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### **EVALUATION OF HYPOLIPIDEMIC ACTIVITY OF COW'S URINE**

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#### ABSTRACT

Hyperlipidemia has been ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart diseases. Major complications of hyperlipidemia are atherosclerotic heart disease, heart attack, stroke, atherosclerosis and hyperlipidemia which are also the primary cause of death. 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, such as Atharva Veda, Charak Samhita, Rajni Ghuntu, Vridhabhagabhatt, Amritasagar, Bhavprakash and Sushrut Samhita, contain beautiful description about these things. We have evaluated the antihyperlipidemic activity of cow urine in rats and in healthy volunteers and results indicated significant activity in both models.

Keywords: Hyperlipidemia, Cow Urine, Heart Disease.

### INTRODUCTION

Cow's urine also called Gomutra is recommended as a healing aid in Ayurveda. It's Treatment and Research Center has conducted a lot of research in the past few years and reached at the conclusion 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, miscarriage and several other diseases [1]. Currently available drugs have been associated with number of side effects. The consumption of synthetic drugs leads to hyperuricemic, diarrhoea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function. As cows urine is naturally available and proved to be beneficiary in many compliations, it is selected to evaluate antihyperlipidemic in high fat diet induced hyperlipidemic rats & humans and also observe the effect on body weights of human volunteers.

### MATERIALS AND METHODS Cow's urine

The urine distillate of Gujarati Indian cow known as Geer cow was used in the study and was procured from the local market. The study was performed after getting a certificate from the Ayurvedic doctor stating that it is free from diseases.

### Dose Preparation and Administration of Standard Atorvastatin

Standard atorvastin at a dose of 5mg/kg<sup>-1</sup> was prepared by suspending bulk atorvasatin in aqueous 0.5% methylcellulose [2].

## ANTI-HYPERLIPIDEMIC ACTIVITY OF COW'S URINE IN RATS

### **Experimental animals**

Adult male Wistar albino rats (Mahaveer Enterprises, Hyderabad, India) of mean weights in the range of 150-200 g were selected and housed in polypropylene cages in a room where the congenial temperature  $27^{\circ}C \pm 1^{\circ}C$  and 12 h light and dark cycles were maintained. The animals are allowed to acclimatize to the environment for seven days and are supplied with a standard pellet diet and water *ad libitum* [2].

### Induction of high fat diet induced hyperlipidemia

High fat diet was used to induce hyperlipidemia in rats supplemented for 21days [3].

### Study design for Antihyperlipidemic Activity

A total of 36 rats were used to evaluate for antihyperlipidemic activity except Group I, all the groups of the rats were supplemented with HFD for 21days to induce hyperlipidemia & parallely treatment was continued along with induction to check the inhibition power of the test drug (Group III &IV),standard drug(Group V) and combinational drug effect of test drug and standard drug (Group VI) was also seen. All these groups effect were compared with untreated HFD fed rats (Group II). Before and after starting the supplementation with HFD, lipid levels and weights of animals were checked and compared.

### Blood sample collection and analysis:

Blood was collected on the 4<sup>th</sup> day after acclimatizing rats to the environment, and on 25<sup>th</sup> day by retro-orbital puncture technique under mild ether anesthesia after 8 h fasting and collected blood samples were allowed to clot for 30 min at room temperature. Blood samples were centrifuged at 3000 rpm for 20 min. Serum was separated and stored at -20°C until biochemical estimations were carried out. Serum samples were analyzed spectrophotometrically for total serum cholesterol (TC), triglyceride (TG) and high density cholesterol (HDL-C) using respective lipoprotein diagnostic kits. Very Low Density Lipoprotein (VLDL), High Density Lipoprotein ratio (HDL-C ratio) and low density lipoprotein cholesterol (LDL-C) were calculated by using the formula [4].

### EFFECT OF COW'S URINE ON BODY WEIGHT AND LIPID PROFILES OF HUMAN VOLUNTEERS

Healthy humans willing to take drug were selected and a total of 10 human volunteers from karimnagar town were included in the present study after taking their consent to participate in the study. The study was performed after explaining the description, purpose and objectives of the study and about cow's urine distillate.

These volunteers were not having any other complications except over weight and obese condition. Before starting the study and after administration of drug, their weights were checked and blood was withdrawn for the estimation of lipid profiles. This was done under proper medical supervision. The treatment with cow's urine was continued for 30days and samples were supplied throughout the study. They were suggested to take drug in the morning at 7am with empty stomach after overnight fasting, and are said to take breakfast after 9.30am. At the end of the treatment, after 30 days, body weights of the volunteers were again checked and blood was again withdrawn to check the effect of cow's urine in them.

