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Original Research Paper

HYPOLIPIDEMIC POTENTIAL OF COW URINE WITH *CARICA PAPAYA* AS A HERBAL DRUG

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ABSTRACT

Phytotherapy today avails itself of the extensive knowledge on its active principles and its chemical and pharmacological characteristics. It seemed, therefore, reasonable and timely to assess the validity of phytotherapeutic products in the adjuvant treatment of hyperlipidemia. Papaya (*Carica papaya* linn**)** is well known for its exceptional nutritional and medicinal properties throughout the world. From the times immemorial, the whole Papaya plant including its leaves, seeds, ripe and unripe fruits and their juice is used as traditional medicine. Cow is a mobile dispensary. It is the treasure of medicines. The cow urine therapy is capable of curing several curable and incurable diseases. Present work aim to check potency of cow urine with freshly prepared hydroalcoholic extract of Carica papaya leaf preparation with potent anti-hyperlipidemic activity. The results of study reveal that the juice and hydroalcoholic extract of Carica papaya with CU when administered to the obese & hyperlipidemic rats causes significant decrease in the body weight, Serum TC, LDL, TG and VLDL level.

Keywords: Cow Urine, Protein, Herbal Drugs.

INTRODUCTION

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.Research studies are being carried out to detect and confirm the action of drugs and natural products that yield better and long-lasting results in terms of weight reduction. In this field, medicinal plants play a very important role. There exists 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. There is a worldwide belief that herbal remedies are safer and less damaging to the human body than synthetic drugs. Therefore laboratories around the world are engaged in screening of plants for biological activities with therapeutics potential. One major criteria for the selection of plant for such a study is traditional healer's claim for its therapeutics usefulness. The traditional Indian medicinal system mentions herbal remedies for the treatment of variety of diseases. The ayurveda has emphasized importance of food in the management of diseases. Even practitioner of modern

system has realized the significance of dietary items, in the form of nutraceutical elements, in the treatment

of chronic diseases.To date, pharmacological treatments do not appear to be effective in producing sustained long-term weight loss and Hyperlipidemic. Therefore, future research is necessary to discover new drug therapies that can be used to reduce the prevalence of hyperlipidemia. Phytotherapy today avails itself of the extensive knowledge on its active principles and its chemical and pharmacological characteristics. Nowadays it is possible to find formulations that maintain the plant-specific characteristics and which undergo microbiological and analytical tests. It seemed, therefore, reasonable and timely to assess the validity of phytotherapeutic products in the adjuvant treatment of hyperlipidemia. Phytotherapy is becoming increasingly popular both for the results it yields in several pathologies, and for a growing sense of mistrust towards conventional medical treatments. However, its popularity is also determined by the desire to find new solutions as a consequence of the new information released by the mass media and industries. It is possible to find on the market medicinal plant products formulated as dry extracts for the preparation of decoction; as total or oil concentrates; tablets and opercles with total phytocomplexes, and less often as hydroalcoholic extracts and tinctures. The products available on the market have been evaluated in relation to the indications, the active principles, the dosage and, therefore, the quantity of active ingredients advised by the producer or the physician. 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.[1] Papaya (*Carica papaya* linn**)** is well known for its exceptional nutritional and medicinal properties throughout the world. From the times immemorial, the whole Papaya plant including its leaves, seeds, ripe and unripe fruits and their juice is used as traditional medicine. Nowadays, Papaya is considered as nutraceutical fruit due to its multifaceted medicinal properties. The prominent medicinal properties of papaya include Anti-fertility, Diuretic, Uretonic, Anti-hypertensive, Hypolipidemic, Anti-helmintic, Wound healing, Anti-fungal, Antibacterial, Antitumor and free radical scavenging activities. Phytochemically, the whole plant contains enzymes (Papain), lycopene, carotenoids, alkaloids, monoterpenoids, flavonoids, mineral and vitamins. This important nutricious fruits feed the body and immune system. In present review article, a attempt is made to compile all the strange facts available about this tasty fruit. This tasty fruit of papaya is popular among family members of all ages for the delicious dishes derived from it.Papaya is a powerhouse of nutrients and is available throughout the year. It is a rich source of three powerful antioxidant vitamin C, vitamin A and vitamin E; the minerals, magnesium and potassium; the B vitamin pantothenic acid and folate and fiber. In addition to all this, it contains a digestive enzyme papain the effectively treats causes of trauma, allergies and sports injuries. All the nutrients of papaya as a whole improve cardiovascular system, protect against heart diseases, heart attacks, strokes and prevent colon cancer. The fruit is an excellent source of beta carotene that prevents damage caused by free radicals that may cause some forms of cancer.[2]

Taxonomical Classification[3]

Domain	Flowering plant
Kingdom	Plantae
Subkingdom	Tracheobionta
Class	Magnoliopsida
Subclass	Dilleniidae
Division	Magnoliophyta
Subdivision	Spermatophyta
Phylum	Steptophyta
Order	Brassicales
Family	Caricaceae
Genus	Carica
Botanical name	*Carica papaya* Linn.

