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Antifertility effect of Sulfasalazine in male albino rats

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ABSTRACT

To evaluate the antifertility activity of the Sulfasalazine by oral administration in male albino rats. Doses of 450 mg/kg/b.wt and 500 mg/kg body weight of Sulfasalazine was administered orally for 60 days to adult male rats. On day 61, the rats were sacrificed and the testis and accessory reproductive organs were removed and weighed. The organs were processed for biochemical estimation and histological work. Treatment with Sulfasalazine resulted in decrease in the weights of testis and accessory reproductive organs. The diameters of testis, seminiferous tubules and Leydig cell nucleus were decreased. The spermatogenic elements, like spermatogonia, spermatocytes and spermatids in the testis were reduced significantly as well as the sperm count in cauda epididymis. There was a significant reduction in the protein, glycogen, DNA and RNA contents and the activity of acid phosphatase in the testis of drug treated rats compared with the control. The cholesterol content and the alkaline phophatase activity were increased significantly in treated rats. The level of testosterone and Follicle stimulating hormone (FSH) were significantly decreased at both treated rats when compared to control. Sulfasalazine arrests spermatogenesis in male albino rats.

KEYWORDS

Sulfasalazine;
Testis;
Accessory organs;
Reproduction;
Spermatogenesis.

INTRODUCTION

Sulfasalazine is a sulfonamide drug, which belongs to the class of drugs called the sulfa drugs. It inhibits bacterial growth by interfering with metabolic process that requires paminobenzoic acid (PABA). Therefore it has been used for many years for the treatment of ulcerative colitis[22]. Ever since a positive correlation was established between sulfasalazine and antifertility in male, a lot of studies have been undertaken to comprehend the exact mechanism by which it does so[10, 14, 18, 20]. However, no precise mechanism has been suggested till date and therefore the drug continues to be enigmatic. Sulfasalazine given orally is metabolized into 5-aminosalicylate and sulfapyridine[16]. It is the sulfapyridine moiety and not 5-aminosalicylate which is responsible for suppressed fertility[6, 14]. In the present study, we have tried to study the long term (60 days) effect of

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sulfasalazine at higher doses level (450 and 500 mg/kg.b.wt.) especially on biochemical estimation and testes histology so as to obtain a histological insight into exact site of sulfasalazine action.

MATERIALS AND METHODS

Test Chemical

Sulfasalazine was procured from the market. (Brand name Sazo marketed by Wallace, India.)

Animals

Colony bred adult healthy male albino rats of Wister strain, weighing 180-200 g were used in the study. The rats were housed in polypropylene cages under standard husbandry conditions (12 hrs light/dark cycle: 25 ± 2°C). Rats were provided water and pellet diet *ad libitum*. The Institutional Ethical Committee for animal care approved the study.

Experimental Design

Male rats of proven fertility were divided into three groups of 6 rats each.

Group I: Rats served as control and received the vehicle (0.5 ml Olive oil /day/rat) for 60days.

Group II: Rats were administered orally Sulfasalazine (450mg/kg b.wt. /day) suspended in Olive oil for 60 days.

Group III: Rats were administered orally Sulfasalazine (500mg/kg b.wt. /day) suspended in Olive oil for 60 days.

Observation

On day 61, the rats were sacrificed by cervical dislocation. The testes, epididymis, seminal vesicle, prostate and Cowper's glands were dissected, freed from surrounding tissue and were weighed quickly to the nearest weight on an electronic balance. Testis from one side of the animal was fixed in Bouin's fluid, embedded in paraffin wax, sectioned at 5 mm and stained in haematoxylin and eosin for histological study. The other testis was used for biochemical estimation, including protein[11], cholesterol[17], glycogen[3] acid and alkaline phosphatase[1] and DNA and RNA[7]. The micrometric measurements like diameters of testis, seminiferous tubules and Leydig cell nucleus were calculated by the method of Deb *et al.*[4]. Spermatogenic elements count was made on 20 randomly chosen round cross sections. The sperm count from cauda epididymis was done by using haemocytometer[21].

Testosterone and FSH

Serum was collected by allowing trunk blood to clot overnight at 4°C, centrifuging at 54 × g at 0°C for 15 min. Serum was collected and stored at -20°C. For each rat, testosterone and FSH levels in serum were measured using fully automated chemiluminescence analyser at Vivek laboratories, Nagarkovil, Tamil nadu, India.

