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Antioxidant and Antimicrobial Activities of Cow Urine

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Abstract: Number of plants and animal derived materials were reported to have antioxidant and antimicrobial activity. The present study relates to such precious and holy animal derived material cow urine, which has these activities. Antioxidant activity was done using two *in vitro* models, DPPH radical scavenging activity and Superoxide scavenging activity. Ascorbic acid was used as the reference standard. The anti microbial activity of cow urine and its distillate was tested by agar well method using the microbes like *Escherichia coli*, *Staphylococcos aureus*, *Staphylococcos epidermitis*, *Bacillus subtilis*, *Klebsiella pneumoniae* and *Proteus vulgaris*. The cow urine and its distillate tested for antioxidant and antimicrobial activities exhibited the mentioned activities and comparatively fresh cow urine was found to be better than its distillate. These results indicate that the cow urine has antioxidant and antimicrobial activities, which supports the claim of traditional practitioners.

Key words: *Bos indicus* % Free radicals % Agar well method % Antimicrobial % Gomutra

INTRODUCTION

Cow, *Bos indicus* is a most valuable animal in all Veda and it is called as the Mother of all. The composition containing cow's excretions, urine, dung, milk, curd and ghee, five ingredients together known as "Panchagawya" is given to women after she delivers a baby. Panchagawya is the main ingredient of many of our ayurvedic preparations [1]. Cow urine one of the ingredients in panchagawya is believed to have therapeutic value. In India cow urine is used by majority of rural population as folklore remedy in almost all the states. Agencies in Gujarat have been marketing the cow urine preparations from multiple outlets, advertising that they are sterilized and completely fresh, with prices ranging from Rs. 20 to Rs. 30 per bottle. Urine therapy was not only used in India, but for several Centuries in many parts of the Globe. As per Ayurvedic literatures gomutra is useful in number of diseases particularly in gulma, kusta, ascitis, filaria, aburda (cancer), etc. Cow urine is also used along with herbs to treat various diseases like fever, epilepsy, anemia, abdominal pain, constipation, etc by the traditional healers [2, 3]. Immunomodulatory [4], hypoglycemic [5] and cardio-respiratory effects [6] of cow urine were established scientifically. Local traditional healers in Mandsaur prescribe cow urine for worm complaints, to develop immunity and to avoid aging. They suggest 10-25 ml of cow urine to be taken in empty stomach for the same. Since free radicals are implicated in the process of aging and presence of inorganic substances in cow urine, our aim was devoted to investigate its antioxidant and antimicrobial properties. According to ancient literatures distillate of cow urine was the one to be used mainly and the distillate was found to exhibit antioxidant effect [3]. So in our present study we have compared fresh cow urine and its distillate for the above-mentioned activities.

MATERIALS AND METHODS

Procurement of cow urine: The urine of Gujarati Indian cow known as Geer cow was used in the study. The study was performed after getting a certificate from the Veterinary doctor stating that it is free from diseases. Fresh urine was collected and filtered. Chemoprofiling confirmed the presence of protein, urea, uric acid, creatinine, phenol, aromatic acids, enzymes like acid phosphatase, alkaline phosphatase, amylase and vitamins [7].

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Antioxidant activity: The antioxidant activity of fresh urine and its distillates was carried out using two methods, DPPH radical scavenging activity and Superoxide scavenging activity [8]. Ascorbic acid was used as the standard.

DPPH radical scavenging activity was measured by spectrophotometric method. To a methanolic solution of DPPH (100 µM, 2.95 ml), 0.05 ml test compound dissolved in methanol was added at different concentrations (1-5 mg ml^{-1}). Equal amount of methanol was added to the control. Absorbance was recorded at 517 nm at regular intervals of 10 minutes for 20 minutes. The percentage reduction was calculated as per the formula

$$\% \text{ Reduction} = \frac{\text{Control absorbance-Test absorbance}}{\text{Control absorbance}} \times 100$$