Figure 1: Papaya plant

Papaya belongs to a group of plant species known as laticiferous plants. These plants contain specialised cells (laticifers), dispersed throughout most plant tissues, that secrete a substance known as 'latex'. Latex is a complex mixture of chemical compounds with diverse chem ical activities. Collectively, these compounds are thought to be involved in defence of the plant against a wide range of pests and herbivores (El Moussaoui *et al.* 2001).

Cow Urine Therapy[4]

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/secrection 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, rheumatoids 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 immunocompetence 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, tartric 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. The aim of present study is to evaluate and compare the anti-hyperlipidemia effects of cow urine & freshly prepared juice and extract of Carica papaya leaf in high fat diet induced hyperlipidemia rat with marketed Atorvastatin.

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 and the study was approved by Institutional Animal Ethics Committee .After above procedures, the study was performed in following manner.

Collection of Cow urine and it's preperations

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 preperations

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

Chemical composition of cow urine and it's preparations

Chemical tests for various constituents of cow urine and its preparations were carried out as per tests given below:

Table 1: Chemical tests for various constituents of cow urine and its preparations

Component	Test	Observation	Gau Ark	Ghan-Wati	Fresh Cow Urine
Urea	TEST FOR UREA: UREASE TEST Sample+ Soya bean meal + Phenol red	Red color was obtained	+ve	+ve	+ve
Chloride	Sample+Conc.HNO_3 +$AgNO_3$	White Precipitate was obtained	+ve	+ve	+ve
Sulphate	Sample +$Bacl_2$	White Precipitate was obtained	+ve	+ve	+ve
Calcium	Sample + Amino oxalate	White Precipitate was obtained	+ve	+ve	+ve
Phosphorus	Sample + Conc. Nitric acid	White Precipitate was obtained	+ve		+ve
Carbohydrate	Sample + molish reagent + H_2SO_4	violet ring at the junction was obtained	-ve	-ve	-ve
Malic acid	2-3 ml sample +Added 2-3 drops 5% $fecl_3$ solution	Yellowish color appearance was obtained	+ve	+ve	+ve

Citric acid	2-3 ml sample + Added one drop dilute NH_4OH & excess $Agno_3$ solution, boil for 15 min. on water bath	White gelatinous Precipitate was obtained	+ve	+ve	+ve
Bile pigment	(PETEN KOFERS TEST) Took 5 ml of cow urine +Dissolved crystal of sucrose +3 ml of conc. Sulphuric acid	Red or reddish purple ring was formed	-ve	+ve	+ve
Ketone bodies	(ROTHER's TEST) Took 5 ml of cow urine + Saturated with solid ammonium sulphate + 2-3 drop of 5% solution of sodium nitroprusside + 2 ml conc. ammonia	No permanent color formed	-ve	-ve	-ve
Creatinine	(JAFFE'S TEST) Took 5 ml of cow urine + 2 ml saturated picric acid + 10 % NAOH	Deep orange color was formed	-ve	-ve	+ve
Protien	(HELLER'S TEST) 3 ml of urine + 3 ml conc. Nitric acid	White Precipitate at the junction was obtained	-ve	+ve	+ve
Ammonia	Took 5 ml of cow urine + red litmus paper	Litmus paper turns to blue	+ve	+ve	+ve
Uric acid	(SCHIFF'S TEST) Moistened a strip of filter paper with Silver nitrate solution & added to it a drop of urine	Black or yellow brown strain formed	+ve	+ve	+ve
Bi- carbonate	3 ml of urine + dilute HCL	Effervescence of co2	+ve	+ve	+ve
Iron	5 ml of test solution , added few drops of 2% potassium Ferro cyanide	Dark blue coloration was obtained	-ve	-ve	+ve
Salisylic acid	Sample +Bromine solution	Cream color Precipitate was obtained	+ve	+ve	+ve
Tartaric acid	2-3 ml 0f test solution ,added one drop dilute NH_4OH & excess 5% $Agno_3$ solution ,boiled for 15 min. on water bath	White gelatinous Precipitate observed	+ve	+ve	+ve

Magnesium	Sample +Ammonium carbonate	White Precipitate was obtained	+ve	+ve	+ve
Succinic acid	In a test tube, took the neutral solutions of the acid, added calcium chloride solutions, shaked & boiled for 2 min. on stretching the sides of the test tube	White Precipitate was obtained	+ve	+ve	+ve
Sulphur	Dilute odiumnitroprusside + sample	Purple color was obtained	+ve	+ve	+ve
Oxalic acid	2ml sample + added few drops 5% lead acetate	White Precipitate was obtained	+ve	+ve	+ve
Potassium	3 ml sample + added few drops of sodium cobalt nitrite solutions	Yellow Precipitate was obtained	+ve	-ve	+ve

Determination pH and Specific gravity of Cow urine and it's preperations

Procedure of pH determination

- At first pH meter was set with reference of stranded buffer of pH 4 and pH 7 and then we adjusted the pH of distilled water and then calculated the pH of samples of cow urine preparations.
- Procedure of specific gravity determination:

The weighing bottle was weighed and then a fix amount of sample of cow urine preparations was filled in weighing bottle and weighed again after this the empty bottle again weighed and then calculated the specific gravity.