### **Statistical Analysis**

Results are expressed as mean  $\pm$  S.D. All the results were compared with control subjects. One-way

analysis of variance (ANOVA) followed by the Dunnet test is used to analyse the results with p < 0.05 considered statistically significant.

### RESULTS

## ANTI-HYPERLIPIDEMIC ACTIVITY OF COW'S URINE IN RATS

To assess the anti – hyperlipidemic activity of cow's urine in rats, HFD was used to induce hyperlipidemia which is characterized by elevated lipid profiles. The effect of cow's urine on the lipid profiles in hyperlipidemic rats was shown in Table 2.

Supplementation with HFD to rats for 21days resulted in significant increases (p< 0.001) of lipid profiles (TC, TG, LDL-C, VLDL-C) and reduction of HDL-C than normal control group indicating the successful induction of hyperlipidemia.

### Comparison of lipid profiles of cow's urine treated groups with untreated hyperlipidemic control group II:

Administration of cow's urine at a dose of 2ml/kg produced a significant (p<0.05) reduction in the elevated TC, TG, LDL-C, VLDL-C levels and were dose dependent, whereas HDL-C levels were significantly increased (p<0.05) with test drug and were dose dependent. This hypolipidemic activity of cow's urine was comparable with standard hypolipidemic drug atorvastatin (Table-2).

Administration of cow's urine at a dose of 5ml/kg produced more significant effect than group III (2ml/kg). Group IV produced a significant (p<0.001) reduction in the elevated TC, LDL-C levels, and significant (p<0.01) reduction in the elevated TG, VLDL levels, whereas HDL-C levels were significantly increased (p<0.01) with test drug and were dose dependent. This hypolipidemic activity of cow's urine was comparable with standard hypolipidemic drug atorvastatin (Table-2).

Treated group V with 5mg/kg of Atorvastatin produced more significant effect than group III (2ml/kg) and group IV (5ml/kg). Group V produced a significant (p<0.001) reduction in the elevated TC, TG, LDL-C, VLDL levels and were dose dependent, whereas HDL-C levels were significantly increased (p<0.001) with standard drug and were dose dependent. This hypolipidemic activity of cow's urine was comparable with Combinational treatment group VI treated with 5ml/kg of test drug (cow's urine) + 5mg/kg of standard drug (atorvastatin).

Combinational treatment group VI with 5m/kg of cow's urine + 5mg/kg of standard drug produced more significant effect than all groups. Group IV produced a significant (p<0.001) reduction in the elevated TC, TG, LDL-C, VLDL levels, whereas HDL-C levels were significantly increased (p<0.01) with combinational treatment and were dose dependent.

This hypolipidemic activity of combinational treatment group was more effective and was comparable with standard hypolipidemic drug atorvastatin.

#### **Body Weights**

The effect of cow's urine on body weights of hyperlipidemic rats were evaluated and results were shown in Table 3 and Figure 1.

With supplementation with HFD, the body weights of animals increased significantly (p<0.001) than normal control group indicating the successful induction of hyperlipidemia (figure.1) whereas, the administration with cow's urine to rats for 21days resulting the significant reduction of these elevated body weights (p<0.001) and was found as dose dependent (figure.1).

These results with cow's urine was comparative with those of standard Atorvastatin. But, when atorvastatin (5mg/kg) combined with cow's urine at a dose of 5ml/kg produced synergistic activity and thereby reducing the body weight (p<0.001).

### HYPOLIPIDEMIC ACTIVITY OF COW'S URINE USING HUMAN VOLUNTEERS

The human volunteers were supplemented with cow's urine distillate for 30days with a dose of 15ml in the morning. The effect of cow's urine on body weight was shown in Table 4.

In human volunteers study, age group from 20 to 43 yrs was included. Body weights from 66 to 104 kgs were included, where they were suffering from overweight and free from diseases. Supplimentation with Cow's urine distillate at a dose of 15ml/day to volunteers did not reduce their body weights significantly. Still on an average they reduced 3kg in 30days with an average value of 750gms for every week which is considered to be healthy, without any changes in their normal life style and without any side effects. Hence, the cow's urine is considered as the body weight reducing agent (Table 5). The effect of cow's urine on the human volunteer's lipid profiles were shown in Table 5. The lipid profiles of healthy volunteers after 30 days treatment did not produce changes still they were reduced. Cow's urine has significantly reduced total cholesterol (p < 0.01), LDL-C (p < 0.05) levels. There is an increase in HDL-C levels on an average of 1.2mg/dl. This shows cow's urine has hypolipidemic activity.

