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Research Article

EVALUATION OF TESTICULAR TOXICITY OF BUTACHLOR (A CHLOROACETANILIDE HERBICIDE) IN RATS

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ABSTRACT

Butachlor, a chloroacetanilide herbicides, widely used in agriculture by farmers was evaluated to determine its effects on testes of rats. Oral administration of butachlor at dose level of 200 mg/kg b.wt./day for 30 day leads to alterations in the testes. Haematological studies showed decrease in erythrocyte count, haematocrit and haemoglobin levels while an increase was noticed in leukocytes count, blood urea and blood sugar. The weight of testes and accessory sex organs decreased significantly. Butachlor administration brought about marked reduction in sperm counts. A significant reduction in glycogen and sialic acid and an increase in protein and cholesterol content of testes were noticed. Histological examination revealed shrunken seminiferous tubules exhibiting loosened epithelium and reduced number of spermatozoa. Fertility test showed 90% negative fertility in treated rats. In addition, acid phosphatase enzyme activity increased significantly whereas alkaline phosphatase enzyme activity showed sharp decline. It also suppressed testosterone, FSH and LH levels significantly. In conclusion, butachlor has deleterious effects on male reproduction.

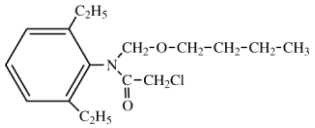
Keywords: Butachlor, Testes, testosterone, FSH, Sperm count.

1. INTRODUCTION

During recent years, intensive use of herbicides has raised increasing concern mainly due to their massive pollution of the environment as these herbicides are directly or indirectly toxic to a wide range of organism [1, 2]. There is increasing evidence that reproductive abnormalities are increasing frequently in both human and animals which is probably related to exposure to toxic contaminations in the environment [3, 4]. Occupational exposures to these chemicals may increase parental risk of infertility and adverse pregnancy outcomes such as spontaneous abortion, preterm delivery, and congenital anomalies [5].

To evaluate the safety of herbicides is of crucial importance because of evidence that some these chemicals are implicated in male sterility, fetal malformations, chromosomal aberrations and cancers [6]. Many herbicide induced androgen deficiency by reducing Leydig cell volume serum concentration of testosterone and the weight of seminal vesicle and epididymis [7]. Exposure of these toxic environmental chemicals increases the risk for sperm abnormalities, decrease fertility, a deficit of male children, growth retardation [8]. It is reported that pesticides may induced pathological changes in the testes and liver of rats [9]. They also alter the reproductive function by altering sperm count and sperm shape, alter sexual behavior or increase infertility in animals and human beings [10, 11].

Butachlor (2-chloro-2', 6'-diethyl-N-(butoxymethyl)aceta--nilide) is a member of chloroacetanilide class of chemistry and is the herbicidal active ingredient in MACHETER EC. This herbicide is used as a pre-emergence control for the undesirable grasses and broadleaf weeds [12].



Butachlor

The consumption of butachlor in Asia alone is more than 4.5 X 10^7 kg per year for weed control [13]. Extensive use of this herbicide over the years has led to the deleterious effects on the soil flora and fauna. Therefore, its environmental impact has been extensively investigated based on toxicity assessment [14]. Butachlor is known to exert genotoxic effect on amphibians and reportedly induce apoptosis in mammalian cells [15]. The toxicity of butachlor is due to not only parent

compound, but also its degradation product such as dialkylquinoneimine [12].

Occupational exposure is associated with low birth weight and causes exfoliative dermatitis, jaundice, increase in liver enzyme, eosinophilia and also produced or displayed a consistent pattern of mutagenic activity [16, 17]. It decreases the percentage of viable motile sperm and sperm velocity and also causes tumor formation in rodents [18]. However, only few attempts have been made to observe the effects of butachlor on male reproductive system. Hence, present investigation was to evaluate the toxic impacts of butachlor on testes.

2. MATERIAL AND METHODS

2.1. Animal models

20 healthy male albino rats, weighing 150-200 gms were used for the present investigations. The animals were kept in clean polypropylene cages covered with chrome plates grills and maintained in an airy room with temperature of (20°C±5°C) with 14:10 L:D cycle. The animals were fed on the standard pellet diet and tap water *ad libitium* and occasionally fed on germinated/sprouted grams and wheat seeds.

2.2. Test material and doses

Technical grade butachlor obtained from "The Herbicides Pvt. Ltd." Jaipur, was used for experimentations. The butachlor was administered to male rats dissolved in olive oil through oral intubations at the dose level of 200 mg/kg b.wt./day for 30 days.

2.3. Experimental procedure

Rats were divided into two groups having ten animals each.

Group I was kept control and given olive oil only.

Group II was treated with butachlor at dose level of 200 mg/kg b.wt/day for 30 day.

