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Effect of gold on stimulation of reproductive function in immature female albino rats

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Significant increase in ovarian and uterine weight and stimulation of ovarian Δ^5-3β- hydroxysteroid dehydrogenase (Δ^5-3β-HSD) activity and elevation of serum estradiol level were observed following gold chloride (0.2 mg/kg body weight/day), sc administration in immature female albino rats. Moreover, normal cyclic changes of estrus were found in vaginal smears of these rats whereas the rats of other groups showed diestrus phase throughout the period of experiment. Histological study of ovary also showed Graafian follicle with ovum in rats treated with 0.2 mg/kg/day of gold proving stimulation of reproductive function, which was not found in the ovarian histological study of other groups including controls. Thus, the results suggest a significant stimulatory effect of gold chloride on female reproductive activity in immature rats. Further, since the above-mentioned changes were evident at a specific dose of gold chloride, the data may have some clinical implications on stimulation and enhancement of fertility in immature female rats.

Keywords: Gold, Reproductive function, Rat

Gold, a valuable noble metal, has been known to be used in health and diseases since thousands of years. In Indian systems of medicine, gold preparations are highly valued and extensively used for tonic and rejuvenating properties[1]. Some gold preparations exhibit analgesic effects in rats and mice[2]. Clinically, gold salts have been used in the treatment of rheumatoid arthritis[3]. Although gold therapy increased gold concentration in adrenal, thyroid, Leydig cells of the testicles and semen[3,4], its specific effects on endocrine and gonadal activities remain obscure. Antitesticular effects of some metallic earth salts including gold chloride were detected in rats and mice over 40 years back[5]. Later it was reported that gold containing Ayurvedic drug showed stimulating effects in some impotent subjects[4]. On the other hand, several metallic earth salts such as lithium[6], lead[7], cadmium[8], copper[9] and arsenic[10] have detrimental effects on male gonads including lowering of testicular and accessory sex organ weight.

Studies on the effect of copper chloride on immature male rats[11] indicated some stimulatory effects on gonad and recent observation on the effects of gold chloride on immature male rats have revealed significant stimulatory effects on testicular activities[12]. Based on the above findings, the present investigation has been undertaken to find out whether gold has got any stimulatory effect on gonadal activities of immature female rats.

Materials and Methods

Chemicals—Gold chloride was purchased from Baird and Tat lock (London), Cross St. Hatton; NAD was purchased from Sigma Chemical, USA.

Animals and treatment—Experiments were performed on immature female Wistar strain albino rats of 25-30 days of age weighing 50-60 g body weight. They were maintained in a light (12L:12D) and temperature (28°±2ºC ambient temperature) controlled animal house and given standard laboratory food and water *ad libitum*.

Rats (18) were, divided in three groups of 6 each. One group of rats was injected (sc) with physiological saline (1 ml/kg body weight/day for 20 days) and designated as control (Group I). The other two groups were injected with 0.1 or 0.2 mg gold chloride/ml sterile distilled water/kg body weight/day for 20 days

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(Groups II and III). All the animals were sacrificed 24 hr after the last injection on 21st day following protocols and ethical procedures. Blood samples for hormone assay were collected from the hepatic vein under light ether anaesthesia. Plasma samples were separated by centrifugation, frozen and stored at –20ºC until assayed.

The ovaries and uterus were dissected out and weighed. One side ovary from each animal was fixed in Bouin's fluid and processed for routine histological studies and the other side of ovary was used for the assay of Δ^5-3β HSD activity.

The vaginal smears of all the animals were collected twice daily and were stained by double staining method.

Measurement of ovarian enzyme and plasma estradiol—Ovarian Δ^5-3β-HSD was assayed by U.V. spectrophotometric measurement of andrstenedione produced from dehydroepiandrosterone (DHA), using the procedure of Talalay[13]. Plasma level of estradiol was assayed by radioimmunoassay technique of Hanning *et al*[14]. Methodological loss during extraction was monitored by adding 10000 cmp {1β,2β-3H (N)} estradiol before extraction with 4 ml of diethyl ether twice. Samples were assayed in duplicate. The antisera to estradiol were purchased from Endocrine Science (Tarzzna, CA, USA) and had 40% cross reactivity with estrone. Free and bound estradiols were separated by using dextran-coated charcoal. The intraassay variation was 6.5%. All the samples were run at one time to avoid interassay variation. Since chromatographic purification of these samples was not performed, the values reported are the sum of estradiol and estrone.

For statistical analysis of the data model 1 ANOVA followed by a multiple comparison two tailed '*t*' test was used to test for differences between control and treated groups[15]. Differences were considered significant when $P< 0.05$.

