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Evaluation of antidiabetic, antioxidant effect and safety profile of gomutra ark in Wistar albino rats

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ABSTRACT:

The effect of Gomutra ark (GoA) on experimental alloxan-induced diabetes in rats was studied. For this purpose, Wistar albino rats of either sex weighing 200-250 g were used. The biochemical parameters like blood sugar, vitamin C, and malondialdehyde release were measured. The safety profile of GoA was evaluated using acute and chronic toxicity studies. GoA significantly lowers blood glucose in diabetic rats although the observed effect was found to be less than glibenclamide. It significantly lowers the level of malondialdehyde and vitamin C in diabetic rats. No toxicity was observed even when cow urine was given 32 times of the study dose in acute toxicity and no significant changes were seen when it was used chronically, which suggests that cow urine is having a very high therapeutic index. This study supports the traditional use of GoA in diabetes and is having a high therapeutic index and is safe for chronic use. However, further studies are needed to elucidate the mechanism of action of Gomutra ark.

KEY WORDS: Alloxan, antidiabetic, antioxidant, glibenclamide, Gomutra ark

INTRODUCTION

In Ayurveda, cow urine (Gomutra) occupies a unique place and has been recognized as water of life or "Amrita".^[1] In Sushruta Samhita, it has been described as the most effective substance of animal origin. In India, drinking of cow urine has been practiced for thousands of years.^[2]

In Panchagavya Ghrita, it is one of the important ingredients. Panchgavya, a term used to describe a formulation constituted with five major substances like urine, milk, ghee, curd, and dung obtained from cow. All the five products possess medicinal properties and are used singly or in combination with some other herbs against many diseases.^[3] This unique kind of the treatment called

as Cowpathy or Panchgavya pathy and it has been reported to be beneficial even for deadly diseases like diabetes, cancer, and AIDS.^[1,4]

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia, glycosuria, hyperlipidaemia, negative nitrogen balance, and sometimes ketonemia.^[5] The widespread pathological changes leads to complications like retinopathy, microangiopathy, and nephropathy.^[6] Most of the oral hypoglycemic agents, currently in use, produce serious side effects like hypoglycemic coma and hepatorenal disturbances. Also safety of most of the presently used drugs is not established during pregnancy.^[7] Hence, there is an urgent need for the search of safer and more effective hypoglycemic agents.

Cow urine has been granted US Patents (No. 6,896,907 and 6,410,059) for its medicinal properties, particularly as a bioenhancer and as an antibiotic, antifungal, and anticancer agent. With regard to the latter, it has been observed to increase the potency of "Taxol" (paclitaxel) against MCF-7, a human breast cancer cell line, in *in vitro* assays (US Patent No. 6,410,059).^[8]

There are so many claims regarding the use of cow urine. Out of these the most important claim is regarding its antidiabetic and antioxidant activity, but only few scientific literatures are available to support this claim.^[9]

Hence, the present study was designed to validate the

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antidiabetic as well as the antioxidant effect of Gomutra ark (GoA) scientifically and also to know if it has any acute and chronic adverse effects and cause any biochemical change in urine parameters.

MATERIALS AND METHODS

Chemicals and equipment

GoA was procured from Govigyan Anusandhan Santhan, Deolapur, Nagpur. Alloxan monohydrate was purchased from sigma chemicals, Mumbai. Glibenclamide of Cipla Company was procured from local medical store. The solvents and chemicals of analytical grade were used and obtained from the Swastik Chemicals Nagpur. The glucometer manufactured by Prestige Company was used.

Animals

Adult Wistar albino rats weighing 200-250 g of either sex were used for the study. The animals were maintained under standard 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 six 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 of the antidiabetic activity of Gomutra ark

The antidiabetic activity of the GoA was assessed using Alloxan monohydrate-induced diabetes in rats. For this purpose, following an overnight fast, animals were injected intraperitoneally with freshly prepared Alloxan monohydrate 2% solution dissolved in 0.9% sodium chloride in a dose of 150 mg/kg body weight.^[10] Fasting blood glucose (FBS) was recorded daily at 9.00 am for 1 week by glucometer; blood was collected from tail vein for this purpose. Animals developed stable hyperglycemia after 4-5 days.

Only those animals with FBS between 200 and 300 mg/dl were selected for the study. They were divided into three groups of six animals in each. The three groups were named as diabetic control, standard, and test group, respectively.

Group 1 (Diabetic control)

Diabetic animals of this group were kept for overnight fasting and their FBS was recorded at around 9 am. This value is considered as FBS value of zero day. Rats were given normal lab food *ad libitum*. The rats were kept for overnight fasting, prior to the day on which the fasting blood sugar

was to be recorded. FBS was recorded on the 1st, 3rd, 7th, 14th, 21st, and 28th day.

