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COW URINE- A PANACEA

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ABSTRACT: Cow urine is considered a Sanjeevani–a medicine capable of curing any disease according to ancient manuscripts from Vedic times. The present study was conducted to validate the medicinal properties of cow urine in a scientific manner. The cow urine sample was procured from Indian indigenous cow Bos indicus from Bombay Panjrapole, Bhuleshwar, Mumbai, and commercial gaumutra from the local market. Various treatments were given to the freshly collected cow urine *viz*. Photoactivated urine (PA), Cow urine distillate (DIS) and Crude cow urine sample (CRU) was used after filtration and centrifugation. *In-vitro* assays for antimicrobial (agar cup method), antioxidant (DPPH assay), anti-hyperglycemic (α-amylase inhibition) and anti-inflammatory (protein denaturation inhibition bioassay) properties of cow urine were carried out. Results confirmed antimicrobial (19 mm zone of clearance), antioxidant (157 µg /ml), anti-hyperglycemic (2.08 µg /ml) and anti inflammatory (23.72µg /ml) and comparable to Chloramphenicol, Ascorbic acid, Acarbose, and Ibuprofen used as standard respectively. The study demonstrates various medicinal properties of cow urine using *in-vitro* assays.

INTRODUCTION: Research in the field of Ayurvedic and natural medicine helps us to make prudent use of traditional knowledge and its application to modern medicine. Cow urine has pharmacological importance. In Ayurveda, the medicinal utility of cow urine has been mentioned in depth. According to Vedic literature and Hindu Mythology cow is the abode of Gods. She is Kamdhenu (desire fulfiller) personified. All qualities of cow urine have been written in Chapter 45 of Sutra Sthan of Sushrut samhita a five thousand years old Ayurvedic manuscript.

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The qualities of cow urine have been mentioned in other Ayurvedic manuscripts such as Charaka Samhita, Rajnighantu, Vriddha vagbhat and Amritsagar (Sushrita Samhita [1].

The importance of cow urine is immense. It has uses in every possible field of biological sciences. The agricultural application comprises of use as a biofertilizer and biopesticide [1]. The therapeutic application includes blood purification, skin diseases, cancer, piles, thyroid, and heart disease [2]. Cow urine is also used as an immune modulator. It promotes T and B cell blastogenesis [3, 4]. Cow urine is used majorly as a bio enhancer to increase the effect of the antibiotics [5, 6,] anti-bacteria [l7,] anti-fungal [8, 9,] anti-tubercular 8, anti-tuberculosis[10], anti-inflammatory 11 and anti-cancer drugs [12, 13]. It has also been granted a US Patent-6410059, 5616593, 5972382, 6896907 and 7235262 for the same [8, 13, 14, 15].

Panchagawya is composed of five ingredients, viz. urine, dung, milk, curd and ghee, obtained from a cow. This Panchagawya is given to women after their delivery. It is also one of the main ingredients of many Ayurvedic preparations [16]. Research has been carried out to test antimicrobial and antioxidant property of cow urine [17, 18, 19, 20, 21, 22, 23]. There have been very few reports on the anti inflammatory use of cow urine [24]. *In-vivo* anti-hyperglycemic activity of cow urine has been carried out [25]. However, there is no report on in vitro anti-hyperglycemic assay. As cow urine is claimed to be a 'Sanjeevani *i.e.*, able to cure all possible ailments, in the present study, an attempt was made to validate the traditional knowledge of Vedas using scientific experimentation. Thus the antimicrobial, antioxidant anti-hyperglycemic and anti-inflammatory activity of cow urine was assessed using standard *in-vitro* assays.

MATERIALS AND METHODS:

Sample Collection: The urine samples of indigenous Gujarati Indian breed - Gir variety of Bos indicus cows were used in the study. The samples were collected from Bombay Panjrapole, Bhuleshwar, Mumbai. The cow breed was identified by veterinarian Dr. Ramesh Pokar.

The urine samples were collected under the guidance of Dr. Pokar. The cows were certified to be free of any diseases. Fresh urine was collected in sterile polyethylene bottles. To remove any debris and precipitated matter, the urine was filtered through Whatman filter paper no. 1. The filtrate was then centrifuged at 4 ºC for 10 min at 10000rpm. Purified cow urine was stored at 4 °C for long-term use. Before evaluation, cow urine was tested for the presence of other pathogens microscopically as well as in broth culture.

