Research Article

Lagenaria siceraria with Cow Urine Evaluated as A Herbal Drug for Hypolipidemic Potential

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ABSTRACT

L. siceraria fruit is traditionally used for its cardioprotective, cardiotonic, general tonic, aphrodisiac and acts as alternate purgative, diuretic, cardiovascular disorder is claimed to be relieved following regular intake of bottle gourd juice for about 4-6 months. The fruits are edible and considered as good source of vitamin C, carotene, vitamin B-complex, pectin and also contain highest choline level- a lipotropic factor. Description was found as Benefits of herbal drugs in our ancient literature of Ayurvedic and Unani medicine. And this literature also revealed the importance of *L. siceraria* as herbal drugs used in various diseases. The cow urine has a unique place in Ayurveda and has been described in 'Sushrita Samhita' and 'Ashtanga Sangraha' to be the most effective substance/secretion of animal origin with innumerable therapeutic values. We used cow urine with combination of L. *siceraria* fruit juice and extract to evaluated Hypolipidemic activity. The results of study reveal that the juice and hydroalcoholic extract of *Lagenaria siceraria* with Cow Urine when administered to the obese & hyperlipidemic rats causes significant decrease in the body weight, Serum TC, LDL, TG and VLDL level.

Keywords: *Lagenaria siceraria,* hypolipidemic potential, cow urine, herbal drug.

INTRODUCTION

Lagenaria siceraria fruit is used as a vegetable in India. The fruit is traditionally used as a cardiotonic, aphrodisiac and general tonic and liver tonic and against liver disorders and pain, anti-inflammatory, expectorant and diuretic agent. Further, antihepatotoxic activity of fruit pulp, analgesic and anti-inflammatory activity of fruit juice and hypolipidimic activity of the fruit have also been evaluated. Recently, the antioxidant activity of ethanolic extract of epicarp and fresh juice of *L. siceraria* fruit has been reported. Cow urine has a unique place in Ayurveda and has been described in 'Sushrita Samhita' and 'Ashtanga Sangraha' to be the most effective substance/secretion of animal origin with innumerable therapeutic values. It has been recognized as water of life or "Amrita'. Various products of cow urine have been suggested as a successful remedy against more than 100 diseases from fever to cancer. Hyperlipidemia is a condition of excess fatty substances called lipids, largely cholesterol and triglycerides in the blood. It is also called hyperlipoproteinemia because these excess lipids travel in the blood attached to proteins. These fatty substances can remain dissolved while in circulation. It is a disorder of lipid metabolism manifestated by elevation of plasma concentrations of the various lipid and lipoprotein fraction, which is the key risks factors for cardio vascular disease (CVD).[1] It is also defined as an elevation of one or more of the following cholesterol esters, Phospholipids or triglycerides. Abnormalities of plasma lipids can result in predisposition to Coronary, cerebrovascular and peripheral vascular arterial diseases and has been reported & has most common cause of death in developed as well as developing nations. Hyperlipidemia refers to elevated levels of lipids and cholesterol in the blood, and is also identified as dyslipidemia to describe the manifestations of different disorders of lipoprotein metabolism. To the best of current knowledge, the treatment of excess weight and hyperlipidemia necessarily requires changes in eating habits and energy expenditure, accompanied by other approaches tailored to the individual patient's needs. The current antihyperlipidemic therapy includes principally statins and fibrates. The former correct the altered blood lipid profile by inhibiting the biosynthesis of cholesterol and the later acts by enhancing the clearance of triglycerides rich lipoproteins. Management of Hyperlipidemia by Phytotherapy.[2] There existence a plethora of knowledge and information and benefits of herbal drugs in our ancient literature of Ayurvedic and Unani medicine. One of the earliest treatises of Indian medicine, the Charaka Samhita (1000 B.C.) mentions the use of over 2000 herbs for medicinal purpose. According to the WHO survey 80% of the populations living in the developing countries rely almost exclusively on traditional medicine for their primary health care needs. Exploration of the chemical constituents of the plants and pharmacological screening may provide us the basis for developing the leads for development of novel agents. In addition, herbs have provided us some of the very important lifesaving drugs used in the armamentarium of modern medicine. To date, pharmacological treatments do not appear to be effective in producing sustained long-term weight loss and Hypolipidemic. Therefore, future research is necessary to discover new drug therapies that can be used to reduce

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the prevalence of hyperlipidemia. Some common medicinal plants that are used either alone or in association, and sold as industrial preparations or by phytotherapists for the treatment of hyperlipidemia.[3]

