

causes severe discolouration and shrivelling of the mustard seeds. Shrivelled seeds lose their weight and oil content considerably. Discolouration without shrivelling though reduces grain weight but does not interfere much with the oil content.

Rape and mustard seeds kept for five months (from middle of April to middle of September) at room temperature ranging from 29 to 38.5°C (natural conditions) or at 35°C or in the natural storage conditions of Punjab became free of *A. brassicae* infection before the start of sowing season. *Alternaria brassicae* although gets eliminated from the seeds yet leaves its bad effects like discolouration and shrivelling. To overcome this, control of the disease in the field especially on the pod stage is very essential because

it directly helps to get a healthy and better quality produce.

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THE EFFECT OF SHORT-TERM TREATMENT OF METHALLIBURE [ICI COMPOUND 33, 828] ON THE HISTOMORPHOLOGICAL AND ENZYMATIC ASPECTS OF TESTIS AND THUMB PAD OF TOAD, *BUFO MELANOSTICTUS*

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ABSTRACT

The short-term treatment with low dose of methallibure (ICI compound 33, 828) to adult male toads (*Bufo melanostictus*) had no effect on testicular histomorphology and spermatogenesis. However, the Leydig cell nuclear diameter as well as 3 β -HSDH and G-6-PDH enzyme activities decreased markedly. This decrease in steroidogenic activity of the Leydig cells was correlated with the regression of thumb pads, which are known to be androgen dependent sex structures in male Anura.

THE antigonadotropic effect of methallibure is well established in mammals¹, but comparative studies on lower vertebrates are scarce²⁻⁷. The present work was undertaken to investigate the effects of low dose of methallibure on spermatogenesis, the steroidogenic activity of the Leydig cells, and the androgen dependent thumb pads of toads, *Bufo melanostictus*.

Adult male *B. melanostictus* obtained during the breeding season (July) from the surrounding areas of Dharwar were used after five days of acclimatization to the laboratory conditions. The first group specimens (10) were injected (ip) with saline to serve as the controls. The second group specimens (10) were injected (ip) with saline suspension of methallibure, biweekly for 20 days. The total dose being 5.25 mg for each of the experimental toad (750 μ g/injection). All toads were autopsied one day after the last injection. The relative testis weights were recorded and representative pieces of testes and thumb pads were fixed in Bouin's fluid for histological and histometric studies⁸.

The remaining pieces of testes were used for the histochemistry of Δ^5 -3 β -hydroxysteroid dehydrogenase (3 β -HSDH) and glucose-6-phosphate dehydrogenase (G-6-PDH) as described earlier⁹.

It is evident from Table I that there is no appreciable effect on the average testis-weight, testis diameter and tubule diameter due to short-term treatment with methallibure. Similarly, no marked alteration in the spermatogenic activity was observed (Table II). However, Leydig cell nuclear diameter decreased (Table I) significantly ($p < 0.01$). Similarly, in treated toads the Leydig cell 3 β -HSDH and G-6-PDH activities decreased (Table I) compared to the controls. The height of epidermis and glandular epithelium of thumb pads also decreased significantly (Table III). The thumb pad epidermis was less papillate and the mucous glands were atrophic in the treated specimens. The present findings are thus in conformity with those reported earlier on other species^{1-5,7}, wherein methallibure was found to cause regression of the secondary

TABLE I
Effect of methallibure on the testis of *B. melanostictus*.

Group	Average testis weight mg/100 g body weight \pm S.E.	Average diameter ($\mu\text{m} \pm$ S.E.)			Leydig cell	
		Testis	Testis tubule	Leydig cell Nucleus	$\Delta^5-3\beta$ -HSDH activity	G-6-PDH activity
Control	215.2 \pm 18.5	1529.8 \pm 79.5	167.3 \pm 4.5	5.5 \pm 0.1	++	+++
Experimental	169.8 \pm 24.7 N.S.	1425.6 \pm 62.8 N.S.	162.0 \pm 8 N.S.	4.8 \pm 0.1 $P < 0.01$	+	++

S.E. = Standard error.

Intensity of reaction is visually graded from (+) to (++++);

p -values calculated by student's t test between control and experimental groups.

N.S. = non significant.

TABLE II
Effect of methallibure on spermatogenetic stages of *B. melanostictus*.

Group	0	I	II	III	IV	V
Control	2.27 \pm 0.39	0.37 \pm 0.08	0.51 \pm 0.14	0.61 \pm 0.19	0.78 \pm 0.13	0.26 \pm 0.06
Experimental	1.87 \pm 0.28 N.S.	0.38 \pm 0.12 N.S.	0.5 \pm 0.13 N.S.	0.66 \pm 0.07 N.S.	0.73 \pm 0.2 N.S.	0.28 \pm 0.05 N.S.

N.S. = Non significant

TABLE III
Effect of methallibure on the thumb pad of *B. melanostictus*

Group	Average height $\mu\text{m} \pm$ S.E.	
	Epidermis	Glandular epithelium
Control	118.8 \pm 4.9	31.4 \pm 0.8
Experimental	95.8 \pm 5.3 $p < 0.02$	22.1 \pm 2.2 $p < 0.01$

S.E. = Standard error.

p -values calculated by student's t test between control and experimental groups.

sex characters in male. However, the low dose of methallibure used in the present study did not significantly influence the testicular histology and histometry during the short-term treatment.

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