

Antifertility effects of Cow urine on seminal quality of mice (Mus musculus)

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ABSTRACT

Objective: A study was undertaken to evaluate the effects of cow urine administration on seminal parameters of male mice. **Methods:** Total 36 mature male mice were selected and divided into two groups: group I (treated group) was fed 0.1 ml of cow urine and group II (control group) was fed same amount of distilled water with similar exposure days (10, 20 and 30 days). After 10 to 30 days of treatment 6 mice of each group were sacrificed. Semen samples were collected from cauda epididymis. Sperm count, sperm motility, sperm mortality, seminal pH and abnormality of spermatozoa were studied. **Results:** The results of this study showed that oral administration of cow urine at 0.1 ml/day significantly ($P < 0.001$) decline the sperm count, sperm motility and seminal pH however, sperm mortality and abnormality of spermatozoa increased significantly ($P < 0.001$) in treated group of mice semen than the control. **Conclusions:** The result suggested that, treatment of cow urine shows antifertility effects by alteration in seminal parameters in treated group of mice. **Key words:** Cow urine, Sperm count, Sperm motility, Abnormality, Antifertility

I. INTRODUCTION

Cow (*Bos indicus*) is a most valuable animal in all Veda and it is called the Mother of all (Jaraldet al, 2008). She is personified as Kamdhenu (desire fulfiller). Cow urine has a special significance in Hindu tradition. It is called Sanjivani and Amrita in Ayurveda. It is included in Panchgavya and is said to be curer of all diseases. Panchgavya is a combination of five Cow products viz. Cow urine (Gomutra), milk (Godugdha), ghee (Goghrit), curd (Godadhi) and dung (Gomaya) (Sahu et al, 2017). The ancient system of medicine (Ayurveda) has mentioned importance of Panchgavya in the treatment of various human diseases. Apart from medicinal uses it is also used in agricultural field as a natural pesticides, fertiliser (Harshadet al, 2017) and in production of Verme compost (Dharma et al, 2005).

Cow urine exhibits the property of Rasayanatwa responsible for modulating various bodily functions including immunity (Chauhan et al, 2001). As per scientific literatures gomutra is useful in number of diseases. It is capable of curing several non-curable disease. It possesses medicinal properties like antimicrobial (Achliya et al, 2004), anticancer (Jain et al, 2010), antibiotic & antifungal (Wate et al, 2011), antioxidant (Jaraldet al, 2008), anti-diabetic ((Jaraldet al, 2008)) and bio enhancer (Ganaie et al, 2010).

From the above studies, it is clear that cow urine has wide range of medicinal properties including antifertility effects in male. Hence attempt has been made to evaluate the efficacy of cow urine as a contraceptive agent in male during different exposure. The present study was undertaken to evaluate the antifertility effects of cow urine on seminal parameters like sperm count, sperm motility, sperm mortality, abnormality of spermatozoa and seminal pH among treated mice with respect to control.

II. MATERIALS AND METHODS

Experimental animal: 36 adult male mice of 12-14 week old and weighing about 25-30 gram were included in the study. These mice were procured from the University Department of Zoology, T.M. Bhagalpur University, Bhagalpur. All mice were maintained under normal husbandry and hygienic condition at $25 \pm 2^\circ$ temperature with proper ventilation.

Experimental design: All mice were divided into 2 groups 1st group (n=18) as treated group while 2nd (n=18) as control group. Treated mice were fed 0.1ml cow urine through oral route while control group were fed same amount of distilled water with similar exposure days (10, 20 and 30 days). 6 mice of both groups were sacrificed after the exposure of 10, 20 and 30 days. The cauda epididymis of both testis were operated out and tinged with 2 ml of normal saline. The suspension were crushed and filtered through a metallic filter to avoid tissue debris.

Seminal parameters: Filtrate was stained with eosin and counting of sperms were done with the help of haemocytometer after the method of Eliasson (1975). Motility of spermatozoa were observed after the methods of Tijee and Oentoeng (1968). Seminal pH was measured by the pH paper.