Table 2: pH and Specific gravity of Cow urine preparations

S.No.	Cow urine preparations	pH of Cow urine preparations	Specific gravity of Cow urine preparations
1.	Fresh cow urine	9.0	1.027
2.	Distillate cow urine (Gau arc)	9.5	0.997
3.	Ganavati	10.0	1.035

Collection of Plant Material

The plant Carica papaya were 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.

Preparation of the Extracts (Carica papaya)

The fresh leaves of Carica papaya was cutted into small pieces, 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 =64.378 gm

Weight of coarse powder to be taken for extraction=20 gm

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

$$\% \text{ Yield} = (64.378-57.550)*100/20$$

$$= 34.14 \text{ \% w/w}$$

Preparation of Leaves Juice

The fresh leaf juice of the *Carica papaya leaves* was obtained by crushing the fresh leaves in the mixer. The crushed leaves was then filtered through muslin cloth and further used for study.

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 Carica papaya

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

Determination of Ash Value Papaya leaf

A porcelain crucible was washed and was dried in oven. After drying, the crucible was weighed accurately and an 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-

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

Weight of porcelain crucible with ash=16.817 gm

Weight of empty porcelain crucible =16.812 gm

Weight of coarse powder to be taken=1 gm

$$= (16.817-16.812)*100/1$$

$$= 0.50 \text{ \% w/w}$$

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”.

Preparation of Normal and High-Fat Diet

The normal and high-fat diet was prepared as following ratio-

Table 3: Composition of Normal and High Fat Diet

S.No.	Ingredients	Amounts in grams	
		For normal diet	For high fat diet
1	whole wheat	67.5 g	50.0 g
2	yellow corn	62.5 g	50.0 g
3	Barley	37.5 g	25.0 g
4	anik spray	37.5 g	37.5 g
5	Sod. Acid phosphate	2.5 g	2.5 g
6	calcium chloride	2.5 g	2.5 g
7	Salt	2.5 g	2.5 g
8	Oil	37.5 g	25.0 g
9	Tablet of vit.B_{12}	1 Tablet	1 Tablet
10	Cholesterol	_	200 mg/kg/day
11	Dalda ghee	_	25.0 g

Diet of above composition was consumed by each animal everyday as follows-

Table 4: Average Normal Diet Consumption by Each Group

Groups	No. of animal	Diet given/day	Avg. Remaining diet	Consumed amt. of diet by each groups	consumed by each animal
I	5	100 gm	4.7 gm	95.3 gm	19.0 gm
II	5	100 gm	8.9 gm	91.1 gm	18.2 gm
III	5	100 gm	7.7 gm	92.3 gm	18.5 gm
IV	5	100 gm	5.6 gm	94.4 gm	18.5 gm
V	5	100 gm	6.9 gm	93.1 gm	18.6 gm
VI	5	100 gm	7.7 gm	92.3 gm	18.5 gm
VII	5	100 gm	9.4 gm	90.6 gm	18.1 gm

Table 5: Average High-Fat Diet Consumption by Each Group

Groups	No. of animal	Diet given/day	Avg. Remaining diet	Consumed amt. of diet by each groups.	consumed by each animal
II	5	100 gm	11.7 gm	88.3 gm	17.7 gm
III	5	100 gm	9.6 gm	90.4 gm	18.0 gm
IV	5	100 gm	8.6 gm	91.4 gm	18.2 gm
V	5	100 gm	9.1 gm	90.9 gm	18.1 gm
VI	5	100 gm	12.0 gm	88.0 gm	17.6 gm
VII	5	100 gm	11.0 gm	99.0 gm	19.8 gm

* In group I, Normal diet was given throughout the study.

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) with Carica papaya leaves juice (CPLJ) in selected dose of 10 ml/kg for next 30 days with normal diet.

Group V- This was fed with the high-fat diet for 30 days and treated with Cow urine (CU) with Carica papaya leaves juice (CPLJ) 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 Carica papaya leaves (CPLE) hydroalcholic extract of 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 Carica papaya leaves (CPLE) 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)

Estimation of Triglyceride Level

Triglyceride level was checked in the different animal groups by using logotech diagnostic kit. Serum was separated and the standard triglyceride reagent (1ml) was added in the samples and then they were allowed to stand for 15 minutes in Incubator and then reading were noted for triglyceride level at 505 nm by auto analyser.