Data processing

Data were expressed in Mean ±SE, if applicable. The significance of difference was analyzed using Student's *t*-test and $P<0.05$; $P<0.05$ was set as significant.

RESULTS

Organ weights

The weights of testes, epididymis, seminal vesicle, prostate and Cowper's gland were significantly ($P<0.01$) reduced in the 500 mg/kg/b.wt treated rats compared with the controls. Seminal vesicle and prostate weights were also reduced significantly ($P<0.05$) in the 450 mg/kg/b.wt treated group when compared to control rats (TABLE 1).

Biochemistry

The protein and DNA levels in both treated groups decreased in significantly ($P<0.01$). There was a sig-

TABLE 1 : Effect of Sulfasalazine on weights (mg/100 g body weight) of reproductive organs.

Group (n=6)	Testes	Epididymis	Seminal vesicles	Prostate	Cowper's glands
Control	1238± 56.54	540± 10.61	680± 39.62	310± 11.6	325± 18.18
Sulfasalazine (450mg/kg/b.wt)	1067± 38.67	468 ± 20.98	587± 54.67**	277± 7.8**	296± 8.91
Sulfasalazine (500mg/kg/b.wt)	1062± 27.0*	448± 30.2*	555± 33.24*	261.6± 5.2*	316± 19.64*

****$P<0.05$, *$P<0.01$, compared with controls.**

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TABLE 2 : Effect of Sulfasalazine on testicular biochemistry.

Group (*n*=6)	Protein (mg/gm)	Cholesterol (mg/gm)	Glycogen (mg/100 mg)	Alkaline phosphatase (µg/lP/mg/hr)	Acid Phosphatase (µg/lP/mg/hr)	DNA (µg/100mg)	RNA (µg/100mg)
Control	243.56±5.90	4.85±0.03	2.0±0.13	6.78±1.17	2.08±0.19	221.0±4.33	386.8±1.83
Sulfasalazine (450 mg/kg/b.wt)	237.51±2.54	4.46±0.09**	1.10±0.03	5.45±0.38**	2.32±0.07	216.4±2.57	383.4±1.62*
Sulfasalazine (400 mg/kg/b.wt)	232.6±6.16*	4.38±0.09*	1.2±0.17**	5.40±0.18*	2.30±0.07*	217.0±1.63*	385±1.41*

****P<0.05, *P<0.01, compared with controls.**

TABLE 3 : Effect of Sulfasalazine on reproductive parameters

Group (*n*=6)	Diameter (µm)			Spermtogenic elements			Cauda epididymal Sperm count
	Testis	Seminiferous tubules	Lyedig cell nucleus	Spermatogonia	spermatocytes	Spermatids	
Control	2773.87±60.43	285±13.19	9.89±0.07	107.0±3.53	124.3±3.32	71.75¡À8.26	13.55±0.81
Sulfasalazine (450mg/ kg)	2787.25±28.28	248.96±11.35**	8.21±0.49**	89.0±1.41*	122.6±4.51**	27.25±5.18*	8.98±0.45**
Sulfasalazine (500mg/ kg)	2759.22±19.50	217.97±9.05*	7.32±0.28*	81.3±1.44*	109.0±3.43*	17.75±0.89*	5.31±0.37*

****P<0.05, *P<0.01, compared with controls.**

nificant (P<0.01) reduction in the RNA at both doses, but more at the higher dose. The glycogen content was decreased in both treated groups, but it was significant (P<0.05) only in the 500 mg/kg/b.wt treated group when compared to control. The acid phosphatase level was decreased in both treated groups, but was significant (P<0.01) only in the 500 mg/kg/b.wt treated rats. There was a significantly increased (P<0.05; P<0.01) in the level of alkaline phosphatase and cholesterol in both treated groups when compared to control (TABLE 2).

Histology

There was no significant change in the diameter of testis, but a significant (P<0.05; P<0.01) reduction in the diameter of the seminiferous tubules and Leydig cell nucleus was observed in both treated groups when compared to control rats. The number of spermatogonia, spermatocytes and spermatids decreased significantly (P<0.05; P<0.01) in both treated groups when compared to normal healthy rats (TABLE 3, Figure 1 & 2).

