Evaluation of antidiabetic and anti-inflammatory activity of fatty extract of *Caprus hircus* milk in rats

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**ABSTRACT**

*Capra hircus* (goat) is one of the domesticated animals used for milk, meat and fibre. Milk is one of the essential products in the human diet, rich in nutritive components. Goat's milk has vitamins, minerals, trace elements, electrolytes, enzymes, proteins, and fatty acids that are easily assimilated by the body. The fatty acids present in *Caprus hircus* milk was revealed to have more beneficial properties to health and used as a traditional medicine for. The present study is aimed to investigate the antidiabetic and anti-inflammatory effect of isopropanol extract of milk of *Caprus hircus*. The antidiabetic activity was evaluated by measuring blood glucose level in rats treated with isopropanol extract of caprine milk and comparing to normal and streptozotocin (STZ) induced rats. The isopropanol extract of *Caprus hircus* produced significant changes in the streptazotocin induced diabetic rats by reducing the glucose levels considerably. isopropanol extract treated group (200 mg/kg b.w) showed the mean value of 157.6±3.656 and 117.6±3.839 respectively as compared with a mean value 317.2±4.244 of diabetic control group. The anti-inflammatory activity was assessed by carrageenan induced paw edema model in rats. There was significant reduction (p< 0.01) in paw diameter in the group that received high dose (200 mg/kg b.w) of isopropanol extract of *Caprus hircus* milk from Mean±SEM of 0.184±0.004 to 0.052±0.003 (71.73%) as compared with the disease control group. The present study concludes that caprine milk extract confirmed promising anti diabetic activity in streptozotocin induced diabetic Wister rats and significant anti-inflammatory properties against carrageenan induced paw edema.

**Keywords:** *Caprus hircus*, fatty acid, antidiabetic, streptozotocin, inflammation, carrageenan, paw edema.

**Introduction**

Diabetes is a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbance of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The disease is associated with microvascular, macrovascular, and metabolic complications. When the renal threshold for glucose reabsorption is exceeded, glucose spills over into the urine (glycosuria) and causes an osmotic diuresis (polyuria), which, in turn, results in dehydration, thirst and increased drinking (polydypsia). Insulin deficiency causes wasting through increased breakdown and reduced synthesis of proteins. Diabetes is a global epidemic with an estimated worldwide prevalence of 246 million people in 2007 and forecasts to rise to 300 million by 2025. The number of people suffering from diabetes all over the world has increased and the disease now kills more people than AIDS. Consequently, diabetes presents a major challenge to healthcare systems around the world. Insulin and
oral hypoglycemic agents, Sulfonylurea, Biguanides and other α-Glucosidase inhibitors, Thiazolidinediones are used to treat diabetes. The all drugs are having the different mechanism of action in disease but they may also produce a number of undesirable effects, which include frequent diarrhea, hypoglycemia, hepatotoxicity, lactic acidosis, dyslipidemia, hypertension, and hypercoagulability. Hence there is a need to search for newer anti-diabetic agents that have therapeutic efficacy with minimum side effects. Management of diabetes without any side effect is still a challenge to the medical community. There is continuous search for alternative drugs. Therefore it is prudent to look for options in natural medicines for diabetes as well. Many traditional plant and animal treatments for diabetes are also used.

Inflammation is a complex biological response of vascular tissues to harmful stimuli including pathogens, irritants or damaged cells. It is a body defense reaction to eliminate or limit the spread of an injurious agent and is characterized by five cardinal signs, redness (rubor), swelling (tumor), heat (calor), pain (dolor) and loss of function (function laesa). The inflammatory process involves a cascade of events elicited by numerous stimuli that include infectious agents, ischemia, antigen-antibody interaction and thermal or physical injury. Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used in the treatment of acute and chronic inflammation, pain and fever. But the greatest disadvantage in presently available synthetic drugs is that they cause gastrointestinal irritation and reappearance of symptoms after discontinuation. Therefore, there is a dire need for screening and development of novel, but better anti-inflammatory drugs and indigenous medicinal products derived from the natural products could be a logical source to find these.

Capra hircus belongs to family Bovidae. It is one of the domesticated animal used for milk, meat and fibre. These products are used therapeutically since ancient time as medicine. Goats produce about 2% of the world's total annual milk supply. The milk generally averages 3.5% butterfat. Studies revealed that goat milk has more beneficial properties to health than cow milk. Goat’s milk has smaller fat globules as well as higher levels of medium chain fatty acids. This means that during digestion, each fat globule and individual fatty acid will have a larger surface-to-volume ratio resulting in a quicker and easier digestion process.