Estimation of HDL

HDL was analyzed by logotech diagnostic kit. Serum was separated and the standard HDL reagent (1ml) was added in the samples and then they were allowed to stand for 15 minutes in Incubator and then they were analysed for HDL level at 505 nm by auto analyser.

Estimation of Cholesterol

cholesterol was analyzed by logotech diagnostic kit. By using cholesterol oxidase phenol 4-aminoantipyrine peroxidase method. Serum was separated and the standard Cholesterol reagent (1ml) was added in the samples and then they were allowed to stand for 15 minutes in Incubator and then reading were noted for Cholesterol level at 505 nm by auto analyser.

Estimation of Liver and kidney function test

Estimation of SGOT

SGOT was analyzed by logotech diagnostic kit Serum was separated and the standard SGOT reagent (1ml) was added in the samples and then they were allowed to stand for 15 minutes in Incubator and then they were analysed for SGOT level at 340s by auto analyser.

Estimation of SGPT

SGPT was analyzed by logotech diagnostic kit. Serum was separated and the standard SGPT reagent (1ml) was added in the samples and then they were allowed to stand for 15 minutes in Incubator and then they were analysed for SGPT level at 340 nm by auto analyser.

Estimation of Serum creatinine

Serum creatinine was analyzed by logotech diagnostic kit. Serum was separated and the standard Serum creatinine reagent (1ml) was added in the samples and then they were allowed to stand for 15 minutes in Incubator and then they were analysed for Serum creatinine level at 505 nm by auto analyser.

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 DISCUSSIONS

Percentage Yield of Hydroalcoholic Extract

The percentage yield of hydroalcoholic extract of *Carica papaya* leaves were found to be as 34.14 % w/w.

Ash Value

The ash value of dry coarse powder of *Carica papaya* leaves were found to be as 0.5 % w/w.

Chemical Evaluation of Extract and Juice of Carica papaya

Table 6: Chemical Evaluation of Extract and Juice of Carica papaya

S. No.	Chemical Constitutes/Test	Inference	
		Extract	Juice
1	Carbohydrate Molisch test Benedict's Test	Present	Present
2	Protein Biuret test	Absent	Present
3	Flavanoid Shinoda test	Present	Present
4	Terpinoid Salkowaski test	Present	Present
5	Glycoside Modified Borntrager's	Present	Present
6	Steroid Libermann Burchard test	Present	Absent
7	Alkaloid Mayer's/Hager's/Wagner's Dragendroff's test	Absent	Absent

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.

Table 7: Chemical constituents detected in cow urine and its preparations

Component	Gau Ark	Ghanvati	Fresh Cow Urine
Urea	+ve	+ve	+ve
Chloride	+ve	+ve	+ve
Sulphate	+ve	+ve	+ve
Calcium	+ve	+ve	+ve
Phosphorus	+ve	+ve	+ve
Carbohydrate	+ve	+ve	+ve
Malic acid	+ve	+ve	+ve
Citric acid	+ve	+ve	+ve
Bile pigment	-ve	+ve	+ve
Ketone bodies	-ve	-ve	-ve
Creatinine	-ve	-ve	+ve
Protien	-ve	+ve	+ve
Ammonia	+ve	+ve	+ve
Uric acid	+ve	+ve	+ve
Bicarbonate	+ve	+ve	+ve
Iron	+ve	-ve	+ve
Salisylic acid	+ve	+ve	+ve
Tartaric acid	+ve	+ve	+ve
Magnesium	+ve	+ve	+ve
Succinic acid	+ve	+ve	+ve
Sulphar	+ve	+ve	+ve
Oxalic acid	+ve	+ve	+ve
Potassium	+ve	-ve	+ve

Determination pH and Specific gravity of Cow urine and its preparations

pH and specific gravity of cow urine and its preparations were determined and result are shown in table below

Table 8: pH and specific gravity determined of cow urine preparations

S. No.	Cow urine preparations	pH of Cow urine preparations	Specific gravity of Cow urine preparations
1	Fresh cow urine	9.0	1.027
2	Distillate cow urine (Gau arc)	9.5	0.997
3	Ganavati	10	1.035

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.

Table 9: Consumption of Normal Diet

Groups	No. of animal	Diet given/day	Avg. Remaining diet	Consumed amt. of diet by each groups.	Consumed by each animal
I	5	100 gm	4.7 gm	95.3 gm	19.0 gm
II	5	100 gm	8.9 gm	91.1 gm	18.2 gm
III	5	100 gm	7.7 gm	92.3 gm	18.5 gm
IV	5	100 gm	5.6 gm	94.4 gm	18.5 gm
V	5	100 gm	6.9 gm	93.1 gm	18.6 gm
VI	5	100 gm	7.7 gm	92.3 gm	18.5 gm
VII	5	100 gm	9.4 gm	90.6gm	18.1 gm

Consumption of High-Fat Diet

The average amount of high fat diet that was consumed by each animal was recorded everyday.

