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Metamitron-induced changes in biochemical profile and cytotoxicity in black Bengal goats

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Abstract

A non-toxic dose of metamitron (triazine herbicide) was determined in black Bengal goats by trial and error. Hematological parameters like total count, Hb percentage, ESR, biochemical parameters like blood sugar, pyruvic acid, LDH, AChE and tissue biochemical parameters like ALT, AST activity, reduced glutathione level and lipid peroxidation level were estimated in black Bengal goats after oral administration of a single dose of metamitron @ 30 mg/kg body weight (b.wt.). Histopathological studies were also carried out in tissue sections of liver, kidney, spleen, thyroid, heart and brain. No significant changes were found in experimental subjects when compared to control indicating that metamitron @ 30 mg/kg b.wt. may be a non-toxic dose to goats and can be used safely in agricultural field.

Keywords: Glutathione, goat, lipid peroxidation, metamitron, toxicity, triazine herbicide

Introduction

Metamitron, a triazine herbicide, is used to control grass and broad-leafed weeds in sugar and fodder beets, in man gold, red beet and certain strawberry varieties (Tomlin, 1997). Because of intensive agricultural practices, use of herbicides along with different inorganic, organic fertilizers and pesticides is increasing day by day. Indiscriminate use of herbicide has been reported to cause potential adverse effects on animal and human beings (Klaassen, 2003). Necessary information is required to be generated in animal system with reference to safety evaluation of metamitron before it is widely advocated for use. Goat has been considered as an experimental animal because its meat and milk are widely consumed by human beings. The free range and semi intensive systems of rearing of goats in India may make it more vulnerable to the possible adverse effects of this herbicide. The left over of metamitron in the meat and milk of the goat may ultimately produce health hazards in human beings. Literature relating to cytotoxicity and effect on biochemical profiles of metamitron is rare in animals, particularly in black Bengal goats. With this idea, the present experiment studied the effects of metamitron [4 amino 3 methyl 6 phenyl 1,2,4 triazine 5(4H) one] on hemogram, biochemical parameters like sugar, pyruvic acid and cytotoxicity in black Bengal goats after administration of a single non-toxic oral dose.

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Materials and methods

Chemicals

Metamitron (technical grade, purity-98%) was supplied by M/s Gharda Chemicals Ltd., Mumbai, India. All of the chemicals used in this study were obtained from from E.Merck (Germany) and Sigma Chemical Co. (USA).

Experimental Animals

Clinically healthy black Bengal male and female goats of approximately 1.5 to 2 years of age, weighing from 12 to 16 kg were used in this experiment. Throughout the experimental period,

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the animals were caged individually in customs made metabolic cages (stainless steel). The temperature of the experimental animal room was maintained at 22°C (±3°C) and artificial lighting facilities, *ad libitum* drinking water, and standard feed were provided (Juliet *et al.*, 1998). Before onset of experiment, the animals were acclimatized to experimental environment for 7 days. Each animal was fasted overnight before administration of metamitron and/or vehicle.

Administration of non-toxic oral dose of metamitron

Single oral non-toxic dose of metamitron (@ 30mg/kg body weight) was administered following the method published earlier (Debnath *et al.*, 2003).

Experimental design

For the present study, four male and four female goats were used, of which one male and one female goat were kept as control. The remaining three male and three female goats were considered to be the experimental animals. Metamitron was administered @ 30 mg/kg b.wt. once orally after suspending in 1% carboxymethyl cellulose (10ml) to each experimental animal. The control group was fed with 10 ml carboxymethyl cellulose suspension (1%).

Collection of blood and tissue samples

Blood samples (8 ml) were collected from the jugular vein of each experimental goat before treatment and thereafter at every 24 hr interval post dosing till the day of sacrifice of the animal. From total blood sample, 2.0 ml blood was kept in EDTA (1 mg/ml) for hemogram study, 1.0 ml was kept in sodium fluoride treated vial (2.5 mg/kg) for glucose estimation and remaining 5.0 ml blood sample was allowed to clot in the refrigerator for separation of serum which was utilized for the estimations of pyruvic acid, acetylcholinesterase and lactic dehydrogenase enzyme activities.