- Various treatments were performed on the cow urine as follows
- Photoactivated urine (PA) was prepared by exposing fresh cow urine to UV rays for 2 h in a sealed glass bottle.
- Cow urine distillate (DIS) was obtained as a commercial sample of the Patanjali brand.
- Crude cow urine sample (CRU) was used after filtration and centrifugation, as mentioned above.
- The above three treatments were used for all the assays.

Antimicrobial Activity: In the present study, the test organisms used were *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* procured from the Department of Life Sciences, University of Mumbai. Chloramphenicol (100 mg/ml) was used as a positive control. The antimicrobial activity of the selected cow urine preparations (PA, DIS, CRU) was performed by agar cup method mentioned by Bauer *et al.* with some modifications [26]. 100 µl of the different urine preparations (PA, DIS, CRU) were added in respective wells along with positive and negative controls. The plates were incubated at 37 ºC for 24 h. The zone of inhibition was measured in mm after 24 h.

Antioxidant Activity: The percentage of antioxidant activity of each substance was assessed by DPPH free radical assay according to Brand-Williams et al. method with some modifications [27]. Ascorbic Acid (standard) of different concentrations (from 25 to 250 µg/ml with an interval of 25 µg/ml) was added. The cow urine samples were reacted with the stable DPPH radical in an ethanol solution. The reaction mixture consisted of samples (PA, DIS, CRU) and 0.1mM DPPH radical solution in ethanol.

The changes in colour (from deep violet to light yellow) after 30 min at 37°C were read at 517 nm using a UV-VIS spectrophotometer (Shimadzu, UV-1800). The mixture of ethanol and DPPH served as control and ethanol as blank. The concentration of the antioxidant property was determined using the standard graph from the slope. As cow urine is a liquid sample, it is difficult to determine the IC_{50} value; hence percentage inhibition was determined. The scavenging activity percentage (AA%) was determined according to Mensor *et al.* [28].

% Inhibition = Absorbance of control –Absorbance of sample/Absorbance of control) × 100

Antihyperglycemic Activity: Anti-hyperglycemic activity of cow urine was determined in vitro with the method used by Adisakwattana *et al.* with modifications [29]. 0.5 ml of Acarbose (standard) of different concentrations (0.5 to 2.5 mg/ml with the

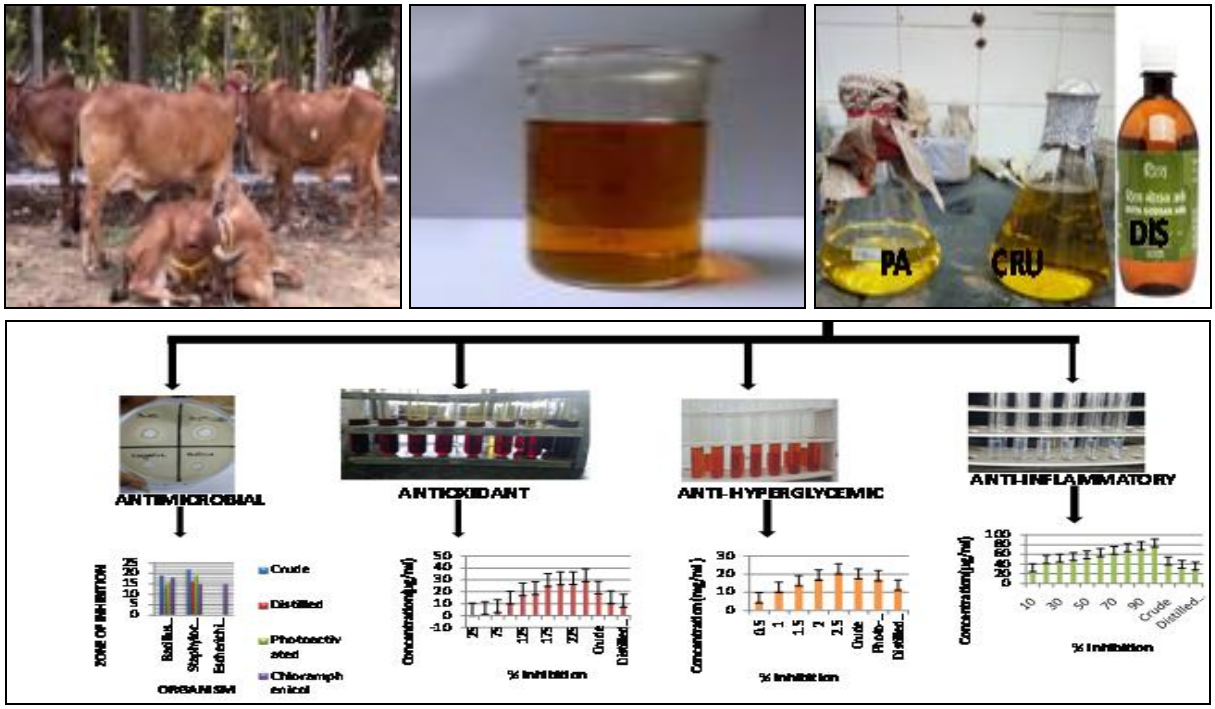
interval of 0.5 mg/ml) was added. In the cow urine tubes, 0.5 ml each of different cow urine preparations (PA, DIS, CRU) were used and processed similar to the standard acarbose. The inhibitory reaction was allowed to take place for 45 min at 37 ºC. A control is prepared undertaking all the steps except 0.5 ml acarbose was replaced by 0.5 ml phosphate buffer pH 6.9. The blank tube contained distilled water and DNSA reagent. The absorbance was read at 546 nm using a UV-VIS spectrophotometer (Shimadzu, UV-1800). The concentration of the anti-hyperglycemic property was determined using the standard graph from the slope. As cow urine is a liquid sample, it is difficult to determine the IC_{50} value; hence percentage inhibition was determined. The absorbance obtained is used to calculate the % inhibition shown by acarbose and cow urine, respectively, with the use of the following formula