At the end of the experimentation all treated rats along with control were weighed, sacrificed under light ether anesthesia. The male reproductive organs were removed, weighed and processed for detail biochemical and histopathological studies.

2.4. Fertility test

The mating exposure test of the animals was performed. They were co-habited with the proestrous female in the ratio 1:3. The vaginal plug and the presence of sperm in the vaginal smear were checked for positive mating. Female were separated and resultant pregnancies were noted when dam gave birth.

2.5. Sperm dynamics

The sperm motility in cauda epididymis and sperm density in testes and cauda epididymis was determined [19].

2.6. Blood analysis

Haemoglobin percentage [20], haematocrit values [21], total erythrocytes count [22], total leukocytes count [22], blood sugar [23] and blood urea [24] were assessed.

2.7. Biochemical parameters

The total protein [25], sialic acid [26], glycogen [27] and cholesterol [28] were assessed. Also, acid and alkaline phosphatase enzymatic activity was determined by Kings Method [29].

2.8. Hormonal immuno assay

Testosterone, leutinizing hormone (LH), and follicle stimulating hormone (FSH) were estimated through chemiluminescence in fully automatic Advia Cemtaus Immuno Assay System.

2.9. Testicular histology

Testes of rats exposed to butachlor and control were fixed in Bouin's fixative for at least 48 h, processed by paraffin wax impregnation method, cut using a rotary microtome at 5 μm thickness, and stained with hematoxylin and eosin (H X E) for light microscopic examination.

2.10. Statistically analysis

The data were analyzed statistically using Student's ''t'' test [30] and the significance of differences was set at $P<0.01$ and $P<0.001$.

3. RESULTS

In the present study, the treated rats showed no significant difference in body weight at the end of the experimentation as compared to control. However, the weight of testes, seminal vesicle ($P\leq0.001$), epididymis and ventral prostate ($P\leq0.01$) were decreased significantly (Fig. 1). A significant decrease in the sperm density in testes and cauda epididymis was observed ($P\leq0.001$) after butachlor treatment (Fig. 2). Also the sperm motility in cauda epididymis was severely impaired ($P\leq0.001$) and fertility test showed 90% negative fertility (Fig. 3).

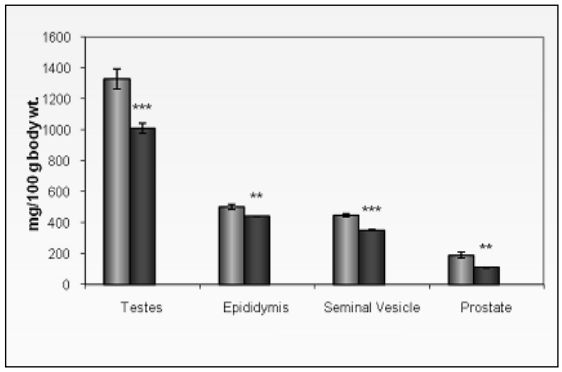


Fig. 1: Change in organ weights after butachlor treatment

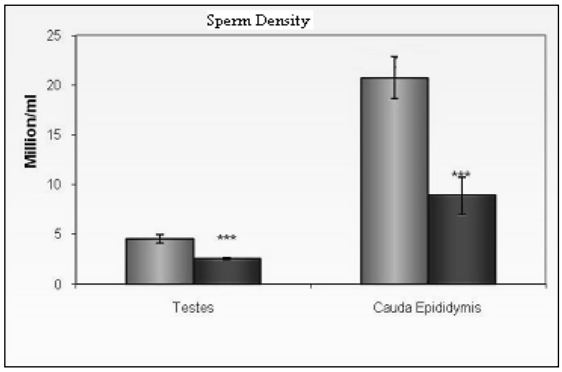


Fig 2: Altered sperm density in testes and cauda epididymis after butachlor treatment

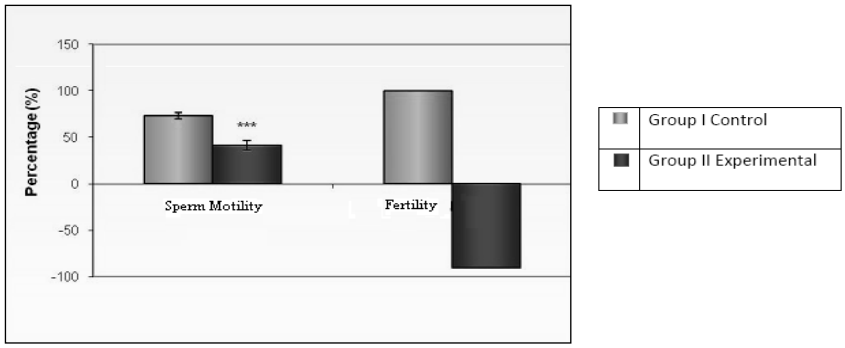


Fig 3: Altered sperm motility in cauda epididymis and fertility test after butachlor treatment

