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EVALUATION OF IMMUNOMODULATORY ACTIVITY OF COW URINE IN DIABETIC RATS

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ABSTRACT

OBJECTIVE: To evaluate the antidiabetic activity of Gomutra in streptozotocin induced diabetic rats. To evaluate the immunomodulatory activity of Gomutra in normal and diabetic rats. To analyse the active compound contributing to immunomodulatory activity in gomutra by FTIR analysis. METHODOLOGY: This study was carried out in the Institute of Pharmacology, Madras Medical College, Chennai during 2014 -2015.FTIR analysis was done at Pharmacovigilance laboratory in chennai. 36 rats were divided into six groups. GROUP I : Normal rats with no medication. GROUP II : Normal rats with Gomutra. GROUP III : Diabetic rats with standard drug. GROUP IV : Diabetic rats with Gomutra. GROUP V : Diabetic rats with Gomutra and standard drug. GROUP VI : Diabetic rats with no medication. Diabetes was induced with streptozotocin at a dose of 50 mg/kg. Antidiabetic activity was studied by measuring the blood glucose level with glucometer. Immunomodulatory activity was determined by Phagocytic Index, Neutrophil adhesion, Haemagglutination titre. Gomutra was analysed by FTIR ,focusing compound Aurum hydroxide and hormones. RESULTS: Gomutra showed a significant reduction in fasting blood glucose in diabetic rats ($p < 0.05$). There was a sigificant increase in mean phagocytic index, neutrophil adhesion($p< 0.01$).There was a lesser haemagglutination titre than groups not receiving gomutra($p < 0.01$).FTIR analysis showed Gomutra contains hormones and not aurum hydroxide.CONCLUSION:Gomutra showed anti diabetic activity in diabetic rats and immunomodulatory activity in normal & diabetic rats. The active compound contributing to immunomodulatory activity is hormones and not Aurum hydroxide.

1. INTRODUCTION

Impaired Immunity is one of the causes for complications of Diabetes mellitus due to deposition of immunological material in vessel walls. Immunological material can be detected by Circulating Immune complexes(CIC). Increased load of CIC in diabetes mellitus is contributed by complexes of insulin with IgG and due to recurrent infections. These will finally result in impaired phagocytic function.[1,2] Hyperglycemia can be treated with Insulin and Oral Hypoglycemic Drugs , but no therapy has been tried for impaired immunity in Diabetes Mellitus. In Sushrita Samhita, Gomutra (Cow urine) has been described as the most effective substance / secretion of animal origin with innumerable therapeutic values. Gomutra (Cow urine) had been evaluated for antidiabetic activity [3]and for immunomodulatory activity [4]. Most of the drugs used are not effective in modifying the affected immunological functions in Diabetes Mellitus. The purpose of this study is to evaluate the effect of gomutra as an immunomodulator in diabetes mellitus.

2. OBJECTIVE

1) To evaluate the antidiabetic activity of cow urine in streptozotocin induced diabetic rats.

2) To evaluate the immunomodulatory activity of cow urine in normal and diabetic rats.

3) To analyse the active compound contributing to immunomodulatory activity in gomutra by FTIR analysis.

2.2 METHODOLOGY

2.3 STUDY PROCEDURE

The study was conducted after obtaining approval from the Institutional Animal Ethics Committee and this protocol met the requirements of national guidelines of CPCSEA (IAEC NO: 2/243) , dated (03/09/2014). All the animals used in the study were procured from the animal house of Madras Medical College, Chennai-600003.

ALBINO RATS

Young Male Adult Wistar Albino rats weighing about 150 to 200grams were used which were obtained from the inbred colony maintained in the Animal House Department, Madras Medical College, Chennai-600003. The study was carried out in the Institute of Pharmacology, Madras Medical College, Chennai-600003 after obtaining Institutional Animal Ethical Committee Approval .The Quantitative analysis by FTIR was done in Pharmacovigilance Laboratory for animal feed and food safety, Madhavaram Milk Colony, Chennai-600051.

GROUPING OF RATS

36 rats were used, which were divided into six groups consisting of 6 animals in each group.