The experimental and control group animals were sacrificed on 5th day post treatment and small portions of hepatic, nephric and cardiac tissues were collected for estimation of alanine (ALT) and aspartate aminotransferase (AST) activities, reduced glutathione level and lipid peroxidation level and kept at –20°C till further use.

Small pieces of liver, kidney, heart, spleen, brain, lung and thyroid gland of experimental groups were collected and preserved in 10% formal saline for histopathological examinations as described by Lillie and Fullmer (1976).

Hematological and biochemical parameters

Hemoglobin level was estimated by indirect acid haematin method described by Coffin (1953) and expressed as gm/dl or gm%. Total erythrocyte count (TEC) and total leucocyte count (TLC) were done by standard methods of Schalm *et al.* (1975). Erythrocyte sedimentation rate was estimated by the method of Wintrobe following the method of Coffin (1953) and expressed as mm 1st hr.

Glucose level was estimated as described by Nelson-Somogyi (1945) and was expressed as mg/dl of blood. Serum pyruvic acid level was measured according to the method of Ladry (1964) and expressed as mg of pyruvic acid/ ml of serum. Serum acetyl cholinesterase activity was studied by the method of Augustinson (1957) and expressed as mg Ach hydrolyzed/ml/30min. Serum lactic dehydrogenase activity was assayed by the method described by Bergmayer *et al.* (1974) and the activity was expressed as mmole NADH oxidized/min/ml.

Tissue biochemical assay

A small portion of cardiac and hepatic tissue was blotted and the fascia was removed. These tissue pieces were separately chopped and minced with the help of scissors and stain less steel blade for homogenization in ice cold normal saline (50mg/ml) in a glass homogenizer with a motor driven Teflon coated pestle (Remi 127A). The homogenates thus obtained were centrifuged at 4000 rpm in a refrigerated (4°C) centrifuge (Remi C-24) and the supernatent was stored at -20°C and subsequently utilized for the biochemical estimations [ALT and AST activities and tissue protein].

A small portion of liver and kidney tissue was processed as described above and homogenized with chilled distilled water (200 mg/ml) in a glass homogenizer (Remi RQ 127A). The crude homogenate of liver tissue only was divided into two parts. To one part of hepatic tissue homogenate and

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kidney homogenate, required amount of trichloro acetic acid (25%) was added in the ratio of 4:1, so that the final concentration of trichloro acetic acid would be 5% for estimation of reduced glutathione.

Another part of liver tissue homogenate (200 mg/ml) was diluted with equal volume of chilled distilled water so that the concentration of tissue homogenate would be 100 mg/ml and was subsequently utilized for the estimation of lipid peroxidation product.

Alanine and aspartate aminotransferase activities of the supernatant fraction of cardiac and hepatic homogenates were measured by the method of Yatazidis (1960) and enzyme activity was expressed as mg pyruvic acid formed/mg protein/hr. For expressing the activities of alanine and aspartate aminotransferase of liver and heart tissues, the protein content of the supernatant fraction of homogenates were estimated by Biuret method (Wooton, 1974). Reduced glutathione content of hepatic and nephric tissues were estimated by the method described by Griffith (1980) and were expressed as μ mole of reduced glutathione/gm tissue. Lipid peroxidation was estimated for hepatic tissue homogenate according to the method described by Nair and Turner (1984) and was expressed as n mole of malonaldehyde/gm tissue.

Statistical analysis

Statistical analysis of data was carried out by using 'Students t test' (Snedecor and Cochran 1994).