Table 10: Consumption of High-Fat Diet

Groups	No. of animal	Diet given/day	Avg. Remaining diet	Consumed amt. of diet by each groups.	Consumed by each animal
II	5	100 gm	11.7 gm	88.3 gm	17.7 gm
III	5	100 gm	9.6 gm	90.4 gm	18.0 gm
IV	5	100 gm	8.6 gm	91.4 gm	18.2 gm
V	5	100 gm	9.1 gm	90.9 gm	18.1 gm
VI	5	100 gm	12.0 gm	88.0 gm	17.6 gm
VII	5	100 gm	11.0 gm	99.0 gm	19.8 gm

Anti-hyperlipidemia Effects

The animals were divided in 7 groups comprising 5 animals each, lipid profile (TC, TG, HDL, LDL and VLDL), biochemical parameter (SGOT, SGPT and CK) and body weight of rats was recorded initially. After 30 days of high fat diet feeding, above parameters and body weight were reanalyzed and considered as 0^{th} day treatment level and at 0^{th} day, all the parameters showed non-significant difference in normal control but there was significant difference in positive control and experimental groups for one or more parameters when compared with initial level of respective groups. The parameters and weight were again evaluated at 15^{th} and 30^{th} day of treatment in experimental groups and compared with 0^{th} day treatment level of respective groups. The level of significance was determined by using student's t-test and ANOVA followed by Dunett's test on Graph-Pad software.

Effect of CU with CPLE, CPLJ on Body Weight of High Fat Diet Induced Obese and hyperlipidemic Rats

The rats when fed with high-fat diet showed marked increase in body weight and lipid profile in all groups except group I which was on normal diet. At the 30^{th} day of treatment, a significant ($P < 0.001$) reduction in body weight was found in the CPLE (200 mg/kg; p.o.) and ATV (10 mg/kg; p.o.) treated groups as compared to the other groups. The weight reduction effect in CPLE (200 mg/kg; p.o.) treated rats was more significant ($p<0.001$) at 15^{th} days of treatment as compared to the standard group ($P <0.01$).

Table 11: Mean Body Weight 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)	145.98±6.11	155.44±4.64^{ns}	159.78±7.06^{ns}	162.5±6.04^{ns}
II	High fat diet (positive control)	144.46±8.50	165.90±3.73^{b}	187.06±4.48^{b}	207.6±11.23^{b}
III	ATV 10 mg/kg	138.5±4.62	157.56±3.27^{a}	149.52±3.03^{c}	141.30±4.54^{b}
IV	CU+CPLJ 10ml/kg	170.06±3.89	189.88±3.51^{a}	187.54±3.52^{ns}	185.36±3.98^{ns}
V	CU+CPLJ 20 ml/kg	139.62±5.79	164.75±7.43^{b}	156.90±8.43^{ns}	149.34±9.04^{c}
VI	CU+CPLE 100 mg/kg	154.14±6.76	178.64±8.13^{b}	175.10±7.57^{ns}	173.22±6.79^{ns}
VII	CU+CPLE 200 mg/kg	150.86±7.66	172.94±9.58^{b}	157.16±3.38^{b}	143.67±5.54^{b}

ATV: Atorvastatin; CPLJ= CP leaf juice,CPLE=CP leaf extract.

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 CPLE & CPLJ on Total Cholesterol level of High Fat Diet Induced Obese & hyperlipidemic rats

The TC level was reduced significantly in all the experimental groups on the 30th day of treatment except CPLJ (10 ml/kg;p.o.) treated group but CPLJ (20 ml/kg;p.o.) showed the highly significant (p<0.0001) TC reduction on the 30th day of treatment. The standard and CPLE (200 mg/kg;p.o.) groups showed the same level of significance at 15th as well as 30th day of treatment and it means that the effect of standard drug was same as the CPLE (200 mg/kg;p.o.).

Table 12: 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 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)	85.5±9.15	96.00±14.84^{ns}	101.76±11.42^{ns}	109.74±12.43^{ns}
II	High fat diet (positive control)	84.7±8.28	200.76±23.94^{a}	280.35±21.47^{b}	304.46±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+CPLJ 10ml/kg	81.54±12.65	220.54±44.40^{b}	205.38±48.60^{ns}	200.69±48.60^{ns}
V	CU+CPLJ 20 ml/kg	87.24±9.20	234.23±50.27^{b}	153.87±6.27^{b}	128.94±6.86^{a}
VI	CU+CPLE 100 mg/kg	92.16±8.01	213.22±39.89^{b}	169±11^{d}	150.23±8.43^{c}
VII	CU+CPLE 200 mg/kg	96.92±8.41	290.36±13.21^{a}	246.14±12.10^{c}	182.66±13^{b}

ATV: Atorvastatin; CPLJ= CP leaf juice,CPLE=CP leaf extract.