Lagenaria siceraria (Molina) Standley syn. *L. leucantha Rusby*; *L. Vulgaris Ser*. (Family: Cucurbitaceae) are commonly known as Bottle gourd, an excellent fruit in the nature having composition of all the essential constituents that are required for normal and good health of humans. It cures pain, ulcers, fever, asthma, and other bronchial disorders. It also cures pain, ulcers, fever, and used for pectoral cough, asthma and other bronchial disorders. *L. siceraria* fruit is traditionally used for its cardioprotective, cardiotonic, general tonic, aphrodisiac and acts as alternate purgative, diuretic, cardiovascular disorder is claimed to be relieved following regular intake of bottle gourd juice for about 4-6 months. The fruits are edible and considered as good source of vitamin C, â-carotene, vitamin B-complex, pectin and also contain highest choline level- a lipotropic factor. Modern phytochemical screening methods showed the presence of triterpenoid cucurbitacins B, D, G, H and reported to contain saponins, essential fixed oils, and vitamins. Decoction of leaves, mixed with sugar given in jaundice. Seeds are nutritive and diuretic, are used in dropsy and as anthelmentic, roots also in the treatment of dropsy. The seeds (wt of 100 seeds, 15 gm) are edible. In china, they are boiled in salt water and eaten as an appetizer.[4]

Taxonomical Classification [5]

- Kingdom: Plantae
- Division: Magnoliophyta
- Class: Magnoliopsida
- Order: Cucurbitales
- Family: Cucurbitaceae
- Genus: *Lagenaria*
- Species: *L. siceraria*
- Part used: Fruit

Cow Urine Therapy

Cow is a mobile dispensary. It is the treasure of medicines. The cow urine therapy is capable of curing several curable and incurable diseases. The holy texts, like Atharva Veda, Charak Samhita, Rajni Ghuntu, Vridhabhagabhatt, Amritasagar, Bhavprakash, Sushrut Samhita contain beautiful description about these things. Cow Urine Treatment and Research Center, Indore has conducted a lot of research in the past few years on patients directly and claimed that it is capable of curing diabetes, blood pressure, asthma, psoriasis, eczema, heart attack, blockage in arteries, fits, cancer, AIDS, piles, prostrate, arthritis, migraine, thyroid, ulcer, acidity, constipation, gynecological problems, ear and nose problems, abortion and several other diseases. Cow urine has a unique place in Ayurveda and has been described in "sushrita samhita" and a ashtanga sangraha to be the most effective substance/secretion of animal origin with innumerable therapeutic value. It has been recognized as water of life or amrita. This kind of alternative treatment as panchgavya therapy or cowpathy has been reported to be beneficial even for dreaded disease like cancer, AIDS, and diabetes. Improvement has been shown or reported with those suffering from flu, allergies, colds, rheumatoid arthritis, bacterial/viral infection, tuberculosis, chicken pox, hepatitis, leucorrhuea, leprosy, ulcer, heart disease, asthma, skin infection, aging, chemical intoxication. Through extensive research studies of cow urine distillated fraction popularly known as ark has been identified as bioenhancer of the activity of commonly used antibiotic, antifungal and anticancer drug. Cow urine enhances the immune competence and improve general health of an individual prevent the free radicals formation and act as anti-aging factor reduce apoptosis in lymphocytes and help them to survive and efficiently repair the damaged DNA and this is effective for cancer therapy. The analysis of cow urine has shown that it contains nitrogen, sulphur, phosphate, sodium, manganese, carbolic acid, iron, silicon, chlorine, magnesium, malic, citric, tartaric and succinic acid, calcium salts, Vitamin A, B, C, D, E, minerals, lactose, enzymes, creatinine, hormones and gold. A person falls ill when there is deficiency or excess of the substances inside the body. The cow urine contains those substances, which are present in the human body. Therefore consumption of cow urine maintains the balance of these substances and cures incurable diseases.

MATERIAL AND METHODS

Material

Before initiation of study, a thorough literature review was done on this particular segment. The authentication of plant was done by Botanical Survey of India, Jodhpur, and Rajasthan. The Study was approved by Institutional Animal Ethics Committee Jodhpur national university, Jodhpur**.** After above procedures, the study was performed in following manner.

Collection of Cow urine and its preparation

The cow urine was collected from Kanhiya Gau shala, Pal Road, Jodhpur and cow urine preparations also collected from there.

Cow urine and it's preparation

Fresh cow urine

Fresh cow urine was collected in the morning, daily from kanhiya Gau shala, Pal Road, Jodhpur

Distillate cow urine (Gau Arc)

Gau arc was prepared by distillation process. Cow urine was boiled in an iron pot to which a vapour condensing device was attached. The vapour through tube was collected in a pot put over cold water.

