

IMMUNOMODULATORY AND ANTIOXIDANT EFFECT OF GOMUTRA ARK IN RATS.

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Introduction

In Sushrita Samhita Gomutra (Cow urine) has been described as the most effective substance / secretion of animal origin with innumerable therapeutic values. In India, drinking of cow urine has been practiced for thousands of years.

All the five products such as urine, milk, ghee, curd and dung obtained from cow possess medicinal properties, and are used singly or in combination with some other herbs against many diseases including Cancer, AIDS and Diabetes.^{1,2} This kind of alternative treatment, termed as "cowpathy".

Immunomodulatory agents are used to either suppress or stimulate the immune responses. Till date no potent immunomodulator medicine is available. Natural products of plant and animal origin offer a vast resource of newer medicinal agents with potential in clinical use.^{3,4} Rasayanas are a group of non toxic natural products preparations which are used to improve the general health by stimulating the body's immunity.

Antioxidant are substances that protect cells against the effects of free radicals. Free radicals are molecules produced due to exposure to tobacco smoke and radiation. Free radicals can

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damage cells and may play a role in heart disease, cancer and other diseases. Although there are claims that cow urine increase antioxidant enzymes still scientific studies are required to support this.⁵

Cow urine patented (U.S. Patents No. 6896907 and 6,410,059) for its medicinal properties recently, particularly for its use along with antibiotics for the control of bacterial infection and fight against cancers.⁶

Practitioners of Ayurvedic medicine from India routinely use cow urine as a remedy to treat many common disorders. In most of the cases the efficacy of this treatment regimen is unproven. However, only few attempts have been made to correlate scientifically the nature of cow urine. There are some studies on the use of cow urine but the studies on Gomutra ark which is prepared by distillation of the cow urine and more palatable due to its lower ammonia content, are scarce.

In order to correlate, ancient and traditional knowledge of use of cow urine with the modern day, scientific methodologies and parameters, we decided to conduct this study, regarding use of Gomutra Ark as Immunomodulatory and antioxidant agent.

Materials and Methods

Chemicals and Equipment

Gomutra *ark* was procured from Govigyan Anusandhan Santhan, Deolapur,

Nagpur. Alloxan monohydrate was purchased from sigma chemicals, Mumbai.. The solvents and chemicals of analytical grade were used and obtained from the Swastik Chemicals Nagpur.

Animals

Adult Wistar albino rats weighing 200-250 gm of either sex were used for the study. The animals were maintained under slandered laboratory conditions (light period of 12 h/day and temperature $27^{\circ} \text{C} \pm 2^{\circ} \text{C}$) with access to water and libitum. The animals were used in groups of 6 for all the studies.

Ethical clearance:

Ethical clearance was taken from Institutional Animal Ethics Committee of the institute where the research was conducted. (MGIMS/IAEC/5/2008)

Evaluation for Immunomodulatory action.^{7,8,9}

Under all the aseptic precautions 10 ml of blood was withdrawn from the carotid vein of sheep and was poured immediately in citrate bulb and mixed well to prevent coagulation. This procedure was done in microbiology department of the Institute under the guidance of the veterinary surgeon.

Measurement of RBC count:

The RBC count of sheep blood withdrawn was measured using Neubauers chamber. Five test tubes, containing 2 ml of sheep blood (taken from the blood kept in citrate bulb) were taken in test tube, which were kept for centrifugation at 2000 rpm for 20 minutes. The RBCs settled at bottom, the plasma from each test tube was withdrawn by using pipette and was discarded (withdrawn in such a way that RBC settled were not disturbed.)

Two groups of rats, containing 6 animals each weighing between 150 to 250 grams were taken. On day 0 both groups were immunized with Sheep RBC (SRBC) 0.5×10^9 cells/ml/100 grams intraperitoneally. The group I (control) was given normal food ad libitum for 21 days, where group II (GoA) was given Gomutra ark at dose of 0.2 ml BD for 21 days along with food ad libitum. On 14th day all rats received SRBC I.P again (booster dose of antigen, blood withdrawn again from sheep on 14th day, the RBC given as done on day 0)

On 21st day, under mild ether anesthesia the blood was withdrawn by using small capillaries from the retro orbital plexuses. Around 2 ml of blood was withdrawn from every rat and was kept in EDTA bulb. It was transferred in test tubes for centrifugation. Centrifugation was done at 2000 rpm for 30 minutes.