Values are expressed in mg/dl 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 level by using t-test XX Results of 15th and 30th day treatment (treated groups) are compared with 0th day treatment (Hyperlipidemia control)

Effect of CU with CPLE & CPLJ on Triglyceride Level of High Fat Diet Induced Obese & hyperlipidemic Rats

There was a significant reduction in TG level in all the experimental groups except CPLJ (10 ml/kg; p.o.) treated group at 30th days of treatment. In present study,CPLE(200 mg/kg;p.o.) and ATV (10mg/kg;p.o.) treated groups showed higher and same significance reduction in TG level at 15th as well as 30th days of treatment.

Table 13: 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)	72.58±15.08	81.74±13.36^{ns}	85.22±10.21^{ns}	94.16±13.30^{ns}
II	High fat diet (positive control)	86.60±11.57	183.02±33.38^{b}	237.88±30.66^{c}	252.96±30.05^{b}
III	ATV 10 mg/kg	109.14±21.17	226.60±39.23^{b}	159.14±19.85^{c}	126.64±13.49^{b}
IV	CU+CPLJ 10ml/kg	76.66±8.16	162.46±49.63^{b}	146.20±44.59^{ns}	143.32±44.11^{ns}
V	CU+CPLJ 20 ml/kg	98.42±21.81	204.38±30.42^{b}	191.48±28.48^{d}	142.88±8^{c}
VI	CU+CPLE 100 mg/kg	81.77±8.93	205.06±39.29^{b}	198.02±38.29^{ns}	185.92±40.19^{d}
VII	CU+CPLE 200 mg/kg	84.62±8.65	252.67±39.42^{a}	175.58±13.40^{c}	148.94±13.54^{b}

ATV: Atorvastatin; CPLJ= CP leaf juice,CPLE=CP leaf extract.

Values are expressed in mg/dl 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 level 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 CPLE & CPLJ on HDL Level of High Fat Diet Induced Obese & hyperlipidemic Rats

There was a significant increase in HDL level except (10 ml/kg;p.o.) group on 30th day of treatment. CPLJ(20 ml/kg;p.o.) treated group showed the highly significant ($p<0.0001$) increase in HDL level at 30th day of treatment as compared to the other experimental groups. CPLE(200 mg/kg;p.o.) and ATV (10 mg/kg;p.o.) treated groups have the same level of significance at 15th as well as 30th days of treatment.

Table 14: 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)	44.58±8.25	46.70±6.92^{ns}	45.12±5.76^{ns}	46.42±5.78^{ns}
II	High fat diet (positive control)	36.98±8.39	33.38±6.92^{d}	32.00±6.59^{ns}	29.46±3.89^{d}
III	ATV 10 mg/kg	46.42±10.18	40.54±9.05^{c}	64.22±5.16^{b}	72.45±8.39^{b}
IV	CU+CPLJ 10ml/kg	41.62±8.80	40.76±5.76^{ns}	44.72±5.46^{ns}	44.56±2.11^{ns}
V	CU+CPLJ 20 ml/kg	33.58±9.57	31.64±7.91^{ns}	60.18±9.34^{b}	67.09±9.63^{a}
VI	CU+CPLE 100 mg/kg	47.2±6.96	42.82±7.36^{d}	46.90±7.72^{c}	53.66±5.29^{c}
VII	CU+CPLE 200 mg/kg	51.74±5.07	49.62±5.98^{ns}	65.49±1.46^{b}	72.82±2.66^{b}

ATV: Atorvastatin; CPLJ= CP leaf juice,CPLE=CP leaf extract.

Values are expressed in mg/dl 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 level 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+CPLE AND CU+CPLJ On LDL Level of High Fat Diet Induced Obese & hyperlipidemic Rats

CPLJ (20ml/kg; p.o.) treated group showed highly significant reduction in LDL level ($p<0.001$) on 15th days of treatment. At 30th day of treatment CPLJ (20ml/kg; p.o.) and CPLE (200mg/kg; p.o.) treated groups showed same level of significance and that was higher than standard group.

Table 15: 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		36.15±16.81^{ns}	41.39±13.68^{ns}	47.28±13.66^{ns}

	(Normal control)	26.18±11.60			
II	High fat diet (positive control)	34.58±8.54	166.16±26.30^{a}	204.56±18^{d}	229.24±11.89^{c}
III	ATV 10 mg/kg	22.84±5.00	160.62±36.46^{a}	102.51±43.94^{ns}	61.54±23.29^{c}
IV	CU+CPLJ 10ml/kg	26.18±17.48	150.08±42.04^{a}	136.02±46.37^{ns}	131.13±44.45^{ns}
V	CU+CPLJ 20 ml/kg	33.75±6.44	161.59±48.21^{a}	55.42±14.76^{b}	33.17±9.07^{b}
VI	CU+CPLE 100 mg/kg	75.01±6.64	133.27±30.40^{b}	85.87±9.73^{d}	62.12±18.65^{c}
VII	CU+CPLE 200 mg/kg	31.73±9.41	191.61±13.70^{a}	147.34±11.4^{c}	80.52±12.60^{b}

ATV: Atorvastatin; CPLJ= CP leaf juice,CPLE=CP leaf extract.