Sperm count

The cauda epididymal sperm count decreased sig-

TABLE 4 : Effect of Sulfasalazine on serum testosterone and FSH

	Testosterone nmol/l	FSH u/L
Control	14.4±2.53	21.87±0.47
Sulfasalazine (450mg/kg/b.wt)	10.01±3.31**	15.01±0.31*
Sulfasalazine (500mg/kg/b.wt)	9.33±2.04*	15.17± 0.45*

****P<0.05, *P<0.01, compared with controls.**

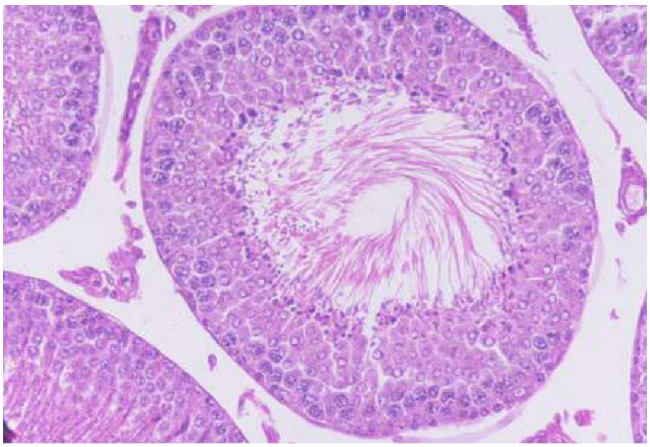

Figure 1 : Transverse section of control rat testis showing normal seminiferous tubules with all types of spermatogenic elements and spermatozoa (X400). Note the healthy Leydig cells (Lc). Spermatocytes (SPC); Spermatogonia (SPG); Spermatozoa (SZ); Spermatids (SPT).

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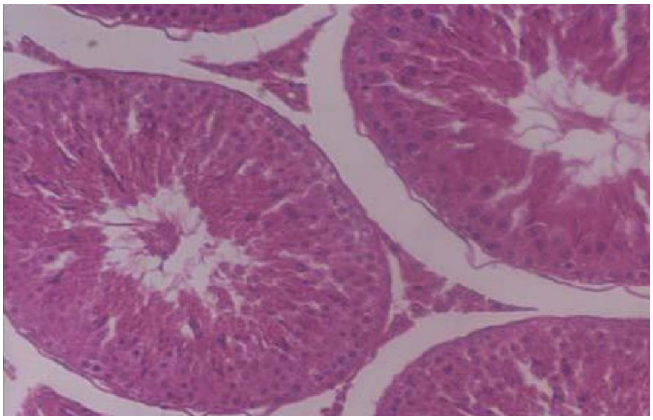

Figure 2. Transverse section of Sulfasalazine treated rat testis showing shrinkage of seminiferous tubules and decreased Leydig cells. Note significant decreases in the spermatogonia (SPG), spermatocytes (SPC) and spermatids and absence of spermatozoa. In spermatocytes nuclear pyknosis (PS) is seen (500mg/kg/b.wt).

nificantly ($P<0.05$; $P<0.01$) in both treated groups when compared to control rats (TABLE 3).

Serum testosterone and FSH

The level of testosterone and FSH were significantly ($P<0.05$; $P<0.01$) decreased at both treated rats when compared to control (TABLE 4).

DISCUSSION

Oral administration of a Sulfasalazine manifested two principal impacts on the male reproductive system of albino rats, antisper-matogenic and antiandrogenic effects. The antispermatogenic effect is reflected in the cessation of spermatogenesis and disintegration of sperm. It is evident that FSH stimulates the development of spermatogonia to spermatocytes and also maintains the spermatogenic process[19] and both FSH and LH/ICSH are necessary for meiosis and development of spermatids[9]. The androgens are necessary to induce meiosis, formation and development of spermatids in response to FSH[8]. The observed reduction in the number of spermatogonia, spermatocytes and spermatids may indicate lowered availability of FSH and LH/ICSH, which are essential for initiation and maintenance of spermatogenesis. It is known that sperm production cannot proceed optimally to completion without a continuous androgen supply[12]. However, the incidence of low sperm count implies Sulfasalazine induced infertility might be the consequence of an array of factors in biochemical events in tissues. The increased cholesterol content of testis after the administration of Sulfasalazine indicated reduced conversion of cholesterol to androgen which is dependent on the availability of LH/ICSH[15]. The glycogen content in the cell indicates energy storage. Sertoli cells and spermatogonia often contain glycogen and secrete substrates from the blood and provide source of reserve carbohydrates for seminiferous tubular cells, and the glycogen level is found to be directly proportional to the steroid hormones[15]. The decreased glycogen content of the testis after the administration of Sulfasalazine may reduce the energy source for spermatogenic activity, which might have resulted in spermatogenic arrest. Acid and alkaline phosphatase is widely distributed in the testis and is important in the physiology of sperm[2]. In the present study the changes in phosphatase activity may be indicative of spermatogenic suppression and/or suppression of exchange of materials between germinal and Sertoli cells[13], it is also indicative of extensive lytic activity. The decrease in the DNA and RNA contents of the testis in the treated rats indicates a poor growth rate.