Results and Discussion

Hematological study

Mean values (with SE) of hemoglobin content of blood at '0'day (pre herbicide) and those of 1, 2, 3, 4 and 5 days post administration were 9.07±0.07, 9.17±0.08, 9.22±0.06, 9.15±0.05, 9.02±0.02 and 9.12±0.04 gm% respectively. The differences of values observed between '0' day and all other days post-treatment were not statistically significant (Table 1). Mean values of erythrocyte sedimentation rate (ESR) during 1st hr at '0' day (pre herbicide) and 1, 2, 3, 4 and 5 days post administration were zero (Table 1). Likewise the total erythrocyte counts were 12.97±0.08, 12.97±0.10, 13.05±0.06, 13.05±0.06, 13.17±0.06 and 13.17±0.02, (x 10^6 / cumm) for '0' day (pre herbicide) and 1, 2, 3, 4 and 5 days post dosing respectively (Table 1). Mean values (with SE) of total leucocyte counts of goat at '0'and 1, 2, 3, 4 and 5 days post administration were 9.50±0.12, 9.32±0.23, 9.50±0.20, 9.40±0.14, 9.65±0.18, and 9.35±0.17 (x10^3/cumm) respectively. It appears that mean values of total erythrocyte and leucocyte count of control and experimental days were almost same indicating no effect of metamitron on total count of erythrocyte and leucocyte.

Biochemical study

Mean blood glucose level in mg/dl (with SE) of '0' day and 1, 2, 3, 4 and 5 days post administration were 47.69±0.83, 48.44±1.13, 48.04±1.27, 46.46±0.76, 47.00±1.83 and

Table 1. Effect of metamitron on haematological parameters of goats (3 male and 3 female) following administration of a single non-toxic oral dose @ 30 mg/kg.

Parameter	Control Day '0'	Day after administration 1	2	3	4	5
Haemoglobin (gm %)	9.07 ±0.07	9.17 ±0.08^{NS}	9.22 ±0.06^{NS}	9.15 ±0.05^{NS}	9.02 ±0.02^{NS}	9.12 ±0.04^{NS}
E. S. R. (mm 1st one hour)	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00
Total Erythrocyte count (10^6/cu mm)	12.97 ±0.08	12.97 ±0.10^{NS}	13.05 ±0.06^{NS}	13.05 ±0.06^{NS}	13.17 ±0.06^{NS}	13.17 ±0.02^{NS}
Total leucocyte count (10^3/cu mm)	9.50 ±0.12	9.32 ±0.23^{NS}	9.50 ±0.20^{NS}	9.40 ±0.14^{NS}	9.65 ±0.18^{NS}	9.35 ±0.17^{NS}

NS=non-significant compared to control value (day '0'). (Mean values of 6 replicates with SE)

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49.74±0.53, respectively. The differences of values between '0' day and all other treatment days separately were not statistically significant (Table 2). Mean serum pyruvic acid level (mg/ml) at '0' day and 1, 2, 3, 4 and 5 days after administration were 194.87±1.00, 192.24±2.17, 193.46±2.32, 192.18±1.45, 194.94±1.60 and 194.61±1.98 respectively. The pre-and post-dosing values were not statistically significant (Table 2). Mean serum lactic dehydrogenase activity (mmole of NADH oxidized/min/ml), at'0' day and 1, 2, 3, 4 and 5 days after administration were 0.067±0.001, 0.067±0.002, 0.069±0.003, 0.070±0.003, 0.072±0.002 and 0.070±0.001 respectively. The differences of values between '0' day and all other days separately were not statistically significant (Table 3). Mean cholinesterase activity (mg Ach hydrolysed/30 min/ml) at '0'day and 1, 2, 3, 4 and 5 days after pesticide administration were 236.20±2.03, 218.48±2.59, 224.17±1.94, 229.07±1.35, 236.31±2.00 and 238.47±0.79 respectively. The differences of values between '0' day and all other days separately were not statistically significant (Table 3). However, during evaluation of maximum non toxic oral dose of metamitron, goats exhibited cholinergic symptoms like bradycardia, increased respiratory rate, frequent urination and defecation at dose level higher than 30 mg/kg b.wt. In addition no significant changes were observed in blood glucose, serum pyruvic acid and serum lactic dehydrogenase activity. However, metamitron reduced the serum cholinesterase activity (Table 3), but the changes were not significant when compared with the control.