Results

Gold chloride at the dose of 0.2 mg/kg significantly increased body weight of the rats in comparison to controls (Table 1). The relative weight of the ovaries and uterus were significantly increased ($P<0.05$) after 0.2 mg/kg dose of gold chloride treatment but not after 0.1 mg/kg dose of treatment (Table 1). There was no significant change in the activity of ovarian Δ^5-3β-HSD after gold chloride treatment at a dose of 0.1 mg/kg body weight/day for 20 days. But the activity of the enzyme was significantly increased in 0.2 mg of gold chloride treated rats in comparison to control group ($P<0.05$) (Table 1). Plasma estradiol level increased significantly in 0.2 mg gold chloride treated animals than the control or 0.1 mg of gold chloride treated group (Table 1).

At the beginning of the experiment all the rats exhibited stage of diestrus (anestrus) at the study of vaginal smear as all of them were immature. Control group animals and the rats treated with 0.1 mg/kg of gold chloride showed continuous diestrus stage throughout the period of treatment. On the other hand, animals treated with 0.2 mg of gold chloride showed the stage of estrus from 16th to 20th days of treatment and this estrus cycle continued regularly for the remaining treatment period in these animals (Table 2).

Histological study of ovary revealed that gold chloride treatment at the dose of 0.2 mg/kg caused stimulatory changes in germinal epithelium and ovarian stroma with graafian follicles at different stage of formation along with matured graafian follicle with ovum in some animals, whereas the

Table 1—Effect of gold chloride on body weight, ovarian and uterine weight, ovarian Δ^5-3β-HSD activity and serum estradiol level in immature female rats

[Values are mean ± SE of 6 rats per group]

Group	Body weight (g)	Ovarian weight % (mg/100 g b.w.)	Uterine weight% (mg/100 g b.w.)	Ovarian Δ^5-3β-HSD activity (units/mg tissue/hr)	Serum estradiol level (pg/ml)
Control	61.55 ± 6.7	57.7 ± 2.5	98.33 ± 4.5	46.55 ± 4.20	38.76 ± 2.50
Gold chloride dose					
0.1 mg/kg/day	66.44 ± 3.3	60.4 ± 2.6	103.2 ± 3.2	50.46 ± 3.53	37.55 ± 4.46
0.2 mg/kg/day	78.56 ± 6.8*	78.5 ± 3.5*	124.56±6.4*	59.50 ± 2.20*	52.45 ± 4.50*

*$P<0.05$ as compared with control ANOVA followed by multiple comparison '*t*' test

Table 2—Vaginal smear chart of rats for the whole period of treatment

Rat	Day of treatment								
	1-12	13	14	15	16	17	18	19	20
1	D	D	D	D	D	D	D	D	D
2	D	D	D	D	D	D	D	D	D
3	D	D	D	D	D	D	D	D	D
4	D	D	D	D	D	D	D	D	D
5	D	D	D	D	D	D	D	D	D
6	D	D	D	D	D	D	D	D	D
7	D	D	D	D	D	D	D	D	D
8	D	D	D	D	D	D	D	D	D
9	D	D	D	D	D	D	D	D	D
10	D	D	D	D	D	D	D	D	D
11	D	D	D	D	D	D	D	D	D
12	D	D	D	D	D	D	D	D	D
13	D	D	LD	PE	E	ME	D	D	PE
14	D	D	PE	E	ME	D	LD	PE	E
15	D	LD	PE	E	ME	ME	D	D	LD
16	D	D	D	D	PE	E	ME	D	D
17	D	D	D	LD	PE	PE	E	ME	D
18	D	LD	PE	E	ME	ME	D	D	PE

D=Diestrus; LD=Late Diestrus; PE=Proestrus; E=Estrus; ME= Metestrus.
Rat 1 to 6 = Group I (Control)
Rat 7 to 12 = Group II (gold chloride at the dose of 0.1 mg/kg/day).
Rat 13 to 18 = Group III (gold chloride at the dose of 0.2 mg/kg/day).

control animals and the animals treated with 0.1 mg of gold chloride did not show the above-mentioned changes (Figs 1, 2).

Discussion

The result shows that gold chloride at a particular dose stimulates gonadal activity of immature female rats. The body weight of the rats treated with 0.2 mg/kg of gold increased significantly from that of control group which indicates advancement towards maturity at an earlier age in this treated group. Probably gold chloride stimulates the pituitary-ovarian axis. In an ovarian steroidogenic event, Δ^5-3β-HSD is a key-regulatory enzyme[16] and increased activity of this after administration of gold chloride indicates an increased gonadotropin secretion as both plasma FSH and LH regulate the activity of the enzymes involved in ovarian steroidogenesis[17]. The weights of the ovary and uterus are the other indicators of plasma gonadotropin and estrogen levels. As ovarian weight is regulated by plasma gonadotropin[18,19] and uterine weight is dependent on ovarian steroids[20], an increase in both of these organ-weight may reflect an increased level of plasma gonadotropin (FSH and LH) and ovarian steroids (estrogen and progesterone). From the present data the increased plasma estradiol level (Table 1) in 0.2 mg gold treated animals directly supports the above mentioned findings of higher ovarian and uterine weight of this group and though plasma gonadotropin level has not been determined in this experiment the increased Δ^5-3β-HSD activity and plasma estradiol level reflect stimulation in plasma gonadotropin level in this group. The plasma progesterone level has also not followed in the present study, but in ovarian steroidogenesis the conversion of pregnenolone to progesterone is dependent on ovarian Δ^5-3β-HSD[21] and the increased activity of this enzyme in 0.2 mg gold treated rats indicate a higher progesterone level in this group.