GROUP I : Normal rats with no medication.

GROUP II : Normal rats with cow urine.

GROUP III : Diabetic rats with standard drug (Glibenclamide).

GROUP IV : Diabetic rats with cow urine.

GROUP V : Diabetic rats with cow urine and standard drug.

GROUP VI : Diabetic rats with no medication.

GOMUTRA COLLECTION

The first early morning voided urine of Cows [*Bos indicus*] was collected from the local sheds belonging to Madras Veterinary College, Periamedu, Chennai.

DRUG DOSE

Cow urine was given at dose of 0.2ml orally BD for 28 days along with food *adlibitum*[5].

Standard Glibenclamide was given at a dose of 0.5mg/kg orally.

2.4 EVALUATION OF ANTIDIABETIC ACTIVITY

INDUCTION OF DIABETES

Preparation of streptozotocin solution: This was done by dissolving the weighed quantity of streptozotocin in 0.1M citrate buffer solution pH 4.5.

Preparation of 0.1M citrate buffer: A weighed quantity of Tri sodium citrate (14.9g) was dissolved in sufficient distilled water to produce 1000 ml and the pH was adjusted to 4.5 using concentrated Hydrochloric acid.

Diabetes was induced in albino rats by the intraperitoneal injection of streptozotocin at a dose of 50mg/kg body weight, into a volume of 1ml/kg. In order to prevent hypoglycemia during the first day after the STZ administration, the rats were given 5 % *w/v* glucose solution orally. Three days after the injection, the blood glucose levels were measured and the animals with blood glucose levels above 250 mg/dl were considered to be diabetic .The animals were stabilized for 5 days and then used for experiments. In all the experiments, rats were fasted for 16 hour prior to STZ injection.[6]

2.5 Blood sampling:

Blood samples were collected retro-orbitally from the inner canthus of the eye under light ether anesthesia using capillary tubes and blood glucose was determined using glucometer.

2.6 EVALUATION OF IMMUNO MODULATORY ACTIVITY

1.Determination of phagocytic index. 2.Neutrophil adhesion test. 3.Hemagglutination Titre.

DETERMINATION OF PHAGOCYTIC INDEX:[CARBON CLEARANCE TEST]

All groups were administered 0.2 ml/animal of carbon suspension I.V. through tail vein on 7th day. Blood samples were collected from retro orbital plexuses immediately at 0 minutes and 15minutes after injection of carbon suspension. After centrifugation, from each sample, 25µl of serum sample was lysed with 2ml of 0.1% acetic acid and absorbance was observed at 675nm. The phagocytic index was calculated by exponential equation.[5]

The results were expressed as phagocytic index:

K = (Ln OD t*15 min*) – (Ln ODt*0 min*) / (t15 min – t*0 min*)

Where, OD t 15 min and OD t *0 min* are the optical densities at 15 min and 0 min respectively.

NEUTROPHIL ADHESION TEST

On the 14th day of drug treatment, blood samples were collected by puncturing the retro-orbital plexus into EDTAvials and analysed for total leucocyte counts (TLC) and differential leucocyte counts (DLC) by fixing blood smears and staining with Field stain I & II-Leishman's stain. After initial counts, blood samples were incubated with 80 mg/ml of nylon fibres for 15 min at 37^0C. The incubated blood samples were again analysed for TLC and DLC. The product of TLC and % neutrophil adhesion gives neutrophil index (NI) of blood sample. Percentage neutrophil adhesion was calculated as shown below[5]

$$\text{Neutrophil adhesion (\%)} = \frac{NIu - NIt}{NIu} \times 100$$

HAEMAGGLUTINATION TITRE

PREPARATION OF SRBC

Under all aseptic precautions 10 ml of blood was withdrawn from the carotid vein of sheep in alsever's solution in Animal house Department ,by the veterinary surgeon. Five test tubes, containing 2 ml of sheep blood (taken from the blood kept in alsever's) were taken in test tube, which was centrifuged at 2000 rpm for 20 minutes. The RBCs settled at bottom was used as Sheep Red Blood Cells(SRBC).