III. RESULTS

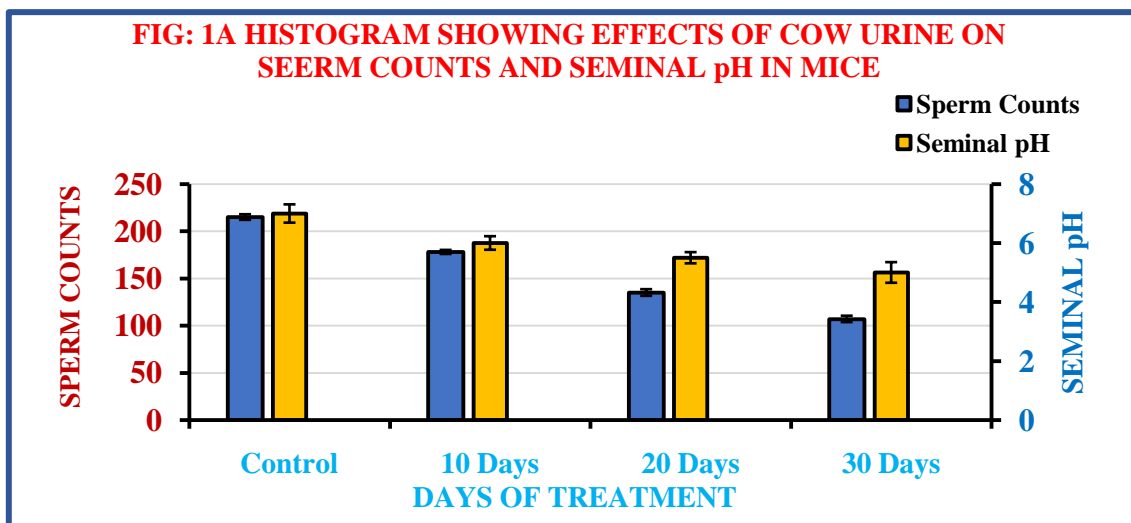
The data represented in **table-1 (fig; 1 & fig; 2)** shows the effect of cow urine administration on physical parameter of semen like sperm count,

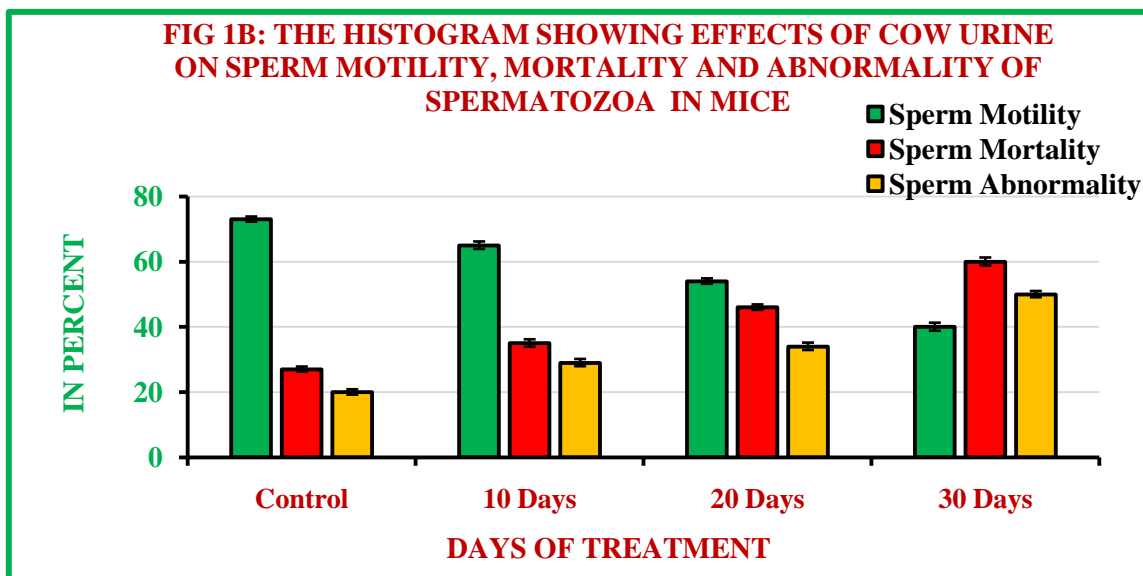
sperm motility, sperm mortality, seminal pH and abnormality of spermatozoa. The administration of cow urine caused significant decline ($P < 0.001$) in sperm counts after 10 to 30 days of treatment when compared with control group of mice. The sperm motility and seminal pH also declined significantly ($P < 0.001$) during 10 to 30 days treatment in treated group of mice than the control. However sperm mortality ($P < 0.001$) and abnormality of spermatozoa increased significantly ($P < 0.001$) in treated group than the control group of mice.