Antiandrogenic action of this drug is reflected in the regression and disintegration of Leydig cells and the degenerative changes in the epididymis, seminal vesicle, prostate and Cowper's gland. As the administration of drug has caused reduction in the spermatogenesis and steroidogenesis, it may alter the sexual behaviour and cause infertility.

We can therefore conclude that testis is the main site of antifertility action of sulfasalazine, where it seems to disrupt the functions of testis, besides acting directly on the sperms leading to abnormal spermatozoa and depressed sperm counts resulting in impaired fertility.

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REFERENCES

[1] O.A.Bessey, O.H.Lowery, M.J.Brock; J.Bio.Chem., **164**, 321-9 **(1946)**.

[2] S.Breton, P.T.S.Smith, B.Lui, D.Brown; ATPase, Nat.Med, **2**, 470-2 **(1996)**.

[3] N.V.Carrol, R.W.Longley, J.H.Roe; J.Biol.Chem., **220**, 583-93 **(1956)**.

[4] C.Deb, M.C.Boral, C.Sarkar; Anat.Rec., **148**, 449-501 **(1964)**.

[5] T.Fukushima, Y.Hamada, M.Komiyama, Y.Matsuno, C.Morii, I.Horii; Reproductive Toxicology, **23(2)**, 153-157 **(2007)**.

[6] T.Fukushima,, M.Kato, T.Adachi, Y.Hamada, M.Horimoto, M.Komiyama, C.Mori, I.Horii; J.Toxicol. Sciences, **85(1)**, 675-682 **(2005)**.

[7] D.Glick; Methods of Biochemical Analysis, **4**, 10 **(1958)**.

[8] P.F.Hall; Testicular steroid synthesis: Organization and regu-lation. In: Knobil E, Neill JD, editors. Physiology of repro-duction, New York: Raven Press, pp. 1335-62 **(1995)**.

[9] T.Haneji, M.Maekawa, Y.Nishimune; Endocrinology, **114**, 801-5 **(1984)**.

[10] A.J.Levi, A.M.Fisher, L.Hughes, W.F.Hendry; Lancet., **2**, 276-278 **(1979)**.

[11] O.H.Lowry, N.J.Rosenbrough, A.L.Farr, R.J.Randall; J.Biol.Chem., **193**, 265-75 **(1951)**.

[12] H.Mohri, D.A.E.Sutur, P.D.E.Brown-Woedman, I.G.White, P.D.Ridely; Nature, pp., 255-75 **(1978)**.

[13] Neena Nair, M.S.Edwards, R.S.Bedwall, R.S.Mathur; Ind.J.Exp.Biol, **25**, 651 **(1987)**.

[14] C.O'Morain, P.Smethurst, C.J.Dore, A.J.Levi; Gut., 25, 1078-1084 **(1984)**.

[15] S.Pathak, A.O.Prakash; Phytother.Res., **3**, 61-66 **(1989)**.

[16] M.A.Peppercorn, P.Goldman; Gastroenterol, **64**, 240-245 **(1973)**.

[17] J.P.Peters, D.D.Vanslyke; Quantitative Clinical Chemistry, Vol. I. Williams and Wilkins, Baltimore **(1946)**.

[18] C.Pholpramool, A.Srikhao; Contraception, **28**, 273-279 **(1983)**.

[19] L.D.Russell, L.E.Alger, L.G.Nequin; Endocrinaol, **120**, 1615-32 **(1987)**.

[20] S.Toovey, E.Hudson, W.F.Hendry, A.J.Levi; Gut., **22**, 445-451 **(1981)**.

[21] World health organization (WHO); Laboratory manual for the examination of human semen and semen-cervical mucus interaction, Cambridge: Cambridge University Press; **(1992)**.

[22] L.Zetzel; New Eng.J.Med, **251**, 610-615 **(1954)**.