After centrifugation the plasma which contained antibodies against SRBC and was collected by using pipette was used, the RBC settled was discarded. The SRBC 0.025 ml, was taken in each well. (the plates containing 8 wells each for visualizing antigen-antibody reactions).

Now 0.025 ml normal saline was poured in first test tube, 0.025 ml of plasma from rats blood was poured in test tube 1, from which 0.025 ml transferred to test tube 2, from where 0.025 ml transferred to test tube 3... and so on. As a result the test tube 1 was having 1:1 dilution, the test tube 2 was having 1:2 dilution, test tube 3 having 1:4, and so on. The 8th test tube having 1:256 dilution (Serial dilution was done).

Total of 10 test tubes were used leading to two fold dilutions in each step. 0.025 ml of plasma containing antibodies was withdrawn from each test tube. It was made to react with

0.025 ml of SRBC, kept in each well of the 10 wells. The plates were incubated at 37degree Celsius for 1 hour. After 1 hour haemagglutination reaction was observed. If there was clumping, it was taken to be as the positive reaction. Haemagglutination reaction positive with highest dilution was taken as the antibody titre.

Evaluation of antioxidant action of the Gomutra ark

The antioxidant action of the Gomutra ark was assessed by using thiobarbiturate acid reactive substances and estimation of the ascorbic acid.

Estimation of thiobarbituric acid reactive substances (TBARS)

The Estimation of thiobarbituric acid reactive substances (TBARS) was assessed by the procedure given by Satoh in 1978, using thiobarbiturate acid reactive substances by TCA-TBA method.¹⁰ The animals were divided into three groups of 6 animals in each. The first group was taken as normal control. Group 2 animals were fed on normal diet *ad libitum* and considered as alloxan induced oxidant injury control. Group 3 animals were given Gomutra ark daily in dose of 1 ml/kg for 30 days. On 30th day, the blood was withdrawn from the retro-orbital plexus and was used for the measuring of the malondialdehyde (MDA) level. MDA value is expressed as Nmole/gm of Hb.

Estimation of ascorbic acid (Vitamin C)

The estimation of the ascorbic acid was assessed by the procedure given by Mc Cormack and Greene.¹¹ The animals were divided into three groups of 6 animals in each. The first group was taken as normal control. The diabetes was

induced in Group 2 and Group 3 by the procedure as mentioned earlier Group 2 animals were fed on normal diet *ad libitum* and considered as alloxan induced oxidant injury control. Group 3 animals were given Gomutra ark daily in dose of 1ml/kg for 30 days. On 30th day, the blood was withdrawn from the retro-orbital plexus and used for the measuring of the vitamin C levels. Vitamin C value is expressed mg/dl of Hb.

RESULTS

Immunomodulatory effect

The antibody titre for the control group was 8.33 ± 6.25 whereas for the group receiving Gomutra ark was 141.33 ± 98.32 . This was statistically significant with $P < 0.05$. (Table 1)

Table 1 : Mean Antibody titres after giving the drugs in rats for 30 days

	Mean Antibody Titre
Group1 (Control)	8.33 ± 6.25
Group 2 (GoA)	$141.33 \pm 98.32^*$

*P < 0.05 when GoA compared with Control.

Antioxidant effect of Gomutra ark.