TABLE-1: Effect of Cow urine on seminal quality of mice

Groups	Sperm Count (x10 ⁴ ml)	Sperm Motility (In %)	Sperm Mortality (In %)	Sperm Abnormality (In %)	Seminal pH
Control (18)	215±2.96	73±0.79	27±0.79	20±0.82	7.0±0.81
10 Days Treatment (6)	178±2.14**	65±1.15*	35±1.15*	29±1.11*	6.0±0.73*
20 Days Treatment (6)	135±1.51**	54±0.83**	46±0.83**	34±1.13**	5.5±0.69**
30 Days Treatment (6)	107±1.37**	40±1.23**	60±1.23**	50±0.96**	5.0±0.52**

Data presented as Mean ± SEM; *, ** shows significance at 0.01 and 0.001 levels with value in control. Number within parenthesis denote number of samples.





IV. DISCUSSION

The results of present study (table no-1, fig; 1 & fig; 2) demonstrated that cow urine administration for 10 to 30 days caused significant ($P < 0.001$) decline in sperm counts in treated group of mice as compared to control group. Sperm count is essential factor for fertility in mice like other mammals as well as human subjects. If sperm count is reduced the fertility of mice is impaired, when treated with cow urine (Ganaieet al, 2011).

In a similar study Kumari et al (2017) and Kumar et al (2017) reported that administration of aqueous extracts of Carica papaya and Aeglemarmelos show significant ($P < 0.001$) decline in sperm counts in treated group of mice than the control respectively. Decline in sperm counts may be due to anti-spermatogenic and anti-androgenic activity of Caricapapaya and Aeglemarmelos. Similar findings were reported by Kambleet al (2017) in which, administration of Achyranthusaspera leaf extract showed arrest of spermatogenesis and inhibit the testicular function in treated mice. This suggests that Cow urine causes decrease in sperm counts by modulating testosterone level in treated group of mice which leads to decline in sperm counts.

In present study, it was observed that motility of spermatozoa declined significantly ($P < 0.001$) in the cow urine treated group of mice than the control. When duration of treatment increases from 10 to 30 days the percentage of sperm motility decreased significantly. Decrease in sperm motility in cow urine treated group of mice is directly responsible for loss of fertility among mice, as slow moving or immotile sperms are

unable to penetrate the cervical mucus to fertilize the ova (Chauhan and Agarwal, 2008). Similar findings were reported by Kumar et al (2017) in which, administration of aqueous leaf extract of Aeglemarmelos showed decrease in sperm motility that leads to infertility among treated mice. Such reduction in motility of spermatozoa may be due to decline in testosterone level, as sperm motility is androgen dependent (Seth et al., 1981). Decline in sperm motility decreases the chances of fusion of male and female gamete in the fallopian tube (Lohiya & Goyal, 1992).

In this study, it is observed that the administration of cow urine for 10 to 30 days causes significant increase ($p < 0.001$) in mortality of spermatozoa in semen of treated group of mice than the control. The increased percentage of mortality of spermatozoa in seminal fluid of treated group of mice may be due to androgen deficiency (Ahmed et al., 2002b). The increase in mortality may be due to lower level of seminal pH, as low pH sperms are more fragile (Pragya et al 2012; Hembromet al, 2011; Das et al, 2018). Lower level of seminal pH create unfavourable environment for sperms that leads to higher sperm mortality among treated mice. If there is higher mortality of sperm, in spite of normal sperm counts, the fertility rate is reduced in mice. At low pH of epididymal fluid bovine spermatozoa show higher mortality (Acott and Carr, 1984).

In this study, cow urine treated mice showed significant ($P < 0.001$) increase in the percentage of abnormal sperms in the semen of treated mice as compared to control group. These abnormal sperms are incapable in capacitation and

fertilization. Hence, where there is higher rate of abnormality in sperms there are lesser chance of fertility. Increase in abnormality of spermatozoa may be due to decrease in surface area of sertoli cells and leydig cells (Chauhan et al, 2009). Increase in abnormality of spermatozoa may be due to decrease in acrosomal integrity (Sunder et al, 2012). The development of spermatozoa is mediated by the testosterone. It helps in maturation of spermatozoa. Any alteration in the level of testosterone in seminal plasma may leads to underdevelopment of spermatozoa (Chauhan et al, 2009). According to Rahman et al (2013) normal sperm morphology is very necessary for the successful fertilization. Abnormal sperm quality and morphology may affect the fertility and that leads to infertility in males. Kumari et al (2017) reported that administration of papaya seed extract affect the morphology of spermatozoa in treated mice. Kumar et al (2017) reported that the aqueous leaf extract of Aegle marmelos caused highly significant increase in abnormality in spermatozoa of mice semen. Due to higher abnormality among spermatozoa caused reduction in fertility rate.