In present study, we have evaluated the beneficial effect of cow's urine for in hypolipidemic activity on evaluation on human volunteers and animals. To assess the anti – hyperlipidemic activity of cow's urine in rats, HFD was used to induce hyperlipidemia characterized by elevated lipid profiles and body weights [3]. These lipid profiles and body weights on parallel treatment with cow's urine were significantly reduced when compared with control groups.

Group	Description and Treatment		
Group 1	Normal Control		
Group 2	Hyperlipidemic Control using High Fat diet		
Group 3	Test drug (2ml/kg) with High Fat diet		
Group 4	Test drug (5ml/kg) with High Fat diet		
Group 5	Standard drug (5mg/kg) with High Fat diet		
Group 6	Test drug (5ml/kg) with(5mg/kg) standard drug and High fat diet		

Table 2. Lipid profiles of animals before and after drug administration

Groups	Total cholester ol	Total cholesterol	HDL-C	HDL-C	Triglycerid es	Triglycerid es	LDL-C	LDL-C	VLDL	VLDL
	Before	After	Before	After	Before	After	Before	After	Before	After
Ι	$167\pm5.13$	175.5 ± 15.3	27.25 ±0.7	39.23±2.9	71.25±1.1	74.93±2.03	125.5±5. 12	121.28±15.3 2	14.25±0.2 3	14.9±0.4
Π	170 .25 ± 15.6	245.71±12 ***	32.5 ±0.77	27.88±2.5 ***	74±7.9	140.23±8.1 ***	122.9±1 6	189.7±14 ***	14.8±1.5	28±1.6 ***
III	$\begin{array}{r} 183 \pm \\ 16.48 \end{array}$	221.15±9 *	29 ±1.26	31.83±2.7*	78.6±14.85	125.46±2.9 2 **	138.2±1 5.2	164.2±8.3 *	15.73±2.9 7	25±0.5 *
IV	$185.6\pm15$	209.3±9.5 ***	30.8 ±4.6	34.21±3.4 **	80.9±6.8	119.48±3.8 **	138.6±1 7.5	151.22±11.6 2 ***	16.18±1.3	23.89±0. 7 **
v	179.8 ±21.9	184.23±6.8 2 ***	31.5 ±1.8	38.8±3.7 ***	75.83±8.61	105±11.29* **	133±23. 5	124.4±8.2** *	15.1±1.7	21±2.25 ***
VI	176.83 ±5.3	175.8±3.1 ***	28.8 ±1.7	36.7±1.4***	79±5.7	99.2±3 ***	132.2±5. 6	119.12±4 ***	15.8±1.1	19.94±0. 6***

All the data are expressed as mean  $\pm$  SD (n=6), \*p = <0.05, \*\*p=<0.01, \*\*\*p=<0.001 vs Group II @ p<0.001 vs Group II, @ p<0.001 vs Group II.

### **Table 1. Grouping of Animals**

Treatment	Group 1 Normal control (NC)	Group 2 Hyperlipidemic control (HLC)	Group 3 Test 2ml/kg	Group 4 Test 5ml/kg	Group 5 Standard Atorvastatin 5mg/kg	Group 6 Combinational treatment: Test 5ml/kg + Atorvastatin 5mg/kg
Before	$182.5 \pm 5$	$202 \pm 5.7$	181.6±13.19	189.16±13.5	$171.5 \pm 22.74$	$192.3 \pm 12.11$
After	$190 \pm 7$	243.7 ± 17 @ ***	189.1 ± 18 ***	164.16 ± 15 ***	$\begin{array}{c} 180 \pm 10 \\ *** \end{array}$	151.25 ± 34.73 ***

Table 3. Body weights (grams) of animals before and after treatment

All the data are expressed as mean  $\pm$  SD (n=6), \*p =<0.05, \*\*p=<0.01, \*\*\*p=<0.001 vs Group II, @ p<0.001 vs Group I.