On 21st day all the rats were immunized with Sheep RBC (SRBC) in the dose of 0.1ml of $1x10^8$, cells/ml/100 grams intraperitoneally. After 10 days of immunization, under mild ether

anesthesia , blood was withdrawn using small capillaries from the retro orbital plexuses. Around 2 ml of blood was withdrawn from every rat and kept in EDTA bulb. It was centrifuged at 2000rpm for 30 minutes. After centrifugation the plasma which contained antibodies against SRBC was used. The SRBC 25µl, was taken in each well of haemagglutination titre plate. (the plates containing 8 wells each for visualizing antigen antibody reactions). Then25µl of normal saline was poured in first well, 50µl of plasma from rats blood was poured in well 1, from which25µl transferred to well 2, from where 25µl transferred to well 3… and so on. Thus serial dilution was done. The plates were incubated at 37^0C for 1 hour. After 1 hour haemagglutination reaction is observed. Clumping, denotes positive reaction. The highest dilution showing positive was taken as the antibody titre.[5]

2.7 STATISTICAL ANALYSIS

The results were expressed in Mean ± S.D. Statistical significance between the groups were analysed using One way ANOVA followed by Student-Newman-Keuls test & One way ANOVA Turkey .

2.8 QUANTITATIVE ANALYSIS OF GOMUTRA

FTIR Analysis was performed in Thermoscientific model: Nicolet is10, OMNIC software-ATR mode.

3.RESULTS

3.1 ANTI DIABETIC ACTIVITY OF GOMUTRA

Table:1

GROUPS	Day 0	Day 7	Day 14	Day 21	Day 28
I	88.5± 3.25	85.3 ±3 3.7	83.16± 2.39	89.16± 4.91	82.16± 4.6
II	90.66 ±8.42	82.16± 8.67	86 ±4.56	87.5 ±7.51	93.66± 5.33
III	274.5 ±7.11	231.83±18.47	177± 18.9	143.16 ±16.45	92.5 ±4.6
IV	234.83 ±13.71	228 ±18.9	209.5± 34.5	172.5 ±27.15	127.55 ±54.65
V	254.33± 18.74	183.16± 12.3	145 ±18.9	117.16 ±15.26	85.5 ±7.15
VI	244.66± 54	294.66±15.63	308.3± 13.10	330.5± 18.97	226.16 ±45.66

The values were expressed in Mean ± S.D.Table 1: depicts the mean fasting blood glucose level of all study groups in units mg/dl.

Figure:1

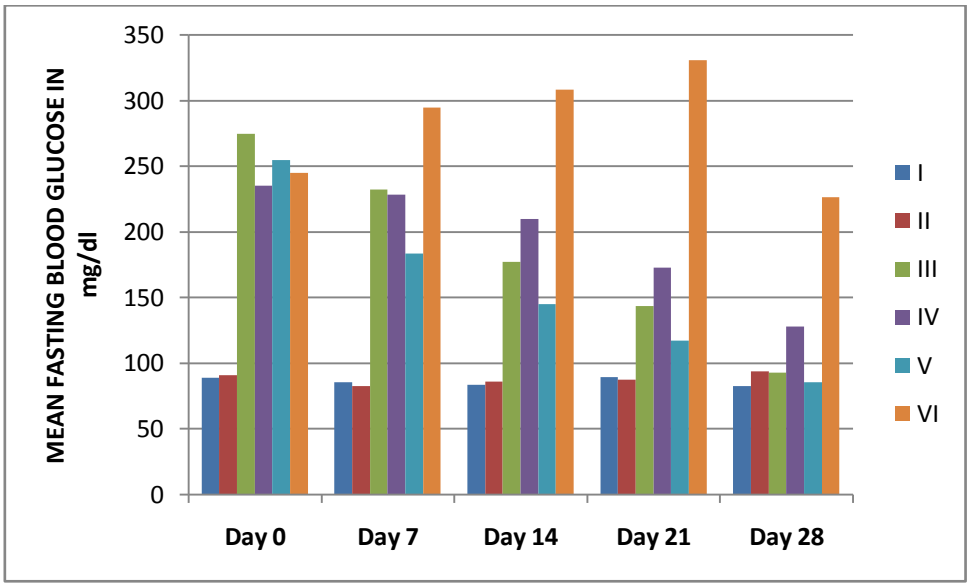


Figure 1: shows the graphical representation of mean fasting blood glucose level of all study groups.