Group 2 (Standard control)

After recording the FBS values of the zero day of this group, these animals were orally fed with Glibenclamide 0.5 mg/kg body weight on the morning of everyday for 30 days. FBS values of the animals of this group were recorded on the 1st, 3rd, 7th, 14th, 21st, and 28th day.

Group 3 (Test group)

After recording the FBS values of the zero day of this group, the animals were given GoA by oral route, using feeding needle, in dose of 1 ml/kg body weight twice a day, for 30 days. FBS values were recorded on the 1st, 3rd, 7th, 14th, 21st, and 28th day.

Evaluation of antioxidant action of the Gomutra ark

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

Estimation of thiobarbituric acid reactive substances

The estimation of thiobarbituric acid reactive substances (TBARS) was done by employing the procedure given by Satoh in 1978, using thiobarbiturate acid reactive substances by TCA-TBA method.^[11] The animals were divided into three groups of six 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 diabetic control. Group 3 animals were given GoA 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/g 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.^[12] The animals were divided in to three groups of six 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 diabetic control. Group 3 animals were given GoA daily in dose of 1 ml/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 as mg/dl of Hb.

Toxicity studies of Gomutra Ark

Acute toxicity study

Male Wistar rats weighing 150-200 g were divided into five groups of six animals each. All groups were given GoA in increasing doses. Group 1 received the GoA at a dose of 2 ml/kg, Group 2 received GoA at a dose of 4 ml/kg, Group 3 received GoA at a dose 8 ml/kg, Group 4 received GoA at a dose of 16 ml/kg, and Group 5 received GoA at a dose of 32 ml/kg. The single dose of GoA was administered orally after overnight fasting. The animals were observed continuously for 2 h, and then occasionally for further 4 h and finally overnight.

Animals were observed for tremors, clonic convulsions, tonic extensions, catatonia, spasticity, opisthotonus, ataxia, sedation, ptosis, respiration. Further photoactometer was used to observe any change in motor activity, whether it is increased or decreased.

Chronic toxicity studies

Animals were divided into two groups of six animals. First group was control and received laboratory food *ad libitum*. The second group was given GoA 1 ml/kg twice daily. The various parameters which were observed were weight, food intake, any gross change in behavior, motor activity. The mean weight of the rats in different groups was recorded after 1 month, 2 months, and 3 months.

RESULTS

Effect of Gomutra ark on Alloxan-induced diabetes

In Group 1 (control group), there was sustained increase in the mean blood glucose level till 28th day after induction of the diabetes by Alloxan. The mean blood glucose level for the diabetic control group on day 0 was 268.33 mg/dl and was 263.5 mg/dl on 28th day.

The Group 2 (Glibenclamide) group, showed a sustained drop in the mean blood glucose levels when compared between 0 day to 28th day. On day 0, it was 257.67 mg/dl, which dropped significantly to 127 mg/dl on 28th day.

The group which received GoA (Group 3) also showed a sustained drop in the mean blood glucose level when compared between 0 day to 28th day. On day 0 it was 258.17 mg/dl, which dropped significantly to 188 mg/d on 28th day. The drop in the mean blood glucose levels obtained by the GoA was less than that of the standard drug Glibenclamide, but when compared to the control the drop in the blood glucose level was statistically significant on 7th, 14th, 21st, and 28th day.

The mean blood glucose fall percentage in the glibenclamide group when compared between 0 and 28th day was 332.42%, and for the group receiving GoA it was 18.68%. Though fall in blood glucose percentage for the group receiving GoA was less than Glibenclamide group this was statistically significant, as the P value was less than 0.05 [Tables 1 and 2].

Antioxidant effect of Gomutra ark

The mean MDA level was expressed in nmoles/g of hemoglobin. In Group 1 (Control), on the day 0, the level of MDA was 86.56 while on the 30th day, the level of MDA was found to be 84.41. In Group 2 (Diabetic control), the levels of the MDA were 126.41 on day 0 and 124.17 on day 30. The group 3 which received GoA, the mean MDA levels was found to be 127.11 on day 0 which was decreased significantly to 112.6 on 30th day [Table 3].