Tissue Biochemical Study

Mean values of alanine aminotransferase activities (mg of pyruvic acid formed/ mg of protein/hr) of liver in control and experimental groups of goats were 7.33±1.07 and 6.38±0.97, respectively and that of cardiac tissues were 258.91±2.82 and 262.93±3.72, respectively (Table 4). In both the tissues, the difference of enzyme activity between control and experimental group was not statistically significant. Mean values of aspartate aminotransferase activities (mg of pyruvic acid formed per mg of protein/hr) of liver

Table 2. Effect of metamitron on some blood biochemical parameters of goats (3 male and 3 female) following administration of a single non-toxic oral dose 30 mg/kg.

Parameter	Control	Day after administration				
	Day '0'	1	2	3	4	5
Blood glucose (mg/ dl)	47.69 ±0.83	48.44 ±1.13^{NS}	48.04 ±1.27^{NS}	46.46 ±0.76^{NS}	47.00 ±1.83^{NS}	49.74 ±0.53^{NS}
Serum pyruvic acid (μg/ ml)	194.87 ±1.00	192.24 ±2.17^{NS}	193.46 ±2.32^{NS}	192.18 ±1.45^{NS}	194.94 ±1.60^{NS}	194.61 ±1.98^{NS}

NS=non-significant compared to control value (day '0'). (Mean values of 6 replicates with SE)

Table 3. Effect of metamitron on some serum enzyme activities of goats (3 male and 3 female) following administration of a single non-toxic oral dose @ 30 mg/kg.

Parameter	Control	Day after administration				
	Day '0'	1	2	3	4	5
Serum lactic dehydrogenase activity (mmole of NADH oxidized/ min/ ml)	0.067 ±0.001	0.067^{NS} ±0.002	0.069^{NS} ±0.003	0.070^{NS} ±0.003	0.072^{NS} ±0.002	0.070^{NS} ±0.001
Serum cholinesterase activity (mg Ach hydrolysed/ 30 min /ml)	236.20 ±2.03	218.48^{NS} ±2.59	224.17^{NS} ±1.94	229.07^{NS} ±1.35	236.31^{NS} ±2.00	238.47^{NS} ±0.79

NS=non-significant compared to control value (day '0'). (Mean values of 6 replicates with SE)

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tissues of control and experimental groups were 45.20±2.45 and 42.07±2.30, respectively and that of cardiac tissues were 234.53±3.27 and 228.65±3.30, respectively (Table 4). Like alanine aminotransferase the difference of activities of aspartate aminotransferase between control and experimental groups was not statistically significant. Average values of reduced glutathione level of liver tissue of control and experimental groups were 4.51±0.12 and 4.35±0.12 m mole/gm tissue, respectively and that of kidney tissues were 2.15±0.08 and 2.08±0.05, respectively (Table 5). In both liver and kidney tissues, the difference of reduced glutathione level between the control and experimental groups was not statistically significant. Average values of lipid peroxidation level of liver tissue of control and experimental groups were 2.68±0.09 and 2.80±0.14 nmole of malonaldehyde/ gm tissues, respectively (Table 5). The difference of lipid peroxidation level between control and experimental groups was not statistically significant.

Histopathological examination

Section of liver tissue of experimental goat showed the evidence of fatty degeneration (Fig.1). Section of kidney showed the evidence of hyaline changes (Fig.2) in the uriniferous tubule. However sections of lung, thyroid gland, spleen and brain did not show evidence of any pathological changes. Except liver and kidney, sections of lung, heart, thyroid, spleen and brain tissue showed no alteration under histopathological examination. Hepatic tissues showed slight fatty changes and section of kidney showed hyaline changes.

Johnson *et al*. (1972) treated grass hay by spraying with atrazine and prometon (traizine herbicide) @ 22.5 and 44.9kg/hectare, respectively and allowed the cattle and sheep to consume it. They did not find any toxic effect on the health of the test animals. They also reported no significant changes in weekly measurement of serum aspartate

Table 4. Effect of metamitron on transaminase activities of liver and heart tissues of goats following administration of a single non-toxic oral dose @ 30 mg/kg.