The mean malondehyde (MDA) level was expressed in nano moles/gram of haemoglobin. In Group 1 (Control), on the day 0, level of MDA was 86.56 while on the 30th day the level of MDA was found 84.41. In Group 2 the levels of the MDA were 126.41 on day 0 and 124.17 on day 30th. The group 3 which received Gomutra ark, the mean MDA levels was found 127.11 on day 0 which was decreased significantly to 112.6 on 30th day. (Table 2)

The mean vitamin C level was expressed in mg/dl of Hb. In Group 1 (Control group), on the day 0 the, level of Vit C was 1.39 while on the 30th day the level of Vit C was found 1.39. In the

Table 2 : MDA levels of different groups expressed in nano moles/gram of Hb.

	Day	Mean ± SD Nmole / gm of Hb
Group 1	0	86.56 ± 9.78
	30	84.41 ± 10.21
Group 2	0	126.41 ± 28.15
	30	124.17 ± 12.6
Group 3	0	127.11 ± 19.71
	30	112.6 ± 20.31*

*P < 0.05 when GoA compared with Diabetic group.

group 2 the levels of the Vit C were 0.52 on day 0 and 0.34 on day 30th. The group which received Gomutra ark, the Vit C level was found 0.41 on day 0 which was decreased significantly to 0.27 on 30th day when compared to Group 2. (Table 3)

Table 3 : Vitamin C levels of different groups expressed in mg/dl of Hb.

	Day	Mean ± SD Mg/dl
Group 1	0	1.39 ± 0.39
	30	1.38 ± 0.54
Group 2	0	0.52 ± 0.34
	30	0.41 ± 0.31
Group 3	0	0.41 ± 0.30
	30	1.27 ± 0.25*

*P < 0.05 when GoA compared with Diabetic group.

Discussion

Since, the ancient times Gomutra ark has been used as immunomodulator in various diseases although we don't have much scientific evidence to support this. The study was carried out to evaluate the Immunomodulatory and antioxidant effect of Gomutra ark.

The antibody titre against the sheep RBC, in the group given Gomutra ark has increased significantly as compared to control. This shows that cow urine has immunomodulatory action.

In a study done by using Panchgavya and Haridradi Ghrita, in which cow urine is one of the component, when administered to rats, it was found that haemagglutinating antibody titre against sheep RBC was significantly raised (P<0.05).^{4,12}

Chauhan and others found that cow urine enhances both cellular and humoral immune responses. This study was done to investigate the blastogenic activity of lymphocytes and effect of in-vivo cow urine treatment so as to find out their potential to mount protective immune response against diseases. There are certain studies which supports this same view^{13,14,15}

The various mechanisms by which a drug can modify immune response are a) modifying cell mediated immune response which is by enhancing B and T cell response, b) by modifying Humoral response which is by enhancing antibodies like IgG and IgM, c) by increase in macrophage induced phagocytosis d) increase in total white blood cells.

Further increase in circulating antibody titre suggests that cow urine may stimulate the humoral immunity. As the antibody titre is increased, cow urine is definitely enhancing humoral immune response although other mechanisms cannot be ruled out.

In a study done by K. Krishnamurthi and othersthey have mentioned the presence of the volatile fatty acids in the cow urine which might act as antioxidant. The volatile fatty acid concentration in redistillate of cow urine has been found to be around 1500 mg/dl.^{4,16}

In our study we also found that Vit C which is an antioxidant is significantly increased and products of oxidation like malondialdehyde is significantly decreased. This effect also might be contributing for the immunomodulatory

effect, because if the DNA damage of any cell is being prevented, than a more better immune response can be generated resulting in immunomodulation.⁵

Gomutra ark is prepared by distillation of the cow urine. As results from Gomutra ark and Cow urine are comparable it appears that the medicinal properties of cow urine are preserved in Gomutra ark also. As Gomutra ark is free from high ammonia content of cow urine it is more palatable and acceptable to the population.

Hence in our study we found that cow urine distillate that is Gomutra Arka has antioxidant potential and this might be because of volatile fatty acids present in cow urine which are known to act as antioxidant

SUMMARY AND CONCLUSION

in conclusion our study suggests that the Gomutra ark has also immunomodulatory and antioxidant effect. Thus, this study supports the traditional use of Gomutra ark although further studies are needed to know the exact mechanism of action of Gomutra ark.

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