In this study, it was observed that treatment of cow urine causes highly significant decline in seminal pH among treated group of mice than control. After 30 days of cow urine treatment, pH of seminal plasma attained minimum level. If the pH of seminal plasma decreases, it become acidic and in acidic pH sperms became highly fragile that resulted into higher rate of mortality of spermatozoa (Turner and Reich 1985). The reduction in seminal pH may be due to blockage in seminal vesicle. Seminal vesicle is an accessory sex gland which secrete alkaline which makes the maximum activity of sperms (Irvine et al, 1994). Zhou et al (2015) reported that pH of seminal plasma may play significant role in maintaining viability and quality of sperms for fertilization. Thus, any alteration in seminal pH caused abnormal sperm function which leads to infertility among treated mice.

V. CONCLUSION

The result obtained from present study shows that cow urine causes decreased sperm count, sperm motility and seminal pH while sperm mortality and abnormality of spermatozoa increased significantly ($P < 0.001$). The normal range of sperm count, sperm motility, seminal pH and abnormality of spermatozoa are essential factor for fertility. Any disturbance of such normal range of seminal quality may affect the fertility of mice.

Thus such type of changes in seminal quality cow urine treated group of mice show antifertility effects among them.

CONFLICT OF INTEREST STATEMENT

The author declares that there is no conflict of interest.

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REFERENCES

- [1]. Jarald E., Edwin S., Tiwari V., Garg R., Toppo E. (2008); Antioxidant and antimicrobial activities of Cow urine. *Global J Pharmacol.* 2(2): 20-22.
- [2]. Sahu R., Lalchand, Gupta R., Rout O.P. (2017); Benefits of Cow urine-a review. *Int. J Recent Adv. Multidis. Res.* 4(9): 2833-2835.
- [3]. Harshad G., Amit N., Nilesh M., Sunanda B., Amrut S. (2017); Gomutra (Cow urine): A multidimensional drug review article. *Int. J. Res. Ayurveda Pharm.* 8(5): 1-6.
- [4]. Dharma K., Rathor R., Chauhan R.S. and Tomar S. (2005a). Panchgavya (cowpathy): An Overview. *Int. J Cow Science.* 1(1): 1-15.
- [5]. Chauhan R.S., Singh B.P., Singhal L.K. (2001); Immunomodulation with Kamdhenu ark in mice. *J Immunol. Immunopathol.* 71: 89-92.
- [6]. Achliya G.S., Megre V.S., Wadodkar S.G., Dorle A.K. (2004); Antimicrobial activity of different fraction of cow urine. *Indian J Nat. Prod.* 20: 14-16.
- [7]. Jain N.K., Gupta V.B., Garg R., Silawat N. (2010); Efficacy of cow urine therapy on various cancer patients in Mandsaur District, India- A survey. *Int. J Green Pharm.* 4: 29-35.
- [8]. Wate S.P., Dhanjode D.P., Duragkar N.J., Tajne M.R. (2011); Antioxidant potential of cow urine and its fractions: A comparative study. *Inter J Uni. Pharm Life Sci.* 1(1): 146-154.
- [9]. Jerald E.E., Edwin, Tiwari V., Garg R., Toppo E. (2008); Antidiabetic activity of cow urine and a herbal preparation