Materials and Methods
Materials
The milk samples of domestic goat, Caprus hircus were collected from intensively-farmed goat population in the Medak region, Andhra Pradesh. The milk was collected in the morning in sterile screw bottles and kept on ice during transportation to the laboratory where milk bottles were stored at 4°C. All reagents used in this study were of analytical grade and obtained from SD Fine-Chem Limited, Hyderabad, India.

Preparation of fatty acid extract of milk from Caprus hircus
The fresh raw milk was collected from a healthy Caprus hircus. The separating funnel and organic solvent method are the chosen apparatus to extract the fat of milk. The fatty acid is extracted using organic solvent isopropanol. The Caprus hircus milk Extract (CHME) obtained is evaluated for qualitative identification of type of constituents present.

Test Animals
Wistar albino rats (weighing 170±10g) of either sex were used in this study. They were procured from National Institute of Nutrition, Hyderabad. The animals were kept in the standard polycarbonate cages and provided with standard rat feed and water ad libitum. The animals were housed under standard environmental conditions with controlled conditions of temperature (23 ± 2 °C), humidity (50 ± 20 %) and 12 hour light-dark cycles. The husk in the cages was renewed twice a week to ensure hygiene and maximum comfort for animals. The animals were acclimatized for 5 days. The experiments were performed according to the current guidelines for the care of the laboratory animals.

Determintation of acute toxicity (LD₅₀)
The acute toxicity for hexane extract of Caprus hircus milk was determined on both sex of wistar rats, maintained under standard conditions. The animals were fasted overnight prior to dosing. Fixed dose method of Schedule Y given by
DCGI was adopted for toxicity studies. The milk extract was administrated orally. The hexane extract of *Caprus hircus* milk exhibiting no mortality at 5000 mg/kg dose, was considered as LD50 cut off dose (safe dose). So 1/50th and 1/25th of that were selected (100 and 200 mg/kg) for the experiment as sub-maximal and maximal dose.

**Ant diabetic activity**

Streptozotocin induced diabetic rats were used for the study of ant diabetic activity.

**Induction of Diabetes to Experimental Rats**[8][9]

Diabetes was induced in the overnight fasted animals by a single intra peritoneal injection of freshly prepared solution of streptozotocin (STZ) 65 mg/kg body weight in 0.1M cold citrate buffer pH 4.5. The animals were allowed to drink 5% glucose solution to overcome the drug-induced hypoglycemia. The control rats were injected with citrate buffer alone as placebo. The animals were considered diabetic if the blood glucose values were >250 mg/dL on the third day after STZ injection.

**Experimental Design**[10][11][12]

The rats were divided into five groups (n = 6). Except group I which served as normal non-diabetic control all other groups were comprised of diabetic rats. Group II served as diabetic (STZ) control. Groups III and IV, received CMHE (100 and 200 mg/kg and group V received standard hypoglycemic drug Metformin (500 mg/kg b.w., p.o.) daily. The blood glucose levels was measured just prior to and 2, 4 and 6h after drug administration. Treatment is continued for 21 consecutive days. The fasting blood glucose levels were estimated on days 0, 1, 7, 14 and 21 using blood glucose test strips with elegance glucometer.

**Anti-inflammatory activity**

Anti-inflammatory activity was evaluated by using carrageenan induced paw edema model.

**Carrageenan-induced paw edema**

Albino rats of either sex weighing 150-200 grams were divided into four groups of six animals each. The dosage of the drugs administered to the different groups was as follows. Group I - Control (normal saline 0.5 ml/kg), Group - II and III – CHME (100 mg/kg and 200 mg/kg, p.o.), Group IV – reference standard antiinflammatory drug (Diclofenac 10 mg/kg, p.o.). All the drugs were administered orally[13]. After one hour of the administration of the drugs, 0.1 ml of 1% W/V carrageenan solution in normal saline was injected into the subplantar tissue of the left hind paw of the rat and the right hind paw was served as the control[14]. The paw volume of the rats were measured in the digital plethysmograph at the end of 0 min, 30min, 60 min, 120 min, 180 min, 240 min, 300 min, 360 min[15].

**Evaluation of antiinflammatory activity**[16]

The percentage increase in paw edema of the treated groups was compared with that of the control and the inhibitory effect of the drugs was studied. The relative potency of the drugs under investigation was calculated based upon the percentage inhibition of the inflammation. Percentage inhibition was calculated using the formula,

\[
\text{Percentage of inhibition of edema} = \left( \frac{Vc - Vt}{Vc} \right) \times 100
\]

Where,

\[Vt = \text{mean paw volume of test group} \]

\[Vc = \text{mean paw volume of control group}.\]

**Serum biochemical parameters**

The blood was used for the estimation of serum biochemical parameters viz. serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), total cholesterol, Urea, Creatinine were estimated by using commercially available reagent. The blood was collected from retro-orbital plexus of the overnight fasted rats under light ether anaesthesia and kept aside for ½h for clotting. Serum is separated by centrifuging the sample at 6000 rpm for 20 min. The serum will be analyzed for total protein, SGPT and SGOT levels[17][18].

