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HISTOPATHOLOGICAL SIDE EFFECTS OF GOSSYPOL ON ADRENAL – PITUITARY AXIS IN MALE AND FEMALE PALM SQUIRREL *FUNAMBULUS PENNANTI* (WROUGHTON)

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ABSTRACT:
Effect of Gossypol on adrenal- pituitary axis was studied. Gossypol a "China Pill" (C_{30} $H_{30}O_8$) is phenolic compound isolated from seed of cotton plant (*Gossypium species*, family Malvaceae). The discovery of this compound by Chinese scientist as male fertility regulating agent (whose action is reversible) is regarded as a breakthrough in the search of male contraceptive. There are also differences of opinion on toxic effects of Gossypol. The clinical trial of this compound in China revealed no side effects. However, side effects such as fatigue, decrease in libido, apetite, dizziness and hypocalcaimic paralysis have been reported by some investigations. In the present investigation attempts have been made to study the histopathological side effects on adrenal-pituitary axis and its mechanism of action.
The experiment was conducted during April and May when the animals (male squirrels) are sexually active. Experimental male animals received Gossypol in two dose, 20 mg/kg B.W./ day and 30 mg/kq. B.W./day for 7 and 5 weeks respectively. At 20 mg. for 7 weeks treatment the cells of zone glomerulosa showed no changes whereas regression and degranulation occurred in 30 mg for 5 weeks treated animals as compared to that of control. In zona fasciculata and zone reticularis Gossypol induced no changes in 20 mg for 7 weeks treated animals, whereas vacuolation and degranulation observed in both of the zones after 30 mg for 5 weeks treatment. Medullary cells show hypertrophy and degranulation in adrenal of both the Gossypol treated groups. ACTH cells of pituitary gland showed no significant changes in 20 mg for 7 weeks treated animals, whereas regressive changes of ACTH Cells were observed in 30 mg for 5 weeks treated animals. Regressive changes in the adrenal cortex appears to be via pituitary gland. Since Gossypol regressed pituitary ACTH cells and deficiency of ACTH causes regression of adrenal cortex. Therefore, it is concluded that regressive changes in the adrenal cortex is via pituitary gland.

Keywords: *Adrenal- Pituitary axis, Gossypol.*

INTRODUCTION:

In the present study Gossypol a "China Pill" (C_{30} $H_{30}O_8$) was used for investigation. The discovery of this compound by Chinese scientist as male fertility regulating agent whose action is reversible, is regarded as a breakthrough in the search of male contraceptive. Antifertility action of Gossypol depends on purity of the compound, dose and duration of treatment. (Kalla, N.R. 1990; Kainz, V.; Frick, J.; Kainz, P. and Kalla, N.R. 1988. Lohiya, N.K.; Sharma, K.; Kumar, M.; Sharma, S. 1990). However, side effects such as fatigue, decrease in libido, apetite, dizziness and hypocalcaimic paralysis have been reported by some investigators, (Oko & Hrudka, 1984; Thomas *et al* 1991). In the present investigation attempts have been made to study the histopathological side effects on adrenal-pituitary axis and its mechanism of action.

METHOD AND MATERIAL:

Gossypol Acetic Acid is used for the study. It will be referred to as Gossypol hereafter. Gossypol was obtained as a gift from WHO. Special programme of Research, Development & Training in Human Reproduction Geneva. The purity of compound was given 99.9% and stored at 4^0C. Gossypol was dissolved in sunflower seed oil. The experiment was conducted during April and May when the male squirrels were sexually active. Detail grouping of squirrels and experimental protocol is given in Table-1 Experimental male animals received Gosypol in two dose, 20 mg/kg B.W./day and 30 mg/ kg. B.W./ day for 7 and 5 weeks respectively.

Pituitary sections were stained with Lead Haematoxylin staining technique as a stain for endocrine cells (Solcia *et al* 1969).

RESULTS AND DISCUSSION

Adrenal of Control Male (Fig. 1 & 4)

The cells of zona glomerulosa were arranged in clusters and cells were round or polygonal with full secretary granules. The cells of zona fasciculata were arranged in cords and were thickly granulated with conspicuous nuclei. The cells of zona reticularis were compactly arranged and the cell walls were fused. The zone was clearly demarcated from medullary zone. Zona medulla was well vascularized and composed of two cell types. Type A cells were large in size, plenty and the other cell types were few and irregular in shape with indistinct nuclei. Cytoplasmic granules were thick.

Adrenal of Experimental Male (Fig. 2, 3, 5 & 6)

At 20 mg for 7 weeks treatment the cells of zona glomerulosa showed no changes whereas regression and degranulation occurred in 30 mg for 5 weeks treated animals as compared to that of control.

In zona fasciculata and zona reticularis Gossypol induced no changes in 20 mg for 7 weeks treated animals, whereas vacuolation and degranulation observed in both of the zones after 30 mg for 5 weeks treatment.

Medullary cells show hypertrophy and degranulation in adrenal of both the Gossypol treated groups

Anterior Pituitary of Control Male (Fig. 7)

ACTH cell were specifically stained with lead haematoxylin & showing normal cytological structure.

Anterior pituitary of Experimental Male (Fig. 8 & 9)

Gossypol induced no significant in changes in ACTH cells of Anterior pituitary gland in 20 mg for 7 weeks treated animals, whereas regressive changes of ACTH cells were observed in 30 mg for 5 weeks treated animals.

