDL receptors causing increased serum LDL levels by altering the activity of hepatic LDL receptor or LDL production rate. Decreased activity of lecithin-cholesterol acyltransferase enzyme due to elevated cholesterol level could be responsible for decrease level of serum HDL in HFD-induced dyslipidemia[24]. Earlier studies also revealed an increase in blood glucose levels and body weight following administration of HFD[22,25]. In line with these literature reports, our preclinical study revealed, an increase in body weight, blood glucose, triglycerides, LDL, total cholesterol and decrease in HDL levels in HFD fed *hfd/veh* group. A lot of variations in the studied parameters have been observed in different earlier studies in HFD rat model. The variations might be due to changes in age group, strain, type of diet and study duration.

A marked dose-dependent decline in body weight was noted in AKC treated rats on HFD. The reduction might be due to decrease in the absorption of fat and/or *Medohara/lekhana karma* (fat lowering/scraping action) of AKC as described in Ayurveda[10]. The hypolipidemic effect of AKC was observed to be similar to as that of rosuvastatin. Some

earlier preclinical studies also showed a direct hypolipidemic potential of *T. arjuna* bark in experimental animal models. A study in diet-induced hyperlipidemia rabbit model showed that treatment of 50% ethanolic extract of *T. arjuna* bark at 100 and 500 mg/kg doses for 60 days reduced total cholesterol and LDL cholesterol. The treatment also reduced LDL/HDL ratio without affecting liver and renal functional and haematological parameters. A reduction in fat deposition in the heart, kidney, and liver was also observed following the extract treatment. The effects were suggested to be due to the occurrence of polyphenolics and flavonoids like, quercetin, pelargonidin, kaempferol and luteolin in *T. arjuna* bark[26]. The hypolipidemic efficacy of 50% v/v ethanol *T. arjuna* bark extract in rat model of HFD has also been reported[27]. Another study proved the lipid lowering effect of 50% ethanol extract of *T. arjuna* bark in type 2 diabetic rats. It showed that 21 days' of the extract treatment improved the oral glucose tolerance, reduced fasting serum glucose, total cholesterol and triglycerides, without affecting the levels serum insulin and liver glycogen[28]. The direct hypolipidemic effect of the *T. arjuna* bark has been suggested to be due to enhanced hepatic cholesterol clearance, lipogenic enzymes down regulation and HMG-CoA reductase enzyme inhibition[29]. Hence, the observed protective effects of the AKC on serum lipids and glucose levels might be due to the suggested properties of the *T. arjuna* bark.

Adipose tissue is a metabolically active immune and endocrine organ. It regulates free fatty acid levels of the plasma and plays a crucial role in systemic metabolic homeostasis by producing adipokines. A major cellular component of adipose tissue includes adipocytes. Obesity is well known to be linked with an enhanced adipocyte size. Initially, the fat accumulates in the adipocytes and once their expansion capacity is over, the accumulated fat undergoes lipolysis to form free fatty acids. The excess of free fatty acids is then delivered to the liver to form triglyceride and very low-density lipoprotein. These changes further lead to increased LDL and decreased HDL through multiple pathways[30,31]. The increased adipocyte thickness observed in our study in the HFD fed vehicle control group suggested increased fat deposition. These observations can also be correlated with the increased serum levels of triglycerides and LDL and reduced HDL in the control group. The AKC treatment reduced the adipocyte thickness along with attenuation in the level of triglycerides, LDL, and HDL. It can be correlated that AKC might have promoted fat breakdown or inhibited fat accumulation. However, more studies are required to understand the precise mechanism of action of AKC on adipocytes.

The results of clinical studies also displayed the hypolipidemic effect of AKC as observed in the preclinical experiments. The patients treated with AKC showed a marked reduction in the serum triglycerides, LDL and total cholesterol compared to their baseline levels, without altering HDL level. Interestingly, despite of reduction in the lipid levels, no change was observed on BMI and fasting glucose in both AKC and rosuvastatin treated groups. The clinical hypolipidemic efficacy of AKC was found to be comparable to as that of rosuvastatin, as insignificant alteration was noted on the studied parameters among both the study groups at the treatment endpoint. An earlier prospective cohort study demonstrated a significant reduction in triglycerides, LDL, total cholesterol, serum C-reactive protein, blood glucose, and an elevated HDL level in dyslipidemic subjects treated for 3 weeks with Arjuna powder (5 g; BD), followed by 4 weeks with Arogyavardhini Vati (500 mg, BD)[32]. The study supported the clinical efficacy of *T. arjuna* bark in dyslipidemic patients. Furthermore, all the other serum biochemical and haematological parameters remained unaltered following treatment with AKC suggesting its clinical safety.

AST and ALT are found in the liver and in case of its injury, levels of these enzymes increases in the blood. Hypolipidemic drugs, like statins have been found to possess considerable hepatotoxic activity, with marked rise in AST and ALT levels[33,34]. Supporting several earlier findings, our preclinical results also showed a significant rise in AST and ALT levels in the HFD fed animals. The levels were even higher in rosuvastatin-treated group. The increased transaminases levels were in accordance with the histopathological investigation of the liver sections that showed sinusoidal dilatational and occasional inflammatory foci in the liver sections. AKC suppressed the induction of transaminases as compared to rosuvastatin. Thus, apart from the hypolipidemic activity, AKC was also found to minimize the associated liver damage, suggesting it to be a relatively safe alternative for dyslipidemia. Interestingly, contrary to the preclinical findings, our clinical results showed a

normalized pool of AST and ALT in the both AKC and rosuvastatin treated groups at the baseline and after the treatment. Some previous clinical studies in different age groups have suggested that during dyslipidemia the elevated liver enzymes level is largely affected by factors like age, weight, type of lipid abnormality, waist circumference and associated disease conditions[35-37]. Hence, a normalized pool of AST and ALT observed in the clinical study might be due to the reported factors, however more scientific studies are needed to understand the exact cause of such variations. It has been observed that in clinical setup, statins at low-to-moderate doses are comparatively safe and there is around 1-3% risk of increase in the serum levels of liver aminotransferases[38,39]. Since, in the clinical study, approved safer dose of rosuvastatin for a short duration was provided, hence this might be the reason for a normalized pool of ALT and AST in the Group II (rosuvastatin-treated) at the baseline and after the treatment.

The results of the both preclinical and clinical experiments supported the hypolipidemic potential of AKC equipotent to as that of rosuvastatin in dyslipidemia. However, a few variations in the levels of some parameters were noted among preclinical and clinical results. Multiple factors can be linked with the observed inconsistencies like, variations in metabolic rate, animals kept on HFD compared to humans that were restricted to the normal lifestyle, physiological and anatomical variations and many more. Furthermore, the role of pharmacogenetics and pharmacogenomics has also been suggested to be some important factors responsible for inconsistent results in the clinical and preclinical studies[40].

Conclusions

The study supported clinical and preclinical efficacy and safety of AKC in managing dyslipidemia. The preclinical results concluded AKC to be safer in a HFD rat model compared to rosuvastatin as it reduced the elevated serum aminotransferases level. The clinical effectiveness of AKC was found to be equipotent to rosuvastatin.

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Conflict of Interest

None declared.

Author Contributions

KT performed formal analysis and original draft writing. VP and MG carried out formal analysis and editing original draft. AM involved in conceptualization and editing original draft. VC and DS performed conceptualization, supervision, writing and editing original draft.

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