% Inhibition = Absorbance of control – Absorbance of sample/Absorbance of control × 100

Anti-inflammatory Activity: *In-vitro* protein denaturation inhibition bioassay was carried by Sakat *et al.* with some modifications [30]. Ibuprofen was used as a standard. Ibuprofen (100 µg/ml) of different concentrations (10 to 100 µg/ml with the interval of 10 µg/ml) was added. The reaction mixture consisted of a sample and 5% w/v BSA. Distilled water was used as a blank. In the cow urine tubes, different cow urine preparations (PA, DIS, CRU) were used and processed as standard Ibuprofen assay. The inhibitory reaction was allowed to take place for 20 min at 37 ºC and then heated at 57 ºC for 3 min.

After cooling the test tubes, 2.5 mL of Phosphate Buffer Saline (PBS, pH 6.3) was added to each tube, and absorbance was read at 660nm on UV-VIS spectrophotometer (Shimadzu, UV-1800). A control tube where no drug was added was maintained. The concentration of the anti-inflammatory property was determined using the standard graph from the slope. As cow urine is a liquid sample, it is difficult to determine the IC_{50} value; hence percentage inhibition was determined. Percentage protein denaturation inhibition for different types of cow urine samples and different concentrations of standards was calculated as follows.

% Inhibition = Absorbance of control –Absorbance of sample/Absorbance of control × 100



Results:

Antimicrobial Activity: Positive results were observed only in Gram-positive organisms. *i.e. B. subtilis* and *S. aureus*. *E. coli* did not show susceptibility to any cow urine samples. The best results were observed in crude cow urine samples followed by photoactivated cow urine and commercial sample **Fig. 1**. The crude cow urine

sample gave a zone of clearance of 19 mm and 22 mm in *B. subtilis* and *S. aureus*, respectively **Table 1** which was higher than the positive control Chloramphenicol (18mm and 15 mm).

TABLE 1: ANTIBACTERIAL POTENTIAL OF DIFFERENT COW URINE PREPARATIONS

Organisms	Sample	Zone of inhibition (in mm)
B. subtilis	Crude	19
	Distilled	14
	Photoactivated	16
	Chloramphenicol	18
S. aureus	Crude	22
	Distilled	16
	Photoactivated	19
	Chloramphenicol	15
E. coli	Crude	No clearance
	Distilled	No clearance
	Photoactivated	No clearance
	Chloramphenicol	15