Residue of cow urine (Ganavati)

This was residue of cow urine after distillation process. Deep iron pan was used and boiled cow urine till it

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become concentrated and salts remained. When the cow urine was concentrated remove it from fire and let it cool.

Collection of plant material

The plant of *Lagenaria siceraria* was collected in the month of March-April from the Jodhpur District of Rajasthan, India. Botanical authentication was confirmed at the Botanical Survey of India, Jodhpur, India. The voucher specimen was deposited in faculty of pharmaceutical sciences for the future reference.

Preparations of hydroalcholic extract (*Lageneria siceraria)*

The fresh fruit of LS was sliced, shade dried and coarsely powdered. A known amount (20g) of the coarse powder was packed in a clean dry soxhlet apparatus. The packed material was extracted with water and ethanol in a 1:1 ratio to obtain hydroalcoholic extract. The completion of extraction was determined by the absence of colours in the side arm of soxhlet apparatus by testing the siphoned solution for absence of any residue on evaporation to dryness. The extract obtained was collected in dry and previously weighed china disk. The solvent was evaporated to dryness on a water bath. After drying the china disk was re-weighed. This procedure was repeated five times to obtained sufficient amount of the extract and yield was calculated as given below-

Weight of china disk= 57.550 gm
Weight of china disk with extract =66.228 gm
Weight of coarse powder to be taken for extraction=20 gm

$$\% \text{ Yield} = \frac{\text{Wt. of china disk with extract} - \text{Wt. of china disk} \times 100}{\text{Wt. of coarse powder to be taken for extraction}}$$

% Yield = (66.228-57.550)*100/20
= 43.39 % w/w

Preparation of fruit juice

The fresh fruit juice of the *L. siceraria* was obtained by crushing the fresh fruits in the mixer. The crushed fruit was then filtered through muslin cloth and further used for study. This procedure was repeated throughout the entire treatment period.

Chemicals

Drugs

- Standard anti-hyperlipidimic by: Atorvastatin
- Other chemical which were used in the study were procured from Loba chem., Ahmadabad. Diagnostic kits (Logotech diagnostic kit) were used in the estimation of biochemical parameters.

Evaluation of chemical constituents of extract and juice of *Legeneria scicereria*

The extract was dissolved in water and fresh juice was prepared and further used for the qualitative chemical evaluation of carbohydrates, Flavonoids, Terpenoids, Glycosides, Protein and Steroids.

Determination of ash value of *Legineria siceraria*

A porcelain crucible was washed and was dried in oven. After drying, the crucible was weighed accurately and one gm of the dry coarse powder of the fruit was taken in crucible and was weighed again. Crucible was heated for 3-4 hour. During this period the powder was converted in to a small quantity of white ash .Crucible with ash was weighed again and the ash value was calculated by following formula-

$$\text{Ash value} = \frac{\text{Wt. of crucible with ash-Weight of empty Crucible} \times 100}{\text{Weight of coarse powder to be taken}}$$

Weight of porcelain crucible with ash=16.819 gm
Weight of empty porcelain crucible =16.812 gm
Weight of coarse powder to be taken=1 gm
= (16.819-16.812)*100/1
= 0.70 % w/w

Selection of animals

Albino rats of Wistar strain were used in the study. The animals were housed under standard environmental conditions with a 12 hour light/dark cycle at the Animal house of the Jodhpur National University, Rajasthan, India. The animals had free access to water ad libitum. The study protocol was approved by the IAEC (Animal Ethical Committee of the Institute), and all procedures were conducted in accordance with the "Guide for the Care and Use of Laboratory Animals".

Anti-hyperlipidemia activity in high-fat diet-induced obese rats

Albino rats were divided into seven groups each comprising five rats. Initially all the animals were given the normal diet for 1 week, period of acclimatization. The Cow urine with herbal combination preparation and standard drug (Atorvastatin) was given after 30 days of High Fat Diet feeding. The Cow urine with herbal combination preparation was given through oro-gastric route.

Group I– This was served as normal control and fed with normal diet throughout the course of study.

Group II- This was served as positive control and fed with high-fat diet throughout the course of study without any treatment.

Group III- This was served as standard and fed with high fat diet for 30 days, treated with Atorvastatin suspension in Tween 80 at dose 10mg/kg; p.o. for next 30 days with normal diet

Group IV- This was fed with the high-fat diet for 30 days and treated with Cow urine(CU) and *Lagenaria siceraria* fruit juice(LSFJ)) in selected dose of 10 ml/kg for next 30 days with normal diet.