Table 1: Changes in blood parameters after butachlor treatment

Treatment	Total erythrocyte count (TEC) million/mm^3	Total leukocyte count (TLC) cells/mm^3	Haemoglobin gm%	Haematocrit %	Blood sugar mg/dl	Blood urea mg/dl
Group I Control (Vehicle only)	6.96± 0.13	4886.62± 313.54	15.20± 0.34	48.15± 1.15	85.64± 2.00	48.02± 1.80
Group II Experimental (200mg/kg bw/day Butachlor for 30 day)	5.69± 0.30 **	5983.57± 30.11 **	12.81± 0.61 **	42.79± 0.38 **	94.03± 0.81**	99.49± 6.41 ***

*= P≤0.05 **=P≤0.01 ***=P≤0.001; Value ±SEM 6 determinations*

A marked decrease in the total erythrocyte count, haemoglobin and haematocrit ($P \leq 0.01$) was observed. Whereas total leukocyte count, blood sugar ($P \leq 0.01$) and blood urea ($P \leq 0.001$) has been significantly increased (Table 1). The study revealed a marked reduction in the sialic acid and glycogen content of testes was observed ($P \leq 0.05$, $P \leq 0.001$) whereas cholesterol and protein contents of testes increased significantly ($P \leq 0.001$, $P \leq 0.01$) (Table 2). A significant decline in levels of testosterone, FSH and LH and alkaline phosphatase activity ($P \leq 0.01$, $P \leq 0.001$) has also been observed whereas acid phosphatase activity increased significantly ($P \leq 0.01$) (Table 3).

Table 2: Biochemical changes in testes after butachlor treatment

Treatment	Protein (mg/g)	Sialic Acid(mg/g)	Cholesterol (mg/g)	Glycogen (mg/g)
Group I Control (Vehicle only)	250.00± 9.94	5.03± 0.03	5.32± 0.64	2.72± 0.09
Group II Experimental (200mg/kgbw/day Butachlor for 30 day)	296.74± 1.10 **	4.79± 0.05	8.19± 0.01 ***	1.56± 0.23 ***

*= P≤0.05 **=P≤0.01 ***=P≤0.001, Value ±SEM 6 determinations*

Table 3: Changes in serum analysis after butachlor treatment

Treatment	Acid Phosphatase KA units	Alkaline Phosphatase KA units	Testosterone (mg/ml)	FSH (mIU/ml)	LH (mIU/ml)
Group I Control (Vehicle only)	5.25± 0.23	63.74 ±2.02	2.9± 0.14	0.66± 0.08	1.8± 0.16
Group II Experimental (200mg/kgb.wt/day Butachlor for 30 day)	6.40± 0.22 **	52.46± 0.01 ***	1.5± 0.37 **	0.42± 0.02 **	0.6± 0.28 **

*= P≤0.05 **=P≤0.01 ***=P≤0.001, Value ±SEM 6 determinations*

3.1. Testicular histology

Histopathological study of the testes of control rats showed normal morphology of seminiferous tubules with all successive stages of spermatogenesis (Fig. 4a). Testes of rats treated with butachlor at dose level of 200 mg/kg b.wt/day for 30 day showed reduced size of seminiferous tubule, complete arrest of spermatogenesis, and increased intertubular space with ruptured interstitial cells in contrast to control group. Lumen is devoid of sperm and filled with cellular debris (Fig. 4b).

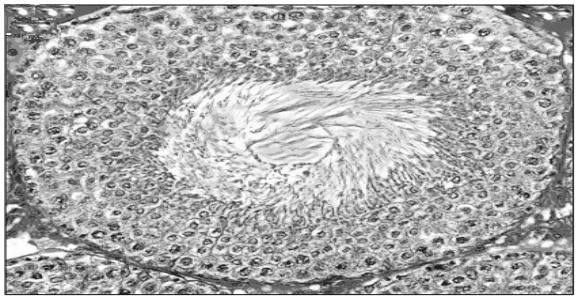


Figure 4: (a) Micro-photograph of control rat testes showing all the successive stages of spermatogenesis i.e. normal morphology of seminiferous tubules. Lumen is filled with sperm. Leydig cells are also present (H X E 200X).