My observations on adrenal- pituitary axis of palm squirrel reveals that low dose of Gossypol caused no histopathological side effects on adrenal cortex and pituitary ACTH cells. On the other hand high dose of Gossypol induced regressive changes in the cells of zona glomerulosa, zona fasciculata, zona reticularis and ACTH cells of anterior pituitary gland. It appears that the regressive changes in the adrenal of squirrel may be due to insufficient production of ACTH hormones by the regressed ACTH cells of anterior pituitary gland. It can be concluded that high dose of Gossypol acts on the adrenal gland via anterior pituitary gland. Direct effects of Gossypol on adrenal gland is ruled out. Some investigators observed no effects of Gossypol on histology and function of adrenal gland (Yuan *et al*, 1980; Zhang *et al*, 1981 and Ye *et al*, 1982). Udoh, (1991) reported that Gossypol induced hypertrophy, degranulation and vacuolation in the cells of adrenal cortex and regression in ACTH cells of anterior pituitary. He concluded that Gossypol acts directly on adrenal but not via hypothalamic pituitary axis.

Mechanism of Action of Gossypol

Regressive changes in the adrenal cortex appears to be via pituitary gland. Since Gossypol regressed pituitary ACTH cells and deficiency of ACTH causes regression of adrenal cortex.

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Table 1 : Experimental design for Gossypol

No. of Animals & Sex	Treatment	Dose mg/ kg B.W. day	Administration	Duration (Weeks)
10 Males (Experimental)	Gossypol	20 mg	I.M.	7
10 Males (control)	Sunflower seed oil (Vehicle)	E.V. (0.1 ml)	I.M.	7
10 Females (Experimental)	Gossypol	30 mg	I.M.	5
10 Females (Control)	Vehicle	E.V. (0.1 ml)	I.M.	5

E.V. = Equivalent volume, I.M. = Intramuscular (injection)

Table 2 : Cell types of Anterior Pituitary and their tinctorial properties

Staining Technique	Cell Types					
Solcia PbH *et al* (1969) PbH (Lead Haema toxylin)	**Type I GH**	**Type II PRL**	**Type III TSH**	**Type IV FSH**	**Type V LH/ICSH**	**Type VI ACTH**
	-	-	-	-	-	Black

Plate I

Effects of Gossypol on Adrenal gland of *Funambulus pennanti* sections are stained with Haematoxylin & Eosin (×290)

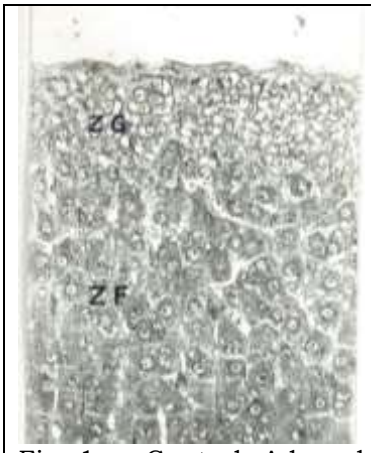

Fig 1 : Control Adrenal showing normal histological structure of zona glomerulosa & Zona Fasciculata

Fig. 2 : Experimental Adrenal low dose – No changes in cells of zona glomerulosa & zona fasciculata

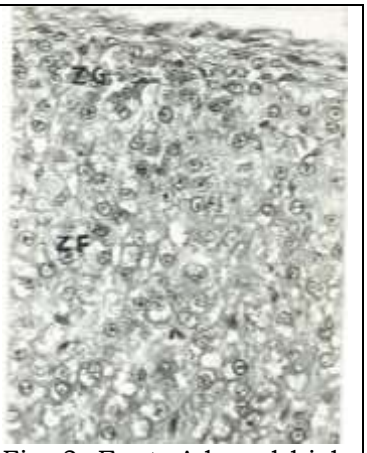

Fig. 3. Expt. Adrenal high dose- Degranulation & vacuolization occurred in the cells of both zona glomerulosa & zona fasciculata

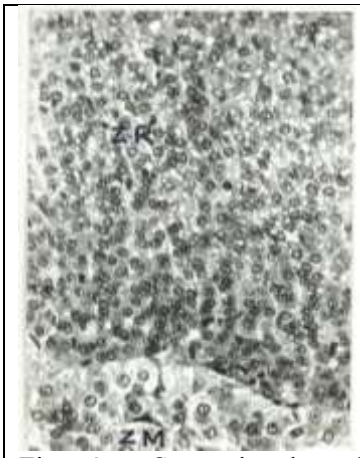

Fig. 4 : Control adrenal showing normal histology of zona reticularis and zona medulla.

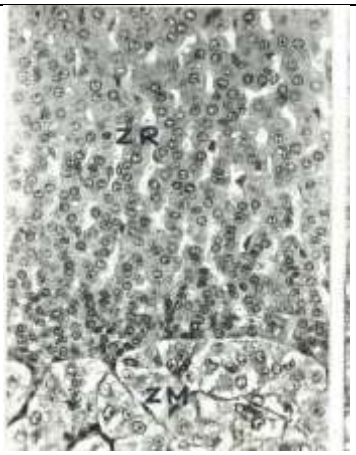

Fig. 5 : Expt. adrenal Low dose showing no changes in zona reticularis and hypertrophy and degranulation in the cells of zona medulla.

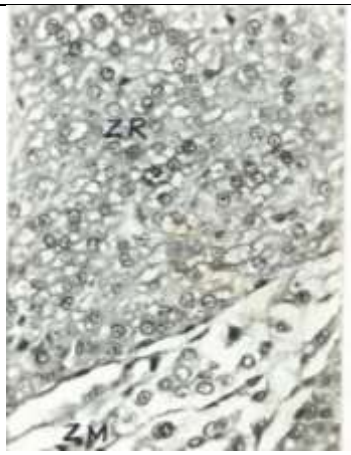

Fig. 6 : Expt. Adrenal High dose- Intense vacuolization seen in the cells of zona reticularis and degranulation in the cells of zona medulla.