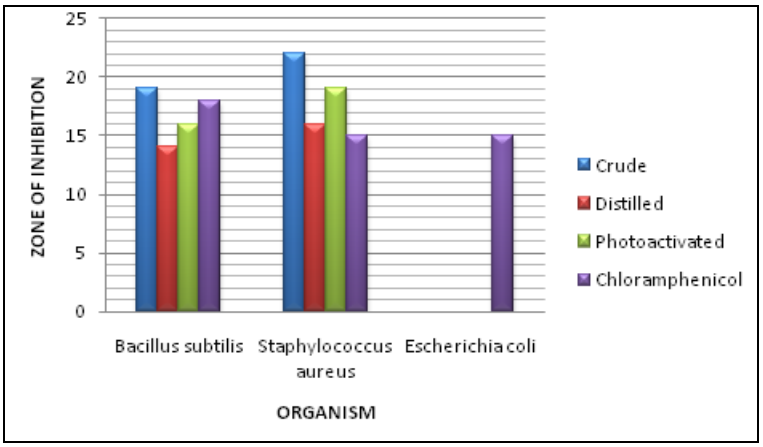


FIG. 1: ZONE OF INHIBITION FOR COW URINE SAMPLES Results are presented as mean ±sd (n=3)

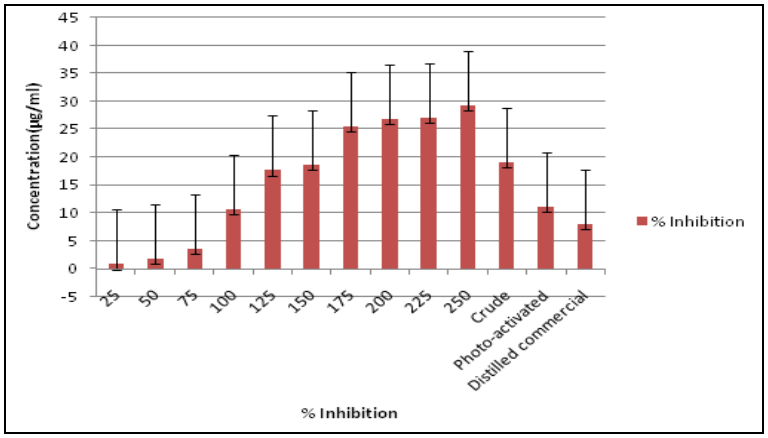


FIG. 2: ANTIOXIDANT ACTIVITY OF COW URINE SAMPLE Results are presented as mean ±sd (n=3)

TABLE 2: ANTIOXIDANT ACTIVITY OF COW URINE SAMPLES

Sample	Concentration(µg/ml)	Absorbance at 517 nm	% Inhibition
Ascorbic acid	25	1.220	0.893±4.75
	50	1.207	1.949±4.75
	75	1.186	3.655±4.75
	100	1.098	10.803±4.75
	125	1.013	17.709±4.75
	150	1.001	18.683±4.75
	175	0.916	25.580±4.75
	200	0.900	26.887±4.75

	225	0.898	27.051±4.75
	250	0.869	8.123±4.75
Cow Urine	Crude	0.998	19.091±4.75
	Photo-activated	1.094	11.129±4.75
	Distilled commercial	1.131	.123±4.758
	25	1.231	-

Antioxidant Activity: The percentage inhibition is a measure of the antioxidant activity is a measure of DPPH scavenging free radical reaction of Ascorbic acid (standard) [18]. The cow urine preparations show positive antioxidant activity. The concentration of cow urine samples in the respective preparations was as follows: PA = 101.87 µg/ml, DIS=80.98 µg/ml and CRU = 157.20 µg/ml respectively from the slope of the standard plot of ascorbic acid inhibition. It is comparable with 150 µg/ml of ascorbic acid. The results show that crude cow urine had the highest inhibitory activity (19.091%). Photo activated cow urine had an intermediate activity of 11.129% where as distilled commercial sample of had the least inhibitory activity of 8.123% **Table 2**, **Fig. 2**. This indicates that cow urine has an antioxidant activity comparable with standard Ascorbic acid.

Antihyperglycemic Activity: The percentage inhibition is a measure of the antihyperglycemic activity of acarbose (standard) and cow urine against α- amylase enzyme. The concentration of cow urine samples (from the slope of standard graph) in the respective preparations was found as: PA=1.96 mg/ml, DIS=1.21 mg/ml and CRU= 2.08 mg/ml and comparable with 2.5 mg/ml of Acarbose. The cow urine preparation showed positive antihyperglycemic activity. Results indicate that crude cow urine had the highest inhibitory activity 18.20%, comparable to 2.5 mg/ml of Acarbose (20.83%) followed by photo-activated 17.22%. The distilled sample showed the lowest activity in **Table 3**, **Fig. 3**. This implies that cow urine has an antihyperglycemic activity comparable with the standard drug Acarbose.