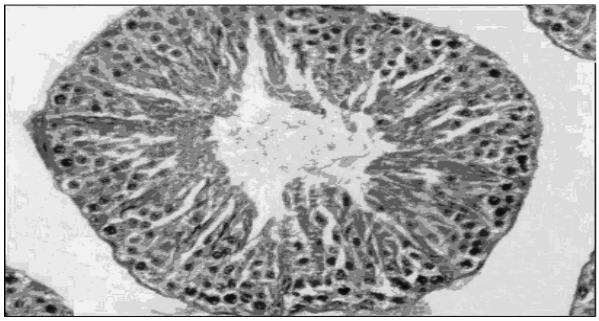


Figure 4: (b) Photograph of testes treated with butachlor 200mg/kg b.wt. for 30 days showing seminiferous tubules with damaged epithelial lining. Spermatogenic elements are very few. Lumun having no spermatozoa, but cellular debris are present.

4. DISCUSSION

Over the last fifty years many human illness and death have occurred as a result of exposure to pesticides. Pesticides are used in agriculture to protect crops but represent at the same time potential risk to farmers and environment [31]. Random use of pesticide damaged not only environment but also affects

all living beings. Pesticides are frequently deliberately released toxic chemical into the environment [32].

The present study revealed that administration of butachlor (200 mg /kg b.wt/day for 30 days) to male rats resulted in testicular toxicity. Administration of butachlor showed a significant decrease in weight of testes and accessory sex organs. The decreased weight of testes may be due to reduced tubular size, spermatogenic arrest and inhibition of steroid synthesis by Leydig cells [33]. The reduction in weight of accessory sex organs may be due to low availability of androgens or antiandrogenic activity of butachlor [34]. Generally, maintenance of weights of accessory reproductive glands depends on testosterone level [35].

The motility of sperm in cauda epididymis indicates less ability of sperm to interact with the oocyte plasma membrane [36]. Decreased sperm density in the epididymis is an indicator of reduced spermatogenesis as a result of the toxicity of any agent [37]. Low caudal epididymal sperm density may be due to alteration in androgen metabolism [38, 39]. Decrease androgen concentration results in decrease sperm production [40, 41] in testes and cauda epididymis. The 90% negative fertility may be attributed to the reduction in motility and density of sperm and altered biochemical milieu of cauda epididymis [42, 43]. Reduction of sperm count, lower motility and lower normal sperm morphology are associated with a higher probability of infertility [44].

Exposure of butachlor results into the reduction of erythrocytes count which may be due to inhibition of production of red blood cells in bone marrow [45]. The decline in erythrocyte counts also may be due to the disruptive action of the insecticides on the erythropoietic tissues as a result of which the viability of the cells might be affected [46]. Leukocytes count increased as pesticide act as chemical stressors, caused slight increase in adrenaline levels [9, 47]. The increased number of leukocytes can occur abnormally as a result of an infection, cancer, or toxic chemical.

Haematocrit percent may be reduced due to decrease in the size of RBC [48]. Increase in the rate of erythrocyte destruction could be the possible reason for reduction in the number and size of erythrocytes [49]. Haemoglobin also reduced due to reduction in the general food intake by rats of no extra iron supply might reason for iron deficiency [50]. A decline in the rate of haemoglobin synthesis occurs during all the stages of maturation of erythrocytes when the supply of iron is inadequate [51]. Blood urea was increased after administration of butachlor which may be due to high concentration of nonprotein nitrogenous substances resulting failure of the body to excrete the metabolic end product of protein [52, 53]. Elevation in the level of blood sugar in blood is due to influence in glucose homeostasis by physiological stress, stimulation of adrenal gland and disturbed metabolism of liver tryptophan as liver play an important role in the glucose homeostasis [54].

Administration of butachlor also changes the biochemical parameters of the reproductive tract. Increased testicular cholesterol concentration may be correlated with its non-utilization by the system leading to a fall in circulating androgen due to antiandrogenic activity [37, 55]. A decrease in testicular glycogen is indicative of decreased number of post-meiotic germ cells (spermatids), a site for glucose metabolism [56]. Reduction in glycogen level by the administration of butachlor inhibits the glycogen synthesis which eventually decreased spermatogensis [57, 58]. Decrease in testicular sialic acid concentration may be due to anti spermatogenic activity or reduced androgen production [59]. Elevation in protein content in testes may be due to hepatic detoxification activity which results in the inhibitory effect on the activity of enzyme involved in the androgen biotransformation [60].

A significant reduction in the alkaline phosphatase activity may be attributed to the decrease osteoblastic activity of bone; since it is formed and present in the osteoblasts [61].The increase in acid phosphatase activity may be due to results of labalization of lysosomal system [9]. Further, reduction in the serum testosterone clearly demonstrated the inhibitory effect of butachlor on secretion of pituitary gonadotropins (FSH and LH) and in turn on testosterone biosynthesis. The low level of testosterone arrests spermatogenesis [62, 63]. FSH and LH affect the development and the function of testes and inhibit the development of spermatogenesis and seminiferous tubules [64]. Hence, from the above results it can be concluded that butachlor exerts testicular toxicity in albino rats.

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