3.2 IMMUNOMODULATORY ACTIVITY OF GOMUTRA IN DIABETIC RATS:

PHAGOCYTIC INDEX:

Table 2:

GROUP	N	Mean ± SD
I	6	1.57±0.62
II	6	6.49±1.24
III	6	3.0223±1.06
IV	6	2.0407±0.96
V	6	4.5801±0.64
VI	6	1.3311±1.01

The values were expressed in Mean ± S.D.

Table 2 : represents the mean Phagocytic index of all study groups.

Figure:2

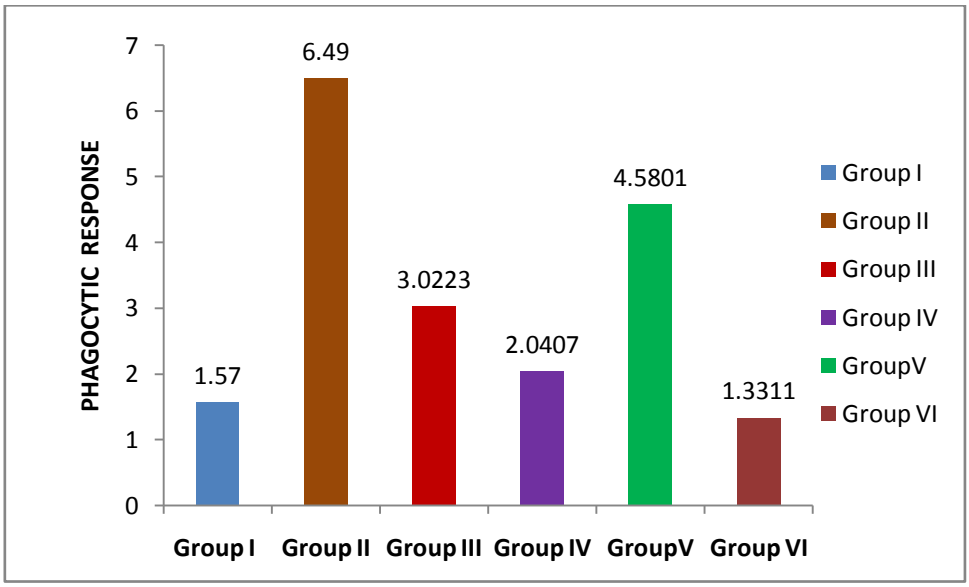


Figure 2: shows the graphical representation of mean phagocytic index of all study groups.

NEUTROPHIL ADHESION TEST:

Table:3

GROUP	N	Mean± SD in %
I	6	10.24±0.20
II	6	18.09±10.57
III	6	13.7560±7.21
IV	6	11.8133 ±7.12
V	6	16.1700±3.33
VI	6	8.9760±10.79

The values were expressed in Mean ± S.D.

Table 3: depicts the mean % Neutrophil adhesion of all study groups.