Since all the rats were immature at the beginning of the experiment, sex cycles (here estrus cycle) were absent in them showing diestrus or anestrus at the vaginal smear study. But the animals treated with 0.2 mg of gold exhibited estrus and other stages of sex cycles (metestrus, diestrus and proestrus) after a certain period of treatment and cyclical changes were continued for the remaining period of experiment

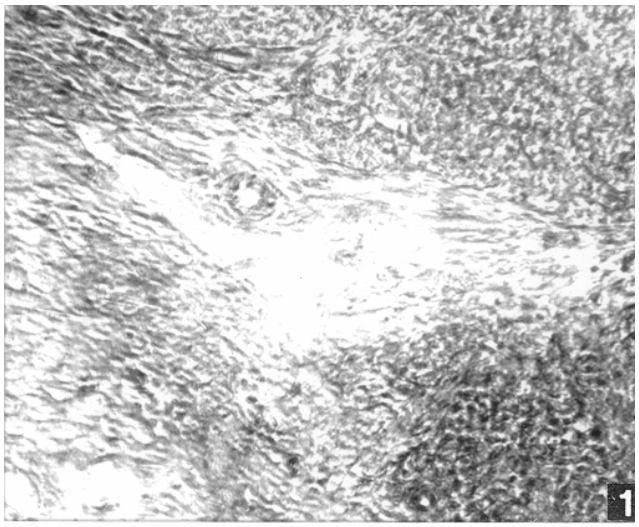

Fig. 1—Ovarian section of vehicle treated control rats or rats treated with gold chloride (at a dose of 0.1 mg/kg body weight) presence of some primordial follicles X-225.

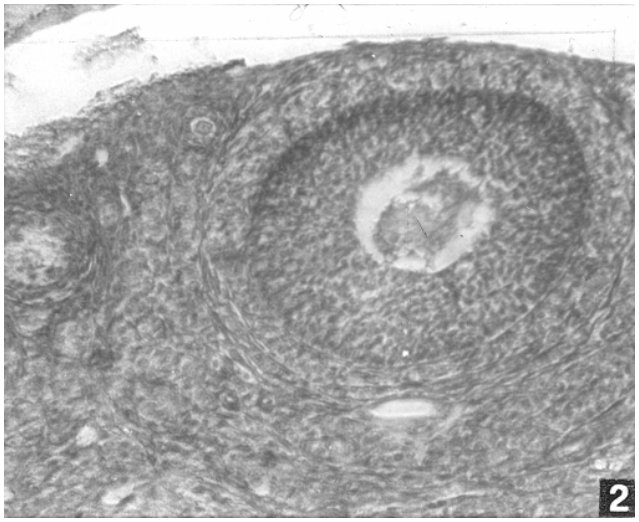

Fig. 2—Ovarian section of rats treated with gold chloride (at a dose of 0.2 mg/kg body weight) presence of graafian follicle near ovarian surface X-225.

(Table 2). This finding further supports the old observations which reveal that circulating estrogen stimulates estrus in female animals in lower vertebrates[22].

The histological study of ovarian sections also exhibited matured graafian follicle containing ovum along with other follicles and germinal epithelium in 0.2 mg gold treated animals (Fig. 2) the finding which was absent in other groups of rats (Fig. 1). Thus the overall picture of gonadal activity of 0.2 mg gold treated rats can be considered as augmented, at the same time the animals of the other two groups remain in non-stimulated condition. But the exact mechanism of these stimulatory changes in female gonads due to external administration of gold chloride is yet to be understood. Other reports with Ayurvedic drug that contains 2.0% gold, 11.5% zinc and 4% calcium along with other microelements have shown some stimulating effects in male reproductive activity[4], although zinc and copper present in the Ayurvedic drug may play an important role in spermatogenesis and fertility[23]. Furthermore, our recent observation of stimulating male gonadal activity by the administration of gold chloride in immature rats[12] supports the results of the present experiment and it can be concluded that administration of gold chloride at a dose of 0.2 mg/kg body weight/day for 20 days can cause stimulation of female reproductive function and early onset of estrous in immature rats. This beneficial effect of gold may have a clinical implication in the improvement of fertility in future.