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Group V- This was fed with the high-fat diet for 30 days and treated with Cow urine (CU) and *Lagenaria siceraria* fruit juice (LSFJ) in selected dose of 20 ml/kg for next 30 days with normal diet.

Group VI - This was fed with the high-fat diet for 30 days and treated with Cow urine (CU) with Hydroalcohlic extract *Lagenaria siceraria* fruit (LSFE) in selected dose of 100 mg/kg for next 30 days with normal diet.

Group VII - This was fed with the high-fat diet for 30 days and treated with Cow urine (CU) with Hydroalcohlic extract *Lagenaria siceraria* fruit (LSFE) in selected dose of 200 mg/kg for next 30 days with normal diet.

Estimation of Body Weight, Serum Lipid Profile and Biochemical Parameters

The body weight of each animal was weighed initially, after 30 days on feeding high-fat diet and finally on 15 and 30 days of normal diet in all groups. The blood sample (1.5 ml) was collected in eppendrof bullets of 2.0 ml through the retro-orbital plexus using capillary. The collected samples were then centrifuged at 10,000 rotations per minutes (rpm) for 10 minutes. Now the supernatant serum was collected and transferred in new eppendrof bullets. In collected serum, blood lipid profile (TC, HDL, LDL and TG) and biochemical parameter (SGOT, SGPT and creatine kinase) were estimated initially, after 30 days of high-fat diet and finally on 15 and 30 days of treatment in experimental and control group. Serum TC, TG, HDL, SGOT, SGPT and creatine kinase were estimated by using commercially available diagnostic kits (Logotech India Pvt. Ltd, Delhi, India) and Autoanalyzer (21 STAR, Adonis Company, Japan). VLDL was calculated as TG/5 and LDL was estimated by using Friedewald et al. formula as follows:

LDL (mg/dl) = TC − (HDL + VLDL)

Serum cholesterol, serum triglycerides, and serum HDL, were estimated by commercially available kits (Logitech diagnostic kit). All biochemical parameters were determined by using autoanalyser (Star – 21 model, adinose company)

Recording of body weight

Body weight of each animal was recorded and on study days 0,15 and 30

Collection of blood

Blood samples were collected in eppendroff bullet on study days 0, 15 and 30 by retro - orbital plexus and serum was separated by centrifugation (for 10 min at 10000 rpm). Separated serum samples were analysed for biochemical parameters.

Statistical analysis

Experimental values are means ± SD of the number of experiments indicated in the legends. Data were evaluated for statistical significance by Student's t-test and ANOVA. P value of 0.05 or less was considered as statistically significant.

RESULTS AND DISCUSSION

Percentage yield of hydroalcoholic extract

The percentage yield of hydroalcoholic extract of *Lagenaria siceraria* fruit was found to be as 43.39 % w/w.

Ash Value

The ash value of dry coarse powder of *Lagenaria siceraria* fruit was found to be as 0.7 % w/w.

Chemical constituents in cow urine and it's preparations

Fresh cow urine was collected from Kanihya guashala in the morning and gauarc and ganavati were also obtained from guashala. Chemical tests to find out various constituents present in cow urine and its preparations were carried out in laboratory as per tests described. Components found in cow urine preparations.

Consumption of Normal Diet

Normal and high fat diet was prepared according to the given composition and all rats were fed with normal diet for 7 day (period for acclimatization). The average amount of normal diet that was consumed by each animal was recorded every day.

Effect of CU with LSFE and LSFJ on Body Weight of High Fat Diet Induced Obese and hyperlipidemic Rats

Table 1: Mean Body Weight at Initial, after High-Fat Diet (0^{th} day) and at 15^{th} and 30^{th} Days of Treatment in each Group

Groups	Treatment	Initial level Mean ± SD	After high fat diet	After treatment of days	
			0th day^{x} Mean ± SD	15th day^{xx} Mean ±SD	30th day^{xx} Mean ±SD
I	Normal Diet (Normal control)	42.58±8.25	44.70±6.92^{ns}	43.12±5.76^{ns}	44.42±5.78^{ns}
II	High fat diet (positive control)	35.98±8.39	32.38±6.92^{d}	31.00±6.59^{ns}	27.46±3.89^{d}
III	ATV 10 mg/kg	44.42±10.18	38.54±9.05^{c}	64.22±5.16^{b}	72.45±8.39^{b}
IV	CU+LSFJ 10ml/kg	39.62±8.80	38.76±5.76^{ns}	41.72±5.46^{ns}	41.56±2.11^{ns}
V	CU+LSFJ 20 ml/kg	33.58±9.57	31.64±7.91^{ns}	60.28±9.34^{b}	67.00±9.63^{a}
VI	CU+LSFE 100 mg/kg	44.2±6.96	39.82±7.36^{d}	43.90±7.72^{c}	50.66±5.29^{c}
VII	CU+LSFE 200 mg/kg	50.74±5.07	49.26±5.98^{ns}	65.50±1.46^{b}	72.84±2.66^{b}