TABLE 3: ANTIHYPERGLYCEMIC ACTIVITY OF COW URINE SAMPLES

Sample	Concentration (mg/ml)	Absorbance at 546 nm	% Inhibition
Acarbose	0.5	1.737	5.03±9.68
	1.0	1.632	10.77±9.68
	1.5	1.567	14.32±9.68
	2.0	1.507	17.60±9.68
	2.5	1.448	20.83±9.68
Cow Urine	Crude	1.496	18.20±9.68
	Photo-activated	1.514	17.22±9.68
	Distilled commercial	1.609	12.02±9.68
Control	-	1.829	-

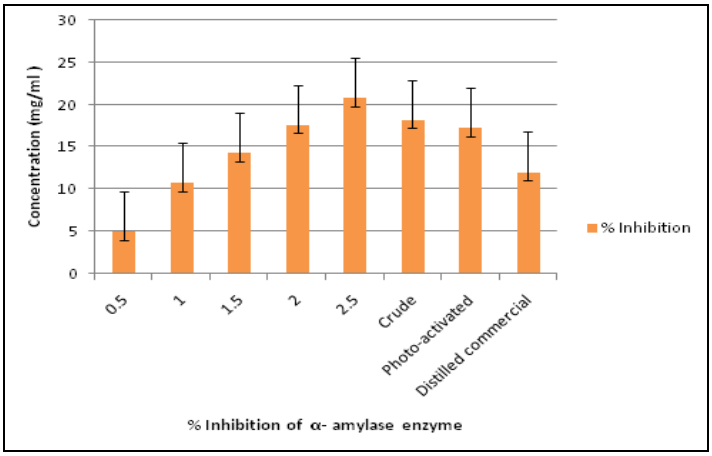


FIG. 3: ANTIHYPERGLYCEMIC ACTIVITY OF COW URINE SAMPLES Results are presented as mean ±sd (n=3)

Anti-inflammatory Activity: The percentage inhibition is a measure of the anti-inflammatory activity of Ibuprofen (positive control) [30]. The cow urine preparations showed positive anti

inflammatory activity. The concentration of cow urine samples in the respective preparations was found PA = 9.49 µg/ml, DIS = 2.39 µg/ml and CRU = 23.72 µg/ml comparable with 20-30 µg/ml of Ibuprofen (from the slope calculated from the standard plot of Ibuprofen). The crude cow urine had the highest inhibitory activity of 39.08% **Table 4** and **Fig. 4**. Photo-activated cow urine had an intermediate activity of 32.18%, whereas the commercial sample had a minimum inhibitory activity of 28.73%. This implies that cow urine is an anti-inflammatory agent but not as potent as the standard drug Ibuprofen.

TABLE 4: ANTI-INFLAMMATORY ACTIVITY OF COW URINE SAMPLES

Sample	Concentration(µg/ml)	Absorbance at 660 nm	% Inhibition
Ibuprofen	10	0.065	25.28±15.42
	20	0.050	42.52±15.42
	30	0.048	44.82±15.42
	40	0.045	48.27±15.42
	50	0.042	51.72±15.42
	60	0.038	56.32±15.42
	70	0.034	60.91±15.42
	80	0.029	66.66±15.42
	90	0.026	70.11±15.42
Cow Urine	100	0.021	75.86±15.42
	Crude	0.053	39.08±15.42
	Photo-activated	0.059	32.18±15.42
	Distilled commercial	0.062	28.73±15.42
Control	-	0.082	

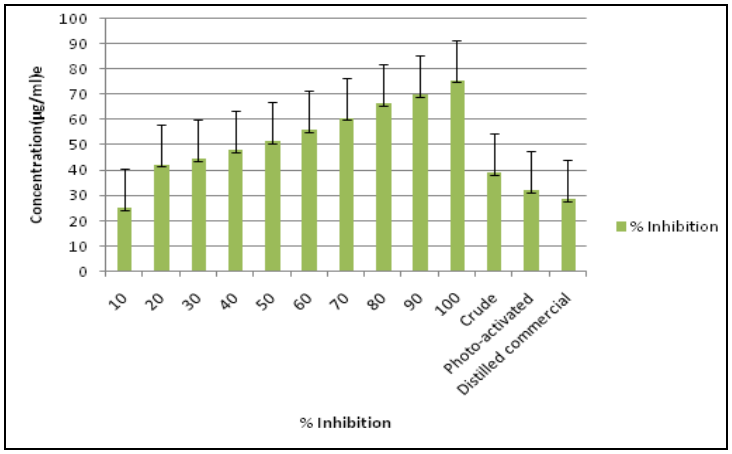


FIG. 4: ANTI-INFLAMMATORY ACTIVITY OF COW URINE SAMPLES. Results are presented as mean ±sd (n=3)