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References

1 Bajaj S, Ahmad I, Raisuddin S & Vohora S B, Augmentation of non-specific immunity in mice by gold preparations used in traditional systems of medicine, *Indian J Med Res,* 113 (2001) 192.

2 Bajaj S & Vohora S B, Analgesic activity of gold preparations used in Ayurveda and Unani Tibb, *Indian J Med Res,* 108 (1998) 104.

3 Manoubi L, Jaafoura M H, Skhiri-Zhoura A, Berry J P & Galle P, Subcellular localization of gold in suprarenal, testicle and thyroid glands after injection of allochrysine in rats, *Cell Mol Biol (Noisy-le-grand)*, 40 (1994) 483.

4 Sharma D C, Jha J & Sharma P, Evaluation of safety and efficacy of a gold containing Ayurvedic drug, *Indian J Exp Biol*, 39 (2001) 892.

5 Kamboj V P & Kar A B, Antitesticular effect of metallic and rare earth salts, *J Reprod Fertil,* 7 (1964) 21.

6 Ghosh P K, Chaudhuri A, Biswas N M & Ghosh D, Effects of lithium chloride on testicular steroidogenic and gametogenic functions in mature male albino rats, *Life Sci,* 46 (1990) 127.

7 Ghosh P, Ghosh D, Sarkar M, Chaudhuri A & Biswas N M, Effects of lead acetate on testicular activity in albino rats, *Indian J Physiol Allied Sci,* 44 (1990) 7.

8 Biswas N M, Sengupta R, Chattopadhyay A, Chaudhuri G R & Sarkar M, Effect of atenolol on cadmium-induced testicular toxicity in male rats, *Reprod Toxicol,* 15 (2001) 699.

9 Chattopadhyay A, Sarkar M, Sengupta R, Roychoudhury G & Biswas N M, Anti-testicular effect of copper chloride in albino rats, *J Toxicol Sci*, 24 (1999) 393.

10 Sarkar M, Roychoudhury G, Chattopadhyay A & Biswas N M, Effect of sodium arsenite on spermatogenesis, plasma gonadotrophins and testosterone in rats, *Asian J Androl,* 5 (2003) 27.

11 Chattopadhyay A, Sarkar M & Biswas N M, Biphasic effect of copper chloride on male reproductive function in immature rats, [Abstract] *Proceedings of 89th Indian Sci Cong,* III (2002) 61.

12 Biswas N M, Chattopadhyay A & Sarkar M, Effects of gold on testicular steroidogenic and gametogenic functions in immature male albino rats, *Life Sci,* 75 (2004) 629.

13 Talalay P, Hydroxysteroid dehydrogenase, in *Methods in enzymology,* edited by S P Colowick and N O Kaplan (Academic Press, New York) 1962, 512.

14 Hanning R, Oreyk P J, Caldwell V B & Behrman H R, Estrogen and its radioimmunoassay, in *Methods of hormone radioimmunoassay*, edited by B M Jaffe & H R Behrman (Academic Press, New York) 1974, 675.

15 Das D, in: *Statistics in biology & psychology*, edited by D Das (Academic Publishers, Calcutta) 1981, 271.

16 Hinshelwood M M, Demter-Arlotto M, Means G D & Simpson E R, Expression of genes encoding steroidogenic enzymes in the ovary, in *Molecular biology of the female reproductive system,* edited by JK Findlay (Academic Press, London) 1994, 129.

17 Murono E P & Payne A H, Testicular maturation in the rat: *In vivo* effect of gonadotropins in steroidogenic enzyme in hypophysectomized immature rats, *Biol Reprod,* 20 (1979) 911.

18 Tagatz G E, Fialkow P J & Smith D, Hypogonadotropic hypogonadism associated with anosmia in the female, *New England J Med,* 283 (1970) 1326.

19 Kulin H E & Reiter E O, Gonadotropins during childhood and adolescence: A review, *Pediatrics,* 51 (1973) 260.

20 Edman C D, The effect of steroid on endometrium, *Seminar Reprod Endocrinol*, 1 (1983) 179.

21 O'Maliey B W & Strott C A, Steroid hormones: Metabolism and mechanism of action, in *Reproductive endocrinology,* edited by S S C Yen, R B Jaffe and R L Barbieri (WB Saunders Co, Philadelphia) 1999, 112.

22 Chatterjee C C, Endocrines, in *Human physiology,* Vol. II, edited by, H Saha, S C Ray, T R Abbot & V G Parulkar (Medical Allied Agency, Calcutta) 2002, 4-266.

23 Wong W Y, Gronen P M, Swinkels D W, Thomas C M, Copius-Peereboon R P, Merkus H M & Steegers-Theunissen RP, The impact of calcium, magnesium, zinc and copper in blood and seminal plasma on semen parameters in men, *Reprod Toxicol,* 15 (2001) 131.