**Statistical Analysis**

The experimental data were the blood sugar levels as compared to the reference drug expressed as mean ± standard error of mean (SEM). in a dose dependent manner. Statistical significance was analyzed by one-way analysis The fasting blood glucose levels of normal, diabetic of variance (ANOVA) followed by followed by LSD multiple comparison test, where the P<0.05 was considered significant.

**Results and Discussion**

The present study evaluated the antihyperglycemic activity of caprine milk fatty extract in streptozotocin (STZ) induced rats. STZ induced
diabetes rats have been widely used as a model for evaluation of antidiabetic activity. STZ is taken up by pancreatic β-cells via glucose transporter GLUT2. The main cause of STZ-induced β-cell death is alkylation of DNA by the nitrosourea moiety of this compound. However, production of NO (Nitric oxide) and reactive oxygen species may also be involved in DNA fragmentation and other deleterious effects of STZ. In this model, diabetes arises from irreversible destruction of the β-islet cells of the pancreas, causing degranulation or reduction of insulin secretion and insulin is markedly depleted, but not absent. The isopropanol extract of Caprus hircus produced significant changes in the streptozotocin-induced diabetic rats by reducing the glucose levels considerably. The prolonged treatment of caprine milk extract on streptozotocin-induced diabetic rats produced consistent reduction in the blood glucose levels. There was significant decrease in blood glucose level of CHME and Standard treated group with mean values of normal control group. On the last (21st) day, there was significant (p<0.0001) decrease in blood glucose level of standard, CHME treated groups with the mean values of 157.6±3.656** and 117.6±3.839** respectively as compared with a mean value 317.2±4.244 of diabetic control group. The blood glucose data obtained clearly indicate that the Caprine milk extract produced significant and consistent antihyperglycemic effect in streptozotocin-induced diabetic rats. The continuous treatment with Caprine milk extract for a period of 21 days produced a significant decrease in the blood glucose levels of the diabetic rats, but not in the normal rats. It is possible that the drug may be acting by potentiating the pancreatic secretion or increasing the glucose uptake. The results of effect of isopropanol extract of Caprus hircus milk on Blood Glucose level before treatment and after treatment are summarised in Table-1.

Serum cholesterol, urea, creatinine, SGOT, SGPT levels are decreased significantly by the isopropanol extract of caprine milk when compared with the diabetic control. The extract exhibited anti-diabetic property in streptozotocin induced rats as exhibited by the blood glucose levels. (Table 2) The study of anti-inflammatory activity of isopropanol extract of Caprus hircus against carrageenan induced paw edema shows that the extract has significant effect on inflammation and markedly reduced the swelling. The percentage reduction in the paw volume in the group of animals treated with Caprine milk extract 100mg was 56.08% and for the 200mg/kg was 71.73% at 4 hours. It shows that the plant extract have significant (P <0.01) anti-inflammatory effect and the results were compared with indomethacin 10mg/kg and show percentage paw volume reduction of 66.30%. (Table 3). Carrageenan induced hind paw edema is the standard experimental model of acute inflammation. Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. Moreover, the experimental model exhibits a high degree of reproducibility. Carrageenan induced edema is a biphasic response. The first Phase is mediated through the release of histamine, serotonin and kinins whereas the second phase is related to the release of prostaglandin and slow reacting substances with peak at 4h.

### Table 1: Effect of Fatty extract of Caprus hircus milk on the blood glucose concentration in streptozotocin induced rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg)</th>
<th>Blood glucose concentration (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>initial</td>
</tr>
<tr>
<td>Normal Control</td>
<td>-</td>
<td>78.40±0.75</td>
</tr>
<tr>
<td>Diabetic control (STZ)</td>
<td>65</td>
<td>279.9±3.94</td>
</tr>
<tr>
<td>CHME I</td>
<td>100</td>
<td>286.0±3.28</td>
</tr>
<tr>
<td>CHME II</td>
<td>200</td>
<td>280.9±4.20</td>
</tr>
<tr>
<td>Standard (Metformin)</td>
<td>500</td>
<td>278.7±4.23*</td>
</tr>
</tbody>
</table>