DISCUSSION:

Antimicrobial Activity: The fresh cow urine showed better antimicrobial activity as compared to photo-activated and distilled cow urine. The crude cow urine sample gave a zone of clearance that was greater than the positive control chloramphenicol (**Table 1**), thereby demonstrating that cow urine has better antibacterial activity as compared to standard broad-spectrum antibiotic Chloramphenicol. *E. coli* did not show susceptibility to any cow urine sample, however zone of clearance (15 mm) in positive control *i.e.* chloramphenicol, indicates that the strain was sensitive to antibiotics. Similar results have been observed by Jarald *et al.*, Rana and De, Shah *et al.* and Ahuja *et al.* against *S. aureus*, *Staphylococcos epidermitis*, *B. subtilis*, *Klebsiella pneumonia*, and *Proteus vulgaris* [18, 21, 23, 31]. In studies carried out by Jarald *et al.* and Ahuja *et al.*, cow urine has antibacterial activity against *E. coli* [18, 31]. The reason may be the susceptibility of the strain used. Thus other strains of *E. coli* should be tested for the same. These findings may be due to the presence in the cow urine of both volatile and non-volatile components. The chemicals like inorganic phosphorus, chloride and dimethylamine may also be involved in hampering bacterial growth [18, 21, 23, 31]. Purification of cow urine by filtration and centrifugation at high speed 10,000 rpm for 45 min at 4 °C has also significantly minimized the presence of microbes and free from

any infection. Photoactivated cow urine showed positive antibacterial activity. Few volatile biogenic compounds (CO_2, NH_3, CH_4, methanol, propanol, and acetone) may have been produced after photo-activation. It may also be due to the change in pH [18, 21, 23, 31]. Urine distillate was not found to be as potent as all the other cow urine preparations. One of the possible reasons may be the removal of toxic components due to distillation. Similarly, the commercial sample might have undergone processing due to which the activities of the natural components maybe have been lost. Only gram-positive organisms were observed to be more sensitive than gram-negative organisms, though. Such findings may be attributable to variations in the composition of the cell wall between these two classes of bacteria in which the outer membrane lipopolysaccharides of gram-negative bacteria may prevent certain environmental substances from entering [23]. As the antibacterial activity in present study is consistent with the reports from other researchers it can be inferred that the cow urine has good antibacterial activity.

Antioxidant Activity: Cow urine plays a significant role in medicine, having conducted a scientific experiment to elucidate the antioxidant function of cow urine. An antioxidant is a chemical moiety that prevents other chemicals from oxidizing and free radicals formation. They neutralize the damaging effects of free radicals, which are usual by-products of cell metabolism. They also protect major cellular components from damage. Free radical reaction in a large array of unrelated biological systems is an important pathway. As seen from the scavenging of DPPH radicals, the results demonstrate that all cow urine samples inhibited the free radicals. The fresh cow urine (157.20 µg/ml) was found to be better than the distillate (80.98 µg/ml). Photoactivated cow urine had a moderate antioxidant activity of 101.87 µg/ml. Cow urine has been found to be effective against free radicals in the present study. From the literature, it is understood that free radicals are involved in various metabolic disorders like diabetes, aging, and cancer. The observed antioxidant property of cow urine samples may provide probable remedial intervention against oxidative stress. The result indicates the antioxidant action is due to the free radical scavenging behaviour of the cow urine components. Similar results were obtained in an *in-vitro* study carried out by Jarald *et al.,* using crude and distillate of cow urine [18]. Lavania *et al. (*2011) carried out an *in-vivo* study on lipid peroxidation, radical scavenging, and level of reduced glutathione and catalase activity using rats [19]. This research provides a thorough validation of free radical scavenging activity of cow urine and lays a comparison between different preparations *i.e.*, crude, photo-activated and distilled. Further analysis like LCMS can be done of cow urine to predict the possible constituent that might have the anti-oxidative property.