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ATV: Atorvastatin; LSFJ : *Lagenaria siceraria* fruits juice; LSFE: Hydroalcholic extract of Lagenaria siceraria fruit. Values are expressed in gm as mean±S.D. (n = 5). Values are statistically significant at $^{a}P < 0.0001$ and $^{b}P < 0.001$, $^{c}P < 0.01$, $^{d}P < 0.05$, ns—non-significant ($P > 0.05$).

X Results of 0th day treatment (Hyperlipidemia control) were compared with initial weight by using t-test

XX Results of 15th and 30th day treatment (treated groups) are compared with 0th day treatment (Hyperlipidemia control) by using ANOVA followed by Dunnett's test.

Effect of CU with LSFE and LSFJ on Total Cholesterol level of High Fat Diet Induced Obese & hyperlipidemic rats

Table 2: Mean TC level at Initial, after High-Fat Diet (0th Day) and at 15th and 30th Days of Treatment in Each Group.

Groups	Treatment	Initial weight (Mean±S.D.)	After high fat diet	After treatment of days	
			0th day^{x} (Mean±SD.)	15th day^{xx} (Mean±SD.)	30th day^{xx} (Mean±SD.)
I	Normal Diet (Normal control)	145.89±6.10	156.44±4.64^{ns}	158.78±7.05^{ns}	161.5±6.04^{ns}
II	High fat di (positive control)	143.46±8.50	164.90±3.73^{b}	186.06±4.48^{b}	207.6±11.23^{b}
III	ATV 10 mg/kg	135.5±4.62	156.56±3.27^{a}	147.52±3.03^{c}	139.30±4.54^{b}
IV	CU+LSFJ 10ml/kg	170.06±3.89	189.88±3.51^{a}	187.54±3.52^{ns}	185.36±3.98^{ns}
V	CU+LSFJ 20 ml/kg	138.62±5.79	163.75±7.43^{b}	156.90±8.43^{ns}	148.34±9.04^{c}
VI	CU+LSFE 100 mg/kg	154.14±6.76	178.64±8.13^{b}	175.10±7.57^{ns}	173.22±6.79^{ns}
VII	CU+LSFE 200 mg/kg	148.86±7.66	172.94±9.58^{b}	155.06±3.38^{b}	141.76±5.54^{b}

Table 3: Mean TG Level at Initial, After High-Fat Diet (0th Day) and at 15th and 30th Days of Treatment in Each Group

Groups	Treatment	Initial level Mean±SD	After high fat diet	After treatment of days	
			0th day^{x} Mean±SD	15th day^{xx} Mean±SD	30th day^{xx} Mean±SD
I	Normal Diet (Normal control)	82.5±9.15	96.00±14.84^{ns}	100.76±11.42^{ns}	109.74±12.43^{ns}
II	High fat diet (positive control)	84.7±8.28	199.76±23.94^{a}	280.35±21.47^{b}	305.46±16.94^{b}
III	ATV 10 mg/kg	85.44±15.27	243.92±48.11^{b}	198.20±45.97^{c}	159.38±23.77^{b}
IV	CU+LSFJ 10ml/kg	80.54±12.65	221.54±44.40^{b}	207.38±48.60^{ns}	200.96±48.60^{ns}
V	CU+LSFJ 20 ml/kg	85.28±9.20	232.32±50.27^{b}	151.78±6.27^{b}	128.94±6.86^{a}
VI	CU+LSFE 100 mg/kg	89.16±8.01	212.22±39.89^{b}	167±11^{d}	148.23±8.43^{c}
VII	CU+LSFE 200 mg/kg	97.92±8.41	290.36±13.21^{a}	246.14±12.10^{c}	182.66±13^{b}

ATV: Atorvastatin; LSFJ: *Lagenaria siceraria* fruits juice; LSFE: Hydroalcholic extract of Lagenaria siceraria fruit.