Values are expressed in mg/dl 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+CPLE AND CU+CPLJ on VLDL Level of High Fat Diet Induced Obese & hyperlipidemic Rats

The VLDL level was reduced in ATV treated group on 15th and 30th day of treatment but the level of significance was at lower side. There was no marked reduction in VLDL level in other experimental groups except CPLE (200 mg/kg; p.o.) treated group which showed a higher degree of significance when compared with standard ATV.

Table 16: 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)	15.71±3.02	16.17±2.67^{ns}	22.55±2.70^{ns}	19.05±2.66^{ns}
II	High fat diet (positive control)	18.14±2.33	36.62±6.68^{c}	47.77±6.13^{b}	50.792±6.01^{b}
III	ATV 10 mg/kg	18.54±3.75	45.10±7.85^{b}	31.82±3.97^{d}	25.52±2.68^{c}
IV	CU+CPLJ 10ml/kg	15.74±1.67	32.68±9.93^{c}	29.64±8.92^{ns}	28.26±8.82^{ns}
V	CU+CPLJ 20 ml/kg	19.84±4.36	41.09±6.08^{b}	39.09±5.70^{ns}	29.77±1.60 ^{d}
VI	CU+CPLE 100 mg/kg	16.17±1.79	41.20±7.86^{b}	39.20±7.66^{ns}	37.38±8.04^{ns}
VII	CU+CPLE 200 mg/kg	16.46±1.73	50.57±7.88^{b}	33.43±2.73^{b}	29.41±2.67^{b}

ATV: Atorvastatin; CPLJ= CP leaf juice,CPLE=CP leaf extract.

Values are expressed in mg/dl 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+CPLE AND CU+CPLJ on SGOT Level of High Fat Diet Induced Obese Rats

There was a significant increase in SGOT level in ATV (10 mg/kg;p.o.) treated group on the 30th day of treatment as compare to 0th and 15th day of treatment. While in other experimental groups the SGOT level was significantly decrease on the 30th day of treatment except CPLJ (10ml/kg; p.o.) treated group (p>0.05). CPLE (200mg/kg; p.o.) treated group showed highly significant reduction in SGOT level on the 30th days of treatment as compare to other groups. The standard marketed preparation ATV showed elevation in SGOT levels after 30th day of treatment indicates its hepato-toxic effect on chronic use.

Table 17: 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)	34.16±20.98	36.80±11.25^{ns}	41.08±10.25^{ns}	42.28±10.69^{ns}
II	High fat diet (positive control)	46.22±5.67	48.64±4.00^{ns}	49.24±3.27^{ns}	51.60±4.11^{ns}
III	ATV 10 mg/kg	38.60±4.00	40.26±4.12^{ns}	46.56±9.46^{ns}	58.60±11.39^{b}
IV	CU+CPLJ 10ml/kg	30.74±10.60	36.34±6.27^{ns}	33.92±7.00^{ns}	31.44±7.90^{ns}
V	CU+CPLJ 20 ml/kg	32.66±10.18	36.36±10.01^{ns}	28.96±9.44^{ns}	21.87±1.53^{d}
VI	CU+CPLE 100 mg/kg	41.16±6.22	44.86±5.24^{d}	37.12±5.09^{ns}	34.66±4.44^{c}
VII	CU+CPLE 200 mg/kg	40.32±8.51	41.46±5.94^{ns}	33.94±8.35^{ns}	24.42±8.47^{b}

ATV: Atorvastatin; CPLJ= CP leaf juice,CPLE=CP leaf extract.

Values are expressed in mg/dl as Mean±SD. (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+CPLE AND CU+CPLJ on SGPT Level of High Fat Diet Induced Obese Rats

There was no significant (p>0.05) increase in SGPT level in the ATV (10mg/kg;p.o.) treated group and other experimental groups showed the significant decrease (p<0.01) in SGPT level at 30th day of treatment except CPLE (100mg/kg;p.o.) treated group.