Anti-hyperglycemic Activity: The use of cow urine as an anti-diabetic agent has been listed in various Ayurvedic manuscripts. A crude cow urine sample with a concentration of 2.08 mg/ml was comparable with 2.0 mg/ml of Acarbose. This implies that cow urine is as potent an anti-hyperglycemic agent as acarbose which is a standard anti-diabetic drug. Similar results were observed in photo-activated and distilled samples and comparable to anti-hyperglycemic activity of acarbose standards at a lower concentration. The difference in the anti-hyperglycemic activity of different cow urine samples maybe due to loss of metabolite components during distillation. The photo-activated cow urine sample may have lost its volatile components and thus showed reduced activity. A number of in vivo experiments were carried out to test the anti-hyperglycemic activity of cow urine, which verified the presence of this activity in cow urine [18, 22, 31]. Cow urine was also found to have anti-hyperglycemic activity as an extraction medium with herbal extracts [32]. However, there were no reports on in vitro analysis of the anti-hyperglycemic activity of cow urine. In our study, different cow urine preparations, *i.e.*, crude, photo-activated, and distilled, showed positive antihyperglycemic activity in comparison to the standard anti-diabetic drug used acarbose. Chemo profiling of cow urine by Gowen lock and McMurray (1988) revealed the presence of several components like protein, aromatic acids, urea, uric acid, creatinine, phenol, and enzymes *viz.* acid and alkaline phosphatase, amylase and vitamins [33]. There is a significant correlation between the total content of phenol and its ability to inhibit α-

amylase in the intestinal pancreatic. Cow urine contains sulfur that may have some activities such as sulphonylureas or may increase insulin receptor sensitivity, or may increase insulin release. Cows are herbivores. The primary and secondary metabolites of these herbs may be excreted out in the urine. Many therapeutic elements have been found in cow urine. Cow urine contains volatile fatty acids, which act as free radical scavengers. Furthermore, in various studies it was found that cow urine has an antioxidant effect. Kwon *et al.,* (2008) indicated that natural components may be effective therapeutic agents for the control of postprandial hyperglycemia with fewer side effects than acarbose [34]. The anti- hyperglycemic effect may be attributed to the antioxidant activity of cow urine by preventing the formation of the free radicals, which may cause damage to the beta cells of the pancreas. Thus crude cow urine is the best sample for therapeutic use as an anti-hyperglycemic agent.

Anti-inflammatory Activity: Anti-inflammatory property is studied as inhibition of albumin denaturation. Protein denaturation is a well-established cause of inflammation [29]. Ibuprofen, a standard anti-inflammatory drug, was used in this assay. Inflammation is the response of living tissues to injury. It involves a complex array of enzyme activation, mediator release, extravasations of fluid, cell migration, tissue breakdown, and repair [35]. Protein degeneration is a well-known cause of inflammation and rheumatoid arthritis [36]. Cow urine samples were effective in inhibiting heat-induced albumin denaturation. A maximum percentage of inhibition of 39.08% was observed in crude cow urine. Results were consistent with Sakat *et al.,* (2010) [29].

Several anti-inflammatory drugs have demonstrated a dose-dependent ability to prevent protein denaturation through thermal induction [37]. Cow urine's ability to lower down protein thermal denaturation may be a contributing factor for its anti-inflammatory activity. The anti-inflammatory activity of cow urine can be due to an unknown component present in it, which can be analyzed and predicted by LCMS. Further development can be done and a potent anti-inflammatory agent with low toxicity and better therapeutic index can be produced.

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CONFLICTS OF INTEREST: The authors declare no conflict of interest.

Author Contributions: Priyanka Lakhani Investigation, Methodology, Data curation, Original draft preparation. Kunj Joshi Investigation, Methodology, Data curation, Original draft preparation. Aditi Kulkarni Conceptualization Suruchi Jamkhedkar Conceptualization, Visualization, Supervision, Original draft preparation.

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CONCLUSION: Ayurveda and modern-day drugs could be used in combination to benefit humanity at the optimum level. In this research, it was scientifically provided evidence that cow urine, when used as an antimicrobial agent, was found to effectively inhibit the growth of microbes. It shows the strong inhibitory activity as an anti-hyperglycemic, antioxidant and anti-inflammatory agent. This can be further used to develop drugs that will be cheaper and which have the easily available raw material. It can be concluded that the Vedic and the Ayurvedic literature hold their strong roots in the manuscripts as well as in the scientific world on the uses of cow urine and its ability to cure many maladies.

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