Figure:3

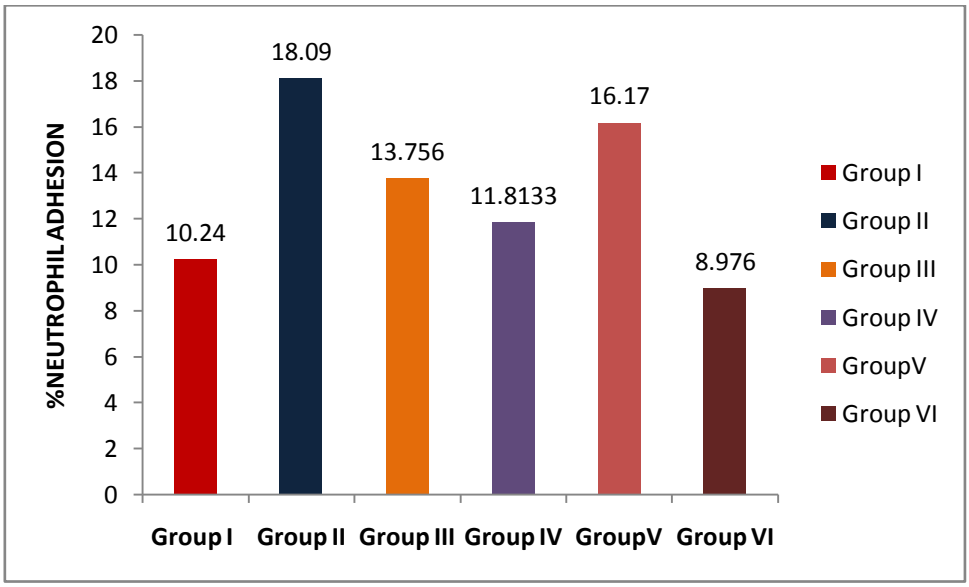


Figure 3: is the graphical representation of mean % Neutrophil adhesion of all study groups.

HAEMAGGLUTINATION TITRE:

Table:4

GROUP	VALUE
I	4± 0.30
II	1± 0.40
III	6± 0.81
IV	2± 0.63
V	3± 0.75
VI	8± 0.98

The values were expressed in Mean ± S.D.

The Table4 : represents the Haemagglutination ranking of all study groups.

Figure:4

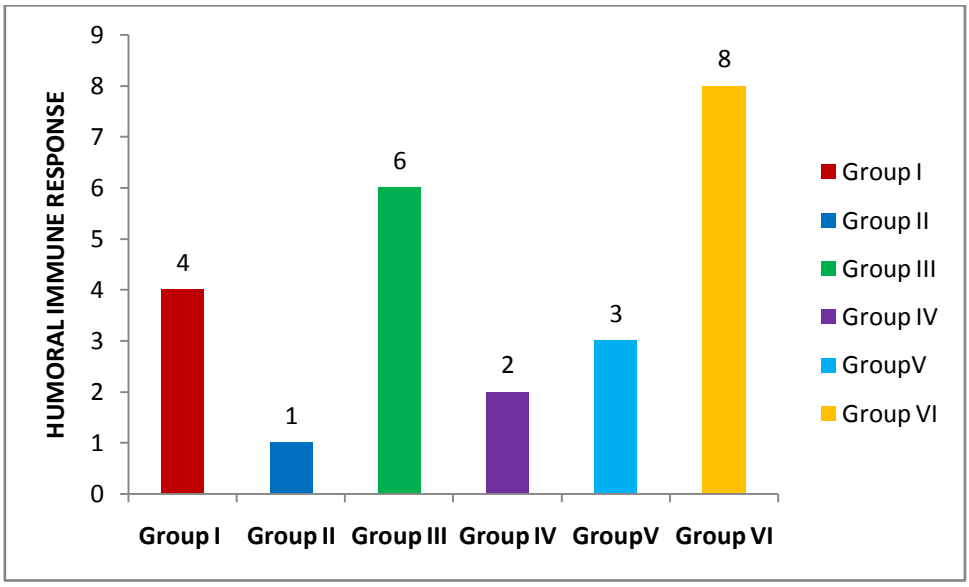
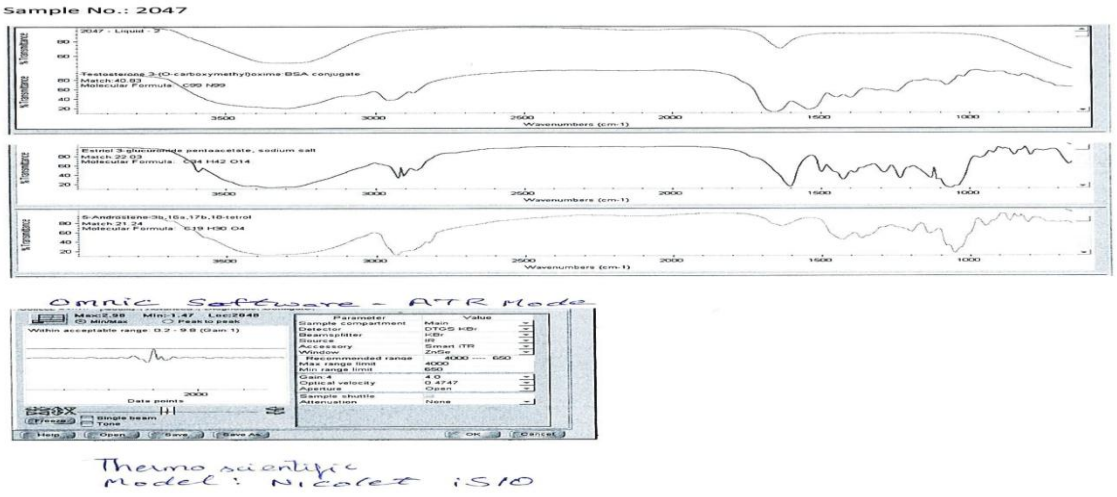