Values are expressed in mg/dl as mean±S.D. (n = 5). Values are statistically significant at $^{a}P < 0.0001$ and

Effect of CU with LSFE and LSFJ on HDL Level of High Fat Diet Induced Obese & hyperlipidemic Rats

Table 4: Mean HDL Level at Initial, After High-Fat Diet (0th Day) and at 15th and 30th Days of Treatment in Each Group

Groups	Treatment	Initial level Mean ± SD	After high fat diet	After treatment of days	
			0th day^{x} Mean ± SD	15th day^{xx} Mean ±SD	30th day^{xx} Mean ±SD
I	Normal Diet (Normal control)	73.58±15.08	80.74±13.36^{ns}	86.22±10.21^{ns}	95.16±13.30^{ns}
II	High fat diet (positive control)	84.60±11.57	182.02±33.38^{b}	237.88±30.66^{c}	252.96±30.05^{b}
III	ATV 10 mg/kg	107.14±21.17	224.60±39.23^{b}	159.14±19.85^{c}	127.64±13.49^{b}
IV	CU+LSFJ 10ml/kg	72.66±8.16	162.46±49.63^{b}	147.20±44.59^{ns}	140.32±44.11^{ns}
V	CU+LSFJ 20 ml/kg	98.42±21.81	204.38±30.42^{b}	189.48±28.48^{d}	142.88±8^{c}
VI	CU+LSFE 100 mg/kg	79.74±8.93	205.06±39.29^{b}	195.02±38.29^{ns}	185.92±40.19^{d}
VII	CU+LSFE200 mg/kg	82.26±8.65	252.76±39.42^{a}	175.85±13.40^{c}	141.94±13.54^{b}

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Effect of CU+LSFE AND CU +LSFJ on LDL Level of High Fat Diet Induced Obese Rats

Table 5: Mean LDL Level at Initial, After High-Fat Diet (0th Day) and at 15th and 30th Days of Treatment in Each Group

Groups	Treatment	Initial level Mean±SD	After high fat diet	After treatment of days	
			0th day^{X} Mean±SD	15th day^{XX} Mean±SD	30th day^{XX} Mean±SD
I	Normal Diet (Normal control)	26.20±11.60	37.15±16.81^{ns}	42.39±13.68^{ns}	48.28±13.66^{ns}
II	High fat diet (positive control)	32.58±8.54	164.16±26.30^{a}	202.56±18^{d}	228.24±11.89^{c}
III	ATV 10 mg/kg	21.48±5.00	159.26±36.46^{a}	101.15±43.94^{ns}	60.45±23.29^{c}
IV	CU+LSFJ 10ml/kg	23.18±17.48	147.08±42.04^{a}	133.02±46.37^{ns}	128.13±44.45^{ns}
V	CU+LSFJ 20 ml/kg	33.76±6.44	161.60±48.21^{a}	55.43±14.76^{b}	33.18±9.07^{b}
VI	CU+LSFE 100 mg/kg	73.01±6.64	131.27±30.40^{b}	83.87±9.73^{d}	60.12±18.65^{c}
VII	CU+LSFE 200 mg/kg	30.73±9.41	190.61±13.70^{a}	148.34±11.4^{c}	81.52±12.60^{b}

Effect of CU+LSFE AND CU +LSFJ on VLDL Level of High Fat Diet Induced Obese Rats

Table 6: Mean VLDL Level at Initial, After High-Fat Diet (0th Day) and at 15th and 30th Days of Treatment in Each Group

Groups	Treatment	Initial level Mean±SD	After high fat diet	After treatment of days	
			0th day^{X} Mean±SD	15th day^{XX} Mean±SD	30th day^{XX} Mean±SD
I	Normal Diet (Normal control)	12.71±3.02	14.15±2.67^{ns}	19.55±2.70^{ns}	17.03±2.66^{ns}
II	High fat diet (positive control)	15.14±2.33	34.60±6.68^{c}	45.77±6.13^{b}	48.792±6.01^{b}
III	ATV 10 mg/kg	18.54±3.75	45.12±7.85^{b}	31.82±3.97^{d}	25.52±2.68^{c}
IV	CU+LSFJ 10ml/kg	12.74±1.67	30.69±9.93^{c}	27.64±8.92^{ns}	26.26±8.82^{ns}
V	CU+LSFJ 20 ml/kg	17.94±4.36	39.08±6.08^{b}	36.09±5.70^{ns}	26.77±1.60^{d}
VI	CU+LSFE 100 mg/kg	14.14±1.79	39.21±7.86^{b}	37.20±7.66^{ns}	35.38±8.04^{ns}
VII	CU+LSFE 200 mg/kg	16.45±1.73	50.55±7.88^{b}	32.43±2.73^{b}	28.41±2.67^{b}

DISCUSSION

Management of hyperlipidemia with the agents devoid of any side effects is still a challenge to the medical system. This has led to an increase in the demand for natural products with antihyperlipidemic activity and fewer side effects. The cow urine with herbal preparations exhibited dose-dependent antidhyperlipidemic property. The antihyperlipidemic effect of these herbal preparations at the different dose is even slightly higher than Atorvastatin 10 mg/kg. Our results are supporting its use as folklore medicine for the treatment of hyperlipidemia and obesity. There was a significant body weight reduction in all experimental group but group treated with LSFE (200 mg/kg/;p.o.) showed more significant reduction on 15th day of treatment.