### Table 4. Names, Age and Body Weights of Healthy Volunteers

S.No	Name of the Volunteer	Age (Yrs)	Before ADM	After ADM	% Reduction	
1	Santosh.B	20	104	98	5.7	
2	Srilatha.D	32	66	63	4.5	
3	Laxman.D	40	98	96.5	1.5	
4	Bhunesh.CH	43	78	76	2.5	
5	Lalitha.CH	38	68	64	5.8	
6	Aparna.D	43	68	66	2.9	
7	Anuradha.D	36	72	69	4.1	
8	Kotilingam.D	42	84	81	3.5	
9	Subhashini.D	38	74	72	2.7	
10	Ramakrishna.CH	40	96	92	4.1	
		AVG	80.8	77.75	3.7	
		±SD	13.9	13.4	1.4	

### **Table 5. Lipid Profiles of Volunteers**

Test Name (mg/dl)	Before ADM	After ADM
Total Cholesterol	164.37 ±19.95	140.5 ± 17 **
HDL-C	$38.4 \pm 4.81$	$39.6 \pm 4.8$
LDL-C	$102.97 \pm 19.8$	88 ± 15.6 *
VLDL-C	$23 \pm 6.4$	$19.4 \pm 5.4$
Triglycerides	$115.47 \pm 32.3$	$98.4 \pm 27.97$

All the data are expressed as mean  $\pm SD(n=10)$ , \*p = <0.05, \*\*p = <0.01, \*\*\*p = <0.001.



#### DISCUSSION

### ANTI-HYPERLIPIDEMIC ACTIVITY OF COW'S URINE IN RATS

On comparison of Group I(Normal control) with Group II (Hyperlipidemic control): Elevation of body weights and lipid levels in Group II (Hyperlipidemic control) after supplementation with HFD for 21days than group I(Normal control) were significant, whereas significant reduction of HDL-C in lipid levels indicates the successful induction of hyperlipidemia in animals HFD (table 4 & 5). Results of Cow's urine treated Groups were compared with untreated group hyperlipidemic control group II: Higher dose treatment group IV shown better than Group III.Group VI produced better results than the Group V which proves combinational treatment is better than standard Atorvastatin drug treatment.

These groups when compared with hyperlipidemic control (Group II), treatment group III, IV, V, VI reduced its weight significantly (p < 0.01 = \*\*\*) from which Group VI proved more beneficial than others.

### II. HYPOLIPIDEMIC ACTIVITY OF COW'S URINE USING HUMAN VOLUNTEERS

The human volunteers were supplemented with cow's urine distillate for 30days with a dose of 15ml in the morning. They were adviced to take cow's urine around at 7am, after overnight fasting and breakfast around at 9.00am every day. In human volunteers study, age group from 20 to 43 yrs were included. Body weights from 66 to 104 kgs were included, where they were suffering from overweight and free from diseases. Body weight of human volunteers after 30 days study didn't not decrease significantly still it is considered to be a healthy as rapid reduction weight may prove to unbeneficiary and may also cause unwanted effects. Still on an average they reduced 3kg in 30days with an average value of 750gms for every week, without any changes in their normal life style and without any side effects.

Lipid profiles of healthy volunteers after 30 days treatment on an average didn't exhibit any significant changes still they were reduced. Cow's urine has significantly reduced total cholesterol (p < 0.01 = \*\*), LDL-C (p < 0.05 = \*). There is an increase in HDL-C

levels on an average of 1.2mgldl. This shows cow's urine has hypolipidemic activity. The results on treatment with cow's urine have proved that lipid profiles and body weights of healthy volunteers and wistar rats have shown good results.

Lipid estimations in the study revealed that cow's urine in combination with atorvostatin has significant (p<0.001) activity in treating hyperlipidemia, when compared with only atorvastatin drug combinational treatment proved to be better. As cows urine is a bio enhancer it enhances the activity of atorvostatin and lowers the required dose, minimize its side effects.In Susrut samhita, several medicinal properties of cow's urine have been mentioned and are known to cause weight loss but research study to prove this activity is not done till now so it has been selected to prove as hypolipidemic drug [5]. 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, such as Atharva Veda, Charak Samhita, Rajni Ghuntu, Vridhabhagabhatt, Amritasagar, Bhavprakash and Sushrut Samhita, contain beautiful descriptions about these animals. Presently, no experiment has been done to prove its activity[1]. A person falls ill when there is deficiency (alteration) of the substances inside the body. The cow urine contains those substances like vitamis, calcium, enzymes, copper etc, which are present in the human body. Therefore, consumption of cow urine maintains the balance of these substances and cures many incurable diseases [6].

#### CONCLUSIONS

In conclusion, cow's urine has been proved to have good action in hypolipidemic state with less side effects. So naturally available cow's urine can serve as a good drug for people with hyperlipidemia. Where atorvastatin with cow's urine combination have given better significant (p<0.001) results than compared with single cow's urine and atorvastatin drugs.

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#### **CONFLICT OF INTEREST: NIL**

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