Figure:4 shows the haemagglutination titre of all study groups

4.FT-IR REPORT

- Absence of Aurum hydroxide
- Presence of Hormones:
- Testosterone
- Testosterone 17 b hemisuccinate:BSA conjugate
- Estriol 3-glucuronide pentaacetate, sodium salt
- 5-Androstane-3b,16a,17b,18-tetrol

Figure:5



5. DISCUSSION

Diabetic rats receiving gomutra had a statistically significant reduction in fasting blood glucose . Group V showed a more significant reduction in fasting blood glucose than Group VI, ($p < 0.05$). Gomutra showed significant immunostimulant effect in carbon clearance test by increasing phagocytic index in a dose dependent manner in both normal rats and diabetic rats. In normal rats, the groups receiving gomutra showed a sigificant increase in mean phagocytic index (6.49 ± 1.24) when compared to group not receiving gomutra (1.57±0.62) ,($p< 0.01$). In diabetic rats, the groups receiving gomutra showed a significant increase in mean phagocytic index (3.02 ± 1.06) , (2.04 ± 0.96) & (4.5801±0.64) when compared to diabetic rats with no medication,(1.3311±1.01),($p < 0.01$). Gomutra showed immunostimulant effect in Neutrophil adhesion test by increasing the percentage neutrophil adhesion. In normal rats, , the groups receiving gomutra showed an increase in mean neutrophil adhesion (18.09±10.57), when compared to group not receiving gomutra (10.24±0.20). In diabetic rats, groups receiving both gomutra and standard showed statistically, significant increase in neutrophil adhesion (16.17±3.33) when compared to group not receiving gomutra (8.97±10.7), ($p < 0.01$). Gomutra has significant immunomodulatory activity in Haemagglutination titre test by decreasing the haemagglutination titre value.The groups receiving cow urine , (1± 0.40), (2± 0.63),(3± 0.75) shows lesser haemagglutination titre than groups which did not receive cow urine therapy (6±0.81) , (8±0.98). From the FTIR analysis report , it showed there was no evidence of presence of AuOH in gomutra .It was evident that gomutra contains only hormones.

6. CONCLUSION

From this study it can be concluded that, Cow urine therapy (Gomutra) in rats shows anti diabetic activity. Cow urine therapy (Gomutra) has significant Immunomodulatory activity in normal & diabetic rats. It is effective in enhancing non-specific immunity as evidenced by the phagocytic activity and neutrophil adhesion test. Cow urine has the potential to inhibit specific immune response (humoral) as evidenced by the haemagglutination titre. FTIR analysis shows gomutra contains active components such as Testosterone, Testosterone 17b hemisuccinate: BSAconjugate, Estriol3 glucuronidepentaacetate, sodiumsalt, 5-Androstane-3b,16a,17b,18-tetrol which may be responsible for its Immunomodulatory activity.

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