Effect of CU+LSFE and CU+LSFJ on SGOT Level of High Fat Diet Induced Obese Rats

Table 7: Mean SGOT Level at Initial, After High-Fat Diet (0th Day) and at 15th and 30th Days of Treatment in Each Group

Groups	Treatment	Initial level Mean±SD	After high fat diet	After treatment of days	
			0th day^{X} Mean±SD	15th day^{XX} Mean±SD	30th day^{XX} Mean±SD
I	Normal Diet (Normal control)	35.14±20.98	37.80±11.25^{ns}	40.06±10.25^{ns}	43.26±10.69^{ns}
II	High fat diet (positive control)	45.18±5.67	47.62±4.00^{ns}	48.22±3.27^{ns}	50.58±4.11^{ns}
III	ATV 10 mg/kg	35.60±4.00	37.26±4.12^{ns}	43.56±9.46^{ns}	57.60±11.39^{b}
IV	CU+LSFJ 10ml/kg	31.71±10.60	37.31±6.27^{ns}	34.89±7.00^{ns}	32.41±7.90^{ns}
V	CU+LSFJ 20 ml/kg	30.66±10.18	35.36±10.01^{ns}	27.96±9.44^{ns}	20.87±1.53^{d}
VI	CU+LSFE 100 mg/kg	38.16±6.22	42.86±5.24^{d}	35.12±5.09^{ns}	32.66±4.44^{c}
VII	CU+LSFE 200 mg/kg	39.32±8.51	40.46±5.94^{ns}	32.94±8.35^{ns}	24.24±8.47^{b}

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Effect of CU+LSFE AND CU+LSFJ on SGPT Level of High Fat Diet Induced Obese Rats

Table 8: Mean SGPT Level at Initial, After High-Fat Diet (0th Day) and at 15th and 30th Days of Treatment in Each Group

Groups	Treatment	Initial level Mean±SD	After high fat diet	After treatment of days	
			0th day^{x} Mean±SD	15th day^{xx} Mean±SD	30th day^{xx} Mean±SD
I	Normal Diet (Normal control)	35.44±14.58	37.56±20.48^{ns}	43.00±19.91^{ns}	46.00±18.33^{ns}
II	High fat diet (positive control)	50.02±12.74	55.72±12.00^{ns}	60.64±9.00^{ns}	64.66±11.90^{ns}
III	ATV 10 mg/kg	46.24±14.11	51.62±9.66^{ns}	54.23±17.11^{ns}	58.6±20.73^{ns}
IV	CU+LSFJ 10ml/kg	39.76±5.20	43.26±11.60^{ns}	37.94±9.29^{ns}	34.8±8.96^{ns}
V	CU+LSFJ 20 ml/kg	27.44.±9.90	38.64±7.08^{ns}	32.46±6.75^{ns}	21.5±5.63^{c}
VI	CU+LSFE 100 mg/kg	38.73±11.61	49.63±6.57^{ns}	44.89±6.67^{ns}	39±8.70^{ns}
VII	CU+LSFE 200 mg/kg	33.76±12.02	45.72±14.23^{d}	40.52±9.58^{ns}	19.82±5.19^{c}

The total cholesterol was reduced with higher significance in LSFE (200 mg/kg/;p.o.) and LSFJ (20 ml/kg/;p.o.) when compared with standard drug. The TG levels were reduced more significantly in LSFE (200mg/kg/;p.o.) in comparison with other groups. HDL levels were increased in all studied groups except LSFJ (10 ml/kg/;p.o.) but was more significantly increased in LSFJ (20 ml/kg/;p.o). The LDL levels were reduced abruptly in groups treated with LSFJ (20 ml/kg/;p.o.) after 15th day of treatment, however after 30th day of treatment the LDL levels were reduced in LSFJ (20 ml/kg/;p.o.) and LSFE (200 mg/kg/;p.o.) with higher significance in comparison with other groups. The VLDL levels reduced more significantly in LSFE (20 mg/kg/;p.o.) when compared with standard group.

The SGOT and SGPT levels were elevated in group treated with standard drug but there was a significant decrease in above levels in group treated with LSFE (200 mg/kg/; p.o.). Though the CK levels were elevated in all studied groups but percentage increase was more in standard group when compared with other studied groups.