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 was 1.39. In the group 2, i.e. the diabetic control group, the levels of the Vit C were 0.52 on day 0 and 0.34 on day 30. The group which

Table 1: Mean values of blood glucose levels and standard deviations in different group of animals

	Day 0 mg/dl	Day 1 mg/dl	Day 3 mg/dl	Day 7 mg/dl	Day 14 mg/dl	Day 21 mg/dl	Day 28 mg/dl
Group 1	268.33 ± 26.27	266.33 ± 24.80	265.67 ± 24.90	262.83 ± 24.67	261.5 ± 23.20	261.83 ± 23.38	263.5 ± 24.65
Group 2	257.67 ± 20.69	227.33 ± 20.10	204.67 ± 31.96*	180.67 ± 31.18*	162.5 ± 27.59*	141.84 ± 15.93*	127.67 ± 13.80*
Group 3	258.17 ± 34.26	232.67 ± 16.43	231.33 ± 22.95	212.67 ± 6.15**	201.67 ± 19.84**	193.16 ± 21.38**	188 ± 21.05**

Results were expressed in Mean ± SD; unpaired student "t" test; *P < 0.05 when Glibenclamide compared with the diabetic group. **P < 0.05 when GoA compared with the diabetic group.

Table 2: Comparison of percentage reduction in blood glucose levels in case of glibenclamide group and group receiving GoA

	Day 1	Day 3	Day 7	Day 14	Day 21	Day 28	Mean
Group 2	11.77%	20.56%	29.88%	36.93%	44.95%	50.45%	32.42%
Group 3	9.87%	10.39%	17.62%	21.88%	25.18%	27.18%	18.68%

Table 3: MDA levels of different groups expressed in nanomoles/gram of Hb

	Day	Mean ± SD nmole/g of Hb
Group 1	0	86.56 ± 9.78
	30	84.41 ± 10.21
Group 2	0	126.41 ± 8.15
	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

Table 5: Signs in different groups when observed for 24 h

Signs	Group 1 (2 ml/kg)	Group 2 (4 ml/kg)	Group 3 (8 ml/kg)	Group 4 (16 ml/kg)	Group 5 (32 ml/kg)
Respiration	N	N	N	N	N
Gait	N	N	N	N	N
convulsions	No	No	No	No	No
Opisthotonus	Ab	Ab	Ab	Ab	Ab
Sedation	NS	NS	NS	NS	NS
Ataxia	NM	NM	NM	NM	NM
Ptosis	Ab	Ab	Ab	Ab	Ab

N: normal, Ab: absent, NS: normal sleep, NM: normal movement.

Table 7: The mean weight of the rats after 1 month, 2 months, and 3 months

	0 month	1 month	2 month	3 month
Group 1	174 ± 22.69	177 ± 25.27	168 ± 11.98	172 ± 18.41
Group 2	184.16 ± 25.38	180.66 ± 17.96	171 ± 15.16	175 ± 17.60

received GoA, the Vit C level was found to be 0.41 on day 0 which was decreased significantly to 0.27 on 30th day when compared to Group 2 [Table 4].

Acute toxicity study

When single dose in various doses 2, 4, 8, 16, and 32 ml/kg were given to various groups, the signs such as tremors, convulsions, catatonia, spasticity, opisthotonus, ataxia, ptosis were not observed even at the dose of 32 ml/kg, which is 32 times more than dose used in the study. Also, respiration was normal when GoA was used in high dose. The mean Photoactometer readings of the control group was found 152.83 ± 33.58, Group 1 was 146.67 ± 34.81, Group 2 was 166.00 ± 48.8, Group 3 was 159.16 ± 57.7, Group 4 was 161.67 ± 47.9, and Group 5 was 140.33 ± 52.3. The differences were found statistically insignificant [Tables 5 and 6].

Chronic toxicity

The mean weight of rats in Group 1 (Control) was found 174 ± 22.69 g at 0 month and 172 ± 18.41 g at the end of 3 months, whereas the mean weight of rats in Group 2 (GoA)

Table 4: 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 the diabetic group

Table 6: the mean of photoactometer readings observed for 10 min after 24 h of receiving GoA

	Mean ± SD
Normal	152.83 ± 33.58
Group 1	146.67 ± 34.81
Group 2	166.00 ± 48.8
Group 3	159.16 ± 57.7
Group 4	161.67 ± 47.9
Group 5	140.33 ± 52.3

Table 8: The photoactometer readings after 1 month, 2 months, and 3 months

	0 month	1 month	2 month	3 month
Group 1	154.34 ± 57.7	146.56 ± 23.3	138 ± 34.5	148 ± 44.7
Group 2	152.83 ± 33.58	161.33 ± 31.71	188.5 ± 63.16	193.66 ± 37.22*

*P < 0.05 when Group 2 compared with Group 1

was found 184.16 ± 25.38 g at 0 month and 175 ± 17.60 g at the end of 3 months. This difference was statistically insignificant [Table 7].