P < 0.05 when compared to control. * P < 0.01. ** P < 0.001 when compared to control. Number of animal / in each group  = 6; Data expressed in mean ± SEM

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Table 2: Effect of Fatty extract of *Caprus hircus* milk on the serum biochemical parameters

<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>vehicle</th>
<th>Diabetic control</th>
<th>standard (Metformin)</th>
<th>Extract (100 mg)</th>
<th>Extract (200 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>81.43 ± 2.3</td>
<td>316.25 ± 2.6</td>
<td>206.59 ± 2.5</td>
<td>148.57 ± 4.8</td>
<td>149.36 ± 3.1</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>128 ± 3.5</td>
<td>224.5 ± 6.7</td>
<td>140.26 ± 4.46</td>
<td>185.25 ± 4.55</td>
<td>154.67 ± 3.60</td>
</tr>
<tr>
<td>Urea</td>
<td>22.0 ± 1.5</td>
<td>78.5 ± 3.47</td>
<td>30.16 ± 2.56</td>
<td>43.22 ± 2.0</td>
<td>32.15 ± 1.44</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.76 ± 0.08</td>
<td>1.72 ± 0.16</td>
<td>0.95 ± 0.10</td>
<td>1.22 ± 0.05</td>
<td>1.04 ± 0.06</td>
</tr>
<tr>
<td>SGPT</td>
<td>73.26 ± 2.60</td>
<td>135.40 ± 7.21</td>
<td>89.50 ± 2.57</td>
<td>110.33 ± 4.05</td>
<td>94.45 ± 5.40</td>
</tr>
<tr>
<td>SGOT</td>
<td>26.45 ± 1.5</td>
<td>65.72 ± 3.84</td>
<td>37.55 ± 1.63</td>
<td>56.64 ± 2.30</td>
<td>48.96 ± 2.67</td>
</tr>
<tr>
<td>Total protein</td>
<td>7.8 ± 0.06</td>
<td>3.9 ± 0.02</td>
<td>8.4 ± 0.10</td>
<td>4.7 ± 0.05</td>
<td>6.5 ± 0.03</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n = 6); * p < 0.001 compared with saline control and ** p < 0.001 compared with STZ control group

Table 3: Effect of Fatty extract of *Caprus hircus* milk on paw edema induced by carrageenan in rats:

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose mg/kg</th>
<th>30 min</th>
<th>Mean paw volume ± SEM (ml)</th>
<th>and % Inhibition (P.I)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>60 min</td>
<td>120 min</td>
<td>180 min</td>
</tr>
<tr>
<td>Normal control</td>
<td>-</td>
<td>0.148 ± 0.00</td>
<td>0.165 ± 0.00</td>
<td>0.174 ± 0.00</td>
</tr>
<tr>
<td>CHME I</td>
<td>100</td>
<td>0.147 ± 0.00</td>
<td>0.090 ± 0.00</td>
<td>0.081 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>4*</td>
<td>0.165 ± 0.00</td>
<td>0.163 ± 0.00</td>
<td>0.173 ± 0.00</td>
</tr>
<tr>
<td>CHME II</td>
<td>200</td>
<td>0.142 ± 0.00</td>
<td>0.078 ± 0.00</td>
<td>0.058 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>5*</td>
<td>0.150 ± 0.00</td>
<td>0.148 ± 0.00</td>
<td>0.156 ± 0.00</td>
</tr>
<tr>
<td>Standard (Diclofenac)</td>
<td>10</td>
<td>0.129 ± 0.00</td>
<td>0.072 ± 0.00</td>
<td>0.060 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>04*</td>
<td>0.136 ± 0.00</td>
<td>0.075 ± 0.00</td>
<td>0.063 ± 0.00</td>
</tr>
</tbody>
</table>

P < 0.05 when compared to control. * P < 0.01. when compared to control. Number of animal / in each group = 6; Data expressed in mean ± SEM

Conclusion

It can be concluded that isopropanol extract of *Caprus hircus* milk has anti-inflammatory activity against carrageenan induced paw edema in rats and a significant anti-hyperglycemic activity against streptazotocin induced diabetic rats. The activity may be due to their content of fatty acids like lauric acid, palmitic acid, stearic acid, oleic acid. This study demonstrates the efficacy of *Caprus hircus* milk as an anti-hyperglycemic agent and anti-inflammatory agent. It also scientifically justifies the use of this milk extract as an anti-edematous agent in folk medicine. However, further studies are required to determine the constituents responsible for its activities and further authenticate its mechanism of action at the cellular and molecular levels.

Conflict of Interests

The authors declare that they have no conflict of interests.

Acknowledgment

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