Superoxide scavenging actyivity was carried out by using alkaline DMSO method. Solid potassium superoxide was allowed to stand in contact with dry DMSO for at least 24 hours and the solution was filtered immediately before use. Filtrate (200 µl) was added to 2.8 ml of an aqueous solution containing nitro blue tetrazolium (56 µM), EDTA (10 µM) and potassium phosphate buffer (10 mM). Sample of urine 1 ml at various concentrations (1-5 mg ml^{-1}) was added and the absorbance was recorded at 560 nm against a control in which pure DMSO has been added instead of alkaline DMSO. The percentage reduction was calculated using the formula

$$\% \text{ Reduction} = \frac{\text{Control absorbance-Test absorbance}}{\text{Control absorbance}} \times 100$$

Statistics: The decolorization was plotted against the sample extract concentration and a linear regression curve was established in order to calculate the IC_{50} (µg ml^{-1}) being the amount of sample necessary to decrease by 50% the absorbance of radicals.

Antimicrobial activity: The antimicrobial activity of cow urine and its distillates was tested by agar well diffusion method for the following strains of microbes like *Escherichia coli* (NCIM 2931), *Staphylococcus aureus* (NCIM 2079), *Staphylococcus epidermitis* (NCIM 2493), *Bacillus subtilis* (NCIM 2063), *Klebsiella pneumoniae* (NCIM 2957) and *Proteus vulgaris* (NCIM 2027). Size of the well was 10 mm and 0.5 ml of urine and its distillate was introduced. Ofloxacin 10 µg ml^{-1} was used as the standard for the study. The petri dishes were then incubated at 37°C for 24 h and the zone of inhibition was measured [9].

RESULTS AND DISCUSSION

The result shows that cow urine and its distillate inhibited the free radicals as seen from scavenging of super oxide and DPPH radicals (Table 1). Comparatively fresh cow urine was found to be more active than its distillate. As far as the antimicrobial study is concerned, the samples, fresh cow urine and its distillate have exhibited antimicrobial activity and comparatively fresh cow urine has exhibited better antimicrobial activity (Table 2). The activity of fresh cow urine was comparable with that of the standard, Ofloxacin.

Keeping in view the enormous role of cow's urine in medicinal and veterinary medicine, a scientific experiment was undertaken to elucidate the antioxidant and antimicrobial activitie of cow urine. Cow urine was found to be effective against free radicals and microbes. An antioxidant is a chemical that prevents the oxidation of other chemicals and the formation of free radicals. They protect key cell components by neutralizing the damaging effects of free radicals, which are natural by products of cell metabolism [10]. Free radical reaction is an important

Table 1: Free radical scavenging activity of cow urine and its distillate by DPPH and NBT method (*in vitro*)

	IC_{50} mg ml^{-1}/*µg ml^{-1}	
Products	NBT method	DDPH method
Fresh cow urine	2.9	3.0
Distillate of cow urine	5.0	5.1
Ascorbic acid	3.0*	2.9*

Table 2: Antimicrobial activity of cow urine and its distillate by well plate method

	Zone of inhibition (mm)		
Organisms	V_1	V_2	V_3
Escherichia coli (NCIM 2931)	23	20	30
Bacillus subtilis (NCIM 2063)	24	21	32
Staphylococcus epidermitis (NCIM 2493)	22	20	28
Staphylococcus aureus (NCIM 2079)	24	18	25
Klebsiella pneumoniae (NCIM 2957)	25	20	28
Proteus vulgaris (NCIM 2027)	23	20	28

V_1-cow urine, V_2-distillate of cow urine, V_3-Ofloxacin

Values are expressed in mean of triplicates

pathway in a wide range of unrelated biological systems. A vast amount of circumstantial evidence implicates free radicals as the mediators of wide range of diseases including diabetes, ageing, cancer, etc [11].

The revealed antioxidant property of cow urine and its distillate may provide potential therapeutic intervention against oxidative threats, both in health and disease. The result suggests that the antioxidant action is attributed to the free radical scavenging activity of the urine components and these components may prevent the process of aging.

According to Linton and Dick [12], phenols are bactericidal to gram positive and gram-negative bacteria. Therefore, presence of phenols in cow urine may be instrumental for its potent antimicrobial nature. Presence of more amount of phenol in cow urine compared to distillate may be the reason for its better activity. The results obtained suggest that the local traditional healers are successfully using cow urine as a medicine may be because of its observed antioxidant activity and much work in this direction has to be done to confirm its utility in higher models.

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