The photoactometer readings in Group 1 (Control) was 151.34 ± 57.7 at the end of 0 month and 148 ± 44.7 at the end of 3 months, whereas for the Group 2 (GoA) the photoactometer reading initially was 152.83 ± 33.58 and was significantly increased to 193.66 ± 37.22 [Table 8].

DISCUSSION

The study was carried out to evaluate the antihyperglycemic and antioxidant effect of GoA. The parameters studied were plasma blood glucose, plasma MDA, and vitamin C levels. In our study, the mean blood glucose level was found to be decreased for the diabetic group receiving GoA daily for 28 days. This shows that the observed anti-diabetic effect is of moderate magnitude and less in comparison to glibenclamide. However, what is important is that GoA produces significant lowering of the blood sugar level and validates the claim that it is effective in diabetes. There are

many mechanisms by which the increased blood sugar level is lowered. Among the probable mechanisms are increase in the glucose transport across cell membrane resulting in increased peripheral glucose utilization, increased glycogen synthesis from glucose, decreased glycogenolysis in liver, increased insulin release from beta cells of islet of Langerhans in pancreas, increase sensitivity of insulin receptors, decreased insulin resistance, and decreased glucose absorption from intestine.

GoA might be acting through one or more than one of the above mechanisms. What might be the mechanism in our study cannot be definitely said. Further studies are required for this purpose; cow urine, contains sulfur which might have some action like sulphonylureas, or it might be increasing sensitivity of insulin receptors, or causing more release of insulin.^[13]

Cow eats various herbs, the metabolite of which can be present in urine. Many useful elements have been found in cow urine. The metabolite found in urine might have antihyperglycemic action. Further in our study we found GoA has an antioxidant effect. Cow urine contains volatile fatty acids which act as antioxidant,^[9] The antioxidant potential might be contributing for the antihyperglycemic effect, by preventing formation of the free radicals which cause damage to the beta cells of pancreas.

Alloxan and the product of its reduction, dialuric acid are reported to generate free radicals. These radicals undergo dismutation to hydrogenperoxide. Thereafter, highly reactive hydrogen radicals are formed by the fenton reaction. The action of these reactive oxygen species (ROS) results in a simultaneous massive increase in cytosolic calcium concentration which may be responsible for the rapid destruction of beta cells.^[14]

Apart from these reactive oxygen species diabetes also increases lipid peroxidation by initiating the beta oxidation of fatty acids by the cell membrane and is mediated by the fatty acyl coenzyme, a oxidase enzyme resulting in membrane function impairment and altering membrane permeability. Increased lipid peroxidation in the diabetic condition results due to increased oxidative stress in the cells as a result of the depletion of the antioxidant scavenging enzymes.^[15]

Hence, from our results, it is suggested that GoA might have a significant protective effect against alloxan-induced type I DM. GoA contains volatile fatty acids like acetic acid 2 propenyl ester, acetic acid methyl ester, 2 2 3 trichloro propionic acid, Butanoic acid-3methyl, propyl ester, 1H

indol-3-acetate, acetic acid phenyl ester, quinoline, which act as an antioxidant. The antioxidant potential might be contributing for the antihyperglycemic effect, by preventing formation of the free radicals which cause damage to the beta cells of pancreas.

In acute toxicity study, no toxicity was observed even when cow urine was given 32 times of the study dose, which suggests that cow urine is having very high therapeutic index. Although no histopathological studies were undertaken, we can say that GoA is safe in animals.

In the chronic toxicity study, there was no significant change in the weight of rats after 3 months of GoA, which suggest that GoA does not cause weight gain.

Further, for the study done for chronic toxicity, the photoactometer readings at the end of 3 months significantly rose when we compare it at 0 month, which suggests that GoA has some possible CNS stimulant action and might be causing increase energy levels.

In some traditional literature, it has been mentioned that cow urine causes a sense of generalized well being, our finding of some increase in the spontaneous motor activity might indicate the same thing in animals, and this sense of wellbeing not causing any weight gain or signs of CNS stimulation is very important.

Hence, from this study, it was found that cow urine has antidiabetic potential which might be due to its antioxidant activity. It is having very high therapeutic index and is very safe.

SUMMARY AND CONCLUSION

From the present study it can be concluded that GoA has a significant anti-diabetic effect which is comparable to standard drug glibenclamide. This activity may be due to the presence of sulfur in the cow urine or due to its antioxidant activity. Thus, this study supports the traditional use of GoA although further studies are needed to know the exact mechanism of action of GoA.

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