- prepared using cow urine. *J Pharma Biol.* 46(10-11): 789-792.
- [10]. Ganaie J.A., Shrivastava V.K. (2010); Effects of gonadotropin releasing hormone conjugate immunization and bio enhancing role of Kamdhenu ark on estrous cycle, serum estradiol and progesterone level in female *Mus musculus*. *Iran. J Reprod. Med.* 8: 70-75.
- [11]. Eliasson, R. (1975): Analysis of semen. In progress in infertility. Eds. S.J. Behrman and R.W. Kistner. Little Brown and company Boston, Vol. (II) in edition chapter -33: PP. 693.
- [12]. Tijee D.Y. and Oentoeng S., (1968), The viscosity of human semen and the percentage of motile spermatozoa. *Fertility, sterility* 19: PP. 562-565.
- [13]. Ganaie J.A., Gautam V., Shrivastava V.K. (2011); Effects of Kamdhenu Ark and Active Immunization by Gonadotropin Releasing Hormone Conjugate (GnRH-BSA) on Gonadosomatic Indices (GSI) and Sperm Parameters in Male *Mus musculus*. *J Reprod. Infertil.* 12(1): 3-7.
- [14]. Kumari S., Kumar R. and Singh V.N. (2017); Antifertility effects of aqueous extract of *Carica papaya* (Linn.) seed on seminal profile of mice (*Mus musculus*). *Int. J Sci. Res.* 6(8): 1963-1965.
- [15]. Kumar R., Kumari S. and Singh V.N. (2017); Antifertility efficacy of aqueous leaf extract of *Aeglemarmelos* (Linn.) on seminal quality of Swiss albino mice. *Int. J Sci. Res.* 6(8): 1392-1394.
- [16]. Kamble A.K., Reddy C., Patil S. (2017); Testicular activity of mice treated with MeOH extract of *Achyranthes asperaleaves*. *J Adv. Med. Sci. and Appl. Tech.* 3(2): 93-100.
- [17]. Chauhan A. and Agrawal M. (2008); Reversible changes in the antifertility induced by *Aeglemarmelos* in male albino rats. *Syst. Biol. In Reprod. Med.* 54(6): 240-246.
- [18]. Seth S.D., Johari N. and Sundaram K.R. (1981); Anti-spermatogenic effect of *Ocimum sanctum*. *Indian J. Exp. Biol.* 19: 975-976.
- [19]. Lohiya N.K. & Goyal R.B. (1992); Antifertility investigation on the crude chloroform extract of *Caricapapaya* Linn. Seeds in male albino rats. *Ind. J Exp. Bio.* 30: 1051-1055.
- [20]. Ahmed M.M., Khan M.Y., Khan A.A. (2002b); Effects of *Ocimum sanctum* (Tulsi) on the reproductive system: An updated review. *Biomed. Research.* 13: 63-67.
- [21]. Pragma S., Hembrom A.R. and Singh V.N. (2012); Antifertility effects of aqueous leaf extract of *Ocimum sanctum* linn. (Tulsi) on seminal profile of mice. *The Bioscan* 7(2): 275-276.
- [22]. Hembrom A.R., Pragma S., Kumar J. and Singh V.N. (2011). Effects of aqueous leaf extract of *Bougainvillea spectabilis* on seminal quality of mice. *J Adv.* 32(2): PP. 119-122.
- [23]. Das P.K., Kumar J., Shubhangi S., Verma A. and Singh V.N. (2018); Antifertility effects of aqueous suspension of *Allium sativum* on seminal profile of Swiss albino male mice. *Int. J. Sci. Res. Vol.* 7(5), 1807-1809.
- [24]. Acott T.S., Carr D.W., (1984): Inhibition of bovine spermatozoa by caudal epididymal fluid: II. Interaction of pH and a quiescence factor. *Biol. Reprod.* 30(4): 926-935.
- [25]. Chauhan A. and Agrawal M. (2009); Assessment of the contraceptive efficacy of the aqueous extract of *Aeglemarmelos* Corr. leaves in male albino rats. *Hum. Fertil. (Comb.)* 12(2): 107-118.
- [26]. Sunder S., kumar A., Gullaiya S., Dubey V. (2012); Antifertility activity of methanolic bark extract of *Aeglemarmelos*(L.) in male Wistar rats. *Daru J. Faculty of Pharm.* 20(1): 94.
- [27]. Rahman M.D., Lee J., Kwon W., Pang M. (2013); Sperm proteomics: Road to male fertility and contraception. *Int. J. Endocrinol.* 1-12.
- [28]. Turner T.T. and Reich G.W. (1985); Caudaepididymal motility: A comparison among five species. *Biol. Reprod.* 32: 120-128.
- [29]. Irvine D.S., Aitken R.J. (1994); Seminal fluid analysis and sperm function testing. *Endocrinology & Metabolism Clinics of North America.* 23(4):725-748.
- [30]. Zhou J., Chen L., Li J., Li H., Hong Z., Xie M., Chen S., Yao B. (2015); The semen pH affects sperm motility and capacitation. *PLOS. One.* 10(7): 1-15.