Table 18: 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)	36.44±14.58	38.56±20.48^{ns}	44.00±19.91^{ns}	47.00±18.33^{ns}
II	High fat diet (positive control)	51.02±12.74	56.72±12.00^{ns}	61.64±9.00^{ns}	65.66±11.90^{ns}
III	ATV 10 mg/kg	49.24±14.11	54.62±9.66^{ns}	57.23±17.11^{ns}	61.6±20.73^{ns}
IV	CU+LSFJ+CPLJ 10ml/kg	41.76±5.20	45.26±11.60^{ns}	39.94±9.29^{ns}	36.8±8.96^{ns}
V	CU+LSFJ+CPLJ 20 ml/kg	27.44.±9.90	38.64±7.08^{ns}	32.46±6.75^{ns}	21.4±5.63^{c}
VI	CU+LSFE+CPLE 100 mg/kg	39.76±11.61	48.66±6.57^{ns}	43.92±6.67^{ns}	41±8.70^{ns}
VII	CU+LSFE+CPLE 200 mg/kg	34.76±12.02	46.72±14.23^{d}	41.52±9.58^{ns}	19.28±5.19^{c}

ATV: Atorvastatin; 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 CPLE and CPLJ on CK Level of High Fat Diet Induced Obese & hyperlipidemic Rats

There was a significant ($p<0.01$) increase in CK level only in ATV (10 mg/kg;p.o) and CPLE (200 mg/kg;p.o.) treated groups on the 30th day of treatment but the percentage increase in ATV (10 mg/kg;p.o.) treated group was more on 30th day of treatment when compared with CPLE (200 mg/kg;p.o.) groups.

Table 19: Mean CK 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)	59.04±13.91	66.52±24.80^{ns}	67.14±22.23^{ns}	72.58±27.17^{ns}
II	High fat diet (positive control)	64.312±28.99	62.78±25.31^{ns}	64.52±20.32^{ns}	69.44±24.47^{ns}
III	ATV 10 mg/kg	63.5±23.15	62.66±25.83^{ns}	88.09±26.57^{ns}	126.14±32.75^{c}
IV	CU+CPLJ 10ml/kg	55.71±23.59	56.62±25.53^{ns}	63.74±26.86^{ns}	82.58.±28.65^{ns}
V	CU+CPLJ 20 ml/kg	46±26.61	51.54±28.04^{ns}	59.60±28.79^{ns}	88.32±34.71^{ns}
VI	CU+CPLE 100 mg/kg	72.14±22.42	79.12±25.51^{ns}	92.74±28.33^{ns}	112.58±22.93^{ns}
VII	CU+CPLE 200 mg/kg	56.04±16.48	58.88±18.62^{ns}	75.86±17.64^{ns}	112.39±27.00^{c}

ATV: Atorvastatin;Values are expressed in UI/L as Mean±SD. (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.

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 CU & CPLE (200 mg/kg/;p.o.) showed more significant reduction on 15th day of treatment. The total cholesterol was reduced with higher significance in CU & CPLE (200 mg/kg/;p.o.) and CU & CPLJ (20 ml/kg/;p.o.) when compared with standard drug. The TG levels were reduced more significantly in CU & CPLE (200 mg/kg/;p.o.) in comparison with other groups. HDL levels were increased in all studied groups except CPLJ (10 ml/kg/;p.o.) but was more significantly increased in CU & CPLJ (20 ml/kg/;p.o). The LDL levels were reduced abruptly in groups treated with CU & CPLJ (20 ml/kg/;p.o.) after 15th day of treatment, however after 30th day of treatment the LDL levels were reduced in CU & CPLJ (20 ml/kg/;p.o.) and CPLE (200 mg/kg/;p.o.) with higher significance in comparison with other groups. The VLDL levels reduced more significantly in CU & CPLE (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 CU & CPLE (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 CU & CPLE (200 mg/kg/;p.o.) and CU & CPLE (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 CU & CPLJ 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 Carica papaya leaves 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 Carica papaya leaves juice(CPLJ) with CU in dose of 10 ml/kg and 20 ml/kg and Carica papaya leaves extract(CPLE) with CU in dose of 100 mg/kg and 200 mg/kg. CPLJ in dose of 20 ml/kg and CPLE in dose of 200 mg/kg showed the most significant results among other preparation in high fat diet induce obese and hyperlipidemic rats. Interestingly CPLE 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. CPLJ 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. CPLJ 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 CPLE with CU (20 ml/kg;p.o.) significantly reduced both TC and LDL level. CPLE with CU (100mg/kg; p.o.) showed less significant results throughout the study so it means that lower dose of hydroalcholic extract *and carica papaya* is not more effective in the treatment of hyperlipidemia and hyperlipidemia.CPLJ with CU (10 ml/kg;p.o.) showed non- significant ($p>0.05$) results throughout the study so it means that carica papaya 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 CPLE and CPLJ 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 CPLE and CPLJ 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 CPLJ and CPLE treated groups showed the hepato-protective property of CP leaves. Finally conclude that specific dose of CPLE and CPLJ can be beneficial to the patients suffering from Hyperlipidemia, obesity 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, cardioprotective and cardiotonic activity of carica papaya leaf with cow urine. A future work on isolation characterization and pharmacological activity of active constituents of carica papaya 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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