Parameter	Liver		Heart	
	Control[a] Group	Experimental[b] Group	Control[a] Group	Experimental[b] Group
Alanine aminotransferase (μg pyruvic acid formed/ mg protein /hr)	7.33 ±1.07	6.38 ±0.97[NS]	258.91 ±2.82	262.93 ±3.72[NS]
Aspartate aminotransferase (μg pyruvic acid formed/ mg protein /hr)	45.20 ±2.45	42.07 ±2.30[NS]	234.53 ±3.27	228.65 ±3.30[NS]

a, Mean of 2 replicates with SE; b, Mean of 6 replicates with SE (experimental goats);
NS=non-significant compared to control group.

Table 5. Effect of metamitron on reduced glutathione and lipid peroxidation levels of tissues of goats following administration of a single non-toxic oral dose @ 30 mg/kg.

Parameter	Liver		Heart	
	Control[a] Group	Experimental[b] Group	Control[a] Group	Experimental[b] Group
Reduced glutathione (μmole /gm tissue)	4.51 ±0.12	4.35 ±0.12[NS]	2.15 ±0.08	2.08 ±0.05[NS]
Lipid peroxidation (n mole of malon aldehyde/ gm tissue)	2.68 ±0.09	2.80 ±0.14[NS]		

a, Mean of 2 replicates with SE; b, Mean of 6 replicates with SE (experimental goats);
NS=non-significant compared to control group.

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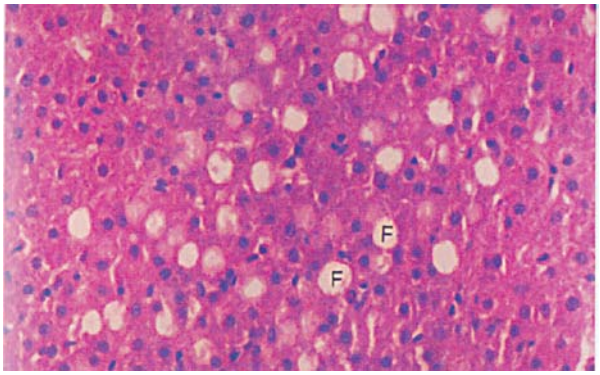


Fig.1. Section of liver of goat on '5' day showing fatty degeneration (F) following administration of a single non-toxic oral dose of metamitron @30 mg/kg body weight, H&E, 450X

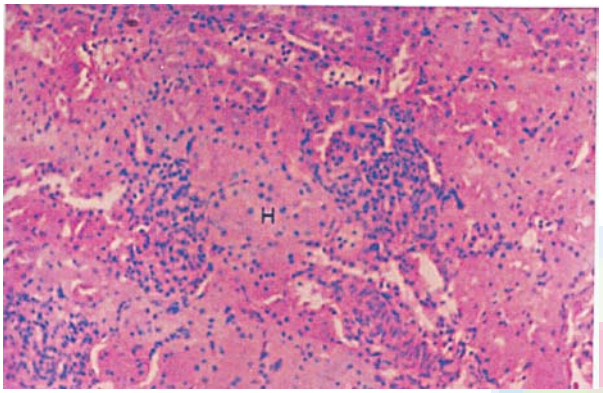


Fig. 2. Section of kidney of goat on '5' day showing hyaline changes (H) in the tubules following administration of a single non-toxic oral dose of metamitron @ 30 mg/kg body weight, H&E, 450 X.

transaminase, alanine transaminase, lactic dehydrogenase, total protein, hemoglobin, packed cell volume and total blood count of cattle and sheep. Similarly no cytotoxicity was reported in liver, kidney, pancreas, spleen, adrenal, thyroid, lung, stomach, small and large intestine and cardiac tissues.

From our results, it is apparent that metamitron at 30 mg/kg b.wt. *per os* cannot produce any toxic symptom and may thus be considered to be safe for use in agricultural practices as a herbicide.

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