The above results reveal that the LSFE (200 mg/kg/;p.o.) and LSFJ (20 ml/kg/;p.o.) are effective in management of obesity and hyperlipidemia in comparison with standard marketed preparation. The possible hepato-toxicity and Rhabdomyolysis side effects were also low in above group when compared with standard drug. However the low dose of LSFJ 10 ml/kg/;p.o. is non-effective in management of obesity and hyperlipidemia.

The effects of the cow urine and herbal preparations on body weight in the obese and hyperlipidemic rats give a significance decrease. The results of the body weight analysis indicate that the body weight of the treated obese and hyperlipidemic rats was found to be significantly (P<0.05) decreased when compared with the normal control group.

The body weight was slightly increased in the normal control group compared to initial weight. Treatment with cow urine and herbal preparations and Atorvastatin prevented increase in body weight and the weight was decreased after the treatment. This shows that cow urine and herbal preparations decrease body weight and hyperlipidemic profile and this may help to maintain normal body weight and normal lipid profile and other biochemical parameters.

CONCLUSION

The results of study reveal that the juice and hydroalcoholic extract of *Lagenaria siceraria* with CU when administered to the obese & hyperlipidemic rats causes significant decrease in the body weight, Serum TC, LDL, TG and VLDL level. In present study four preparations were taken i.e. *Lagenaria siceraria* fruit juice (LSFJ) with CU in dose of 10 ml/kg and 20 ml/kg and hydroalcoholic extract of *Lagenaria siceraria* (LSFE) with CU in dose of 100 mg/kg and 200 mg/kg. LSFJ in dose of 20 ml/kg and LSFE in dose of 200 mg/kg showed the most significant results among other preparation in high fat diet induce obese and hyperlipidemic rats. Interestingly LSFE with CU (200 mg/kg; p.o.) showed more significant (p<0.001) reduction in body weight at 15th as well as 30th day of treatment as compare to standard and other groups. It also showed highly significant (p<0.001) reduction in TC, TG, VLDL and LDL level and increase in HDL level at 30th day of treatment and these results are resemble to the standard drug.

LSFJ with CU (20ml/kg; p.o.) showed very significant reduction (p<0.0001) in total cholesterol and increase in HDL level at 30th day of treatment as compare to standard and other groups. This is important in treatment of hypercholesterolemia particularly where low HDL is the most prevalent lipoprotein for abnormality. LSFJ with CU (20ml/kg; p.o.) also showed the more significant reduction (p<0.001) in the LDL level at 15th as well as 30th day of treatment as compare to other groups. This is useful in the treatment of atherosclerosis because high level of TC and most importantly LDL level are the predictors of atherosclerosis and LSFE with CU (20 ml/kg;p.o.) significantly reduced both TC and LDL level. LSFE with CU (100mg/kg; p.o.) showed less significant results throughout the study so it means that lower dose

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of hydroalcholic extract of *Lagenaria siceraria* is not more effective in the treatment of hyperlipidemia and hyperlipidemia. LSFJ with CU (10 ml/kg;p.o.) showed non-significant ($p>0.05$) results throughout the study so it means that lower dose of *Lagenaria siceraria* juice not effective in the treatment of hyperlipidemia and obesity. Additionally the biochemical parameters such as SGOT, SGPT and CK were also studied to evaluate the side effect of the LSFE and LSFJ with respect to the standard drug (ATV-10 mg/kg; p.o). Percentage increment in CK level was more significant in ATV (10 mg/kg;p.o.) treated group on the 30th day of treatment as compare to other groups. SGOT and SGPT level was decrease in LSFE and LSFJ treated groups at both 15th and 30th days of treatment while in the ATV (10 mg/kg;p.o.) treated group the level of SGOT and SGPT was increase. Increased muscle enzymes (SGOT, SGPT and CK) level showed the higher incident of rhabdomyolysis in ATV (10 mg/kg;p.o.) treated group while reduction in SGOT and SGPT level and less increment in CK level in LSFJ and LSFE treated groups showed the hepato-protective property of LS fruit. Finally conclude that specific dose of LSFE can be beneficial to the patients suffering from Hyperlipidemia, hyperlipidemia and atherosclerosis without compromising with wanted but unavoidable side effects of established marketed preparation like statins. The present study helps to support the traditionally claimed antihyperlipidemia, cardio protective and cardiotonic activity of *Lagenaria siceraria* fruits with cow urine. A future work on isolation characterization and pharmacological activity of active constituents of *Lagenaria siceraria* fruit extract and juice is required for further beneficial exploitation which was not done in current study due to time limit of designed protocol.

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