Indian J Physiol Pharmacol 1993; 37(3) : 204-208

BEHAVIOURAL AND BIOCHEMICAL CHANGES PRODUCED BY REPEATED ORAL ADMINISTRATION OF THE INSECTICIDE ENDOSULFAN IN IMMATURE RATS

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(Received on September 29, 1992)

Abstract : In order to study the response of rats to repeated administration of the insecticide, endosulfan during the period of growth to maturity, food intake, body weight gain, Spontaneous Motor Activity (SMA) and Muscle Coordination (MC) were determined at regular intervals in male immature Wistar rats treated with a tolerated dose of (2 mg/kg/day) orally for 90 days. Twentyfour h after the termination of the treatment, organ weight and protein concentrations were determined. The convulsive action of picrotoxin (4 mg/kg, ip) was tested in another endosulfan-treated group. Food consumption and body weight gain decreased parallely. No changes occurred in the body tissues but for liver which was enlarged and its protein, glutamic oxaloacetic transminase and glutamic pyruvic transaminase concentrations increased. The MC was unaffected. A stimulation of SMA occurred several days (75-90) after commencing treatment and these animals responded greatly than control animals to the convulsive action of picrotoxin. These findings indicated that although endosulfan produced anorexia, there were no signs of undernourishment and motor impairment in these animals. Its toxic actions were confined chiefly to the liver and central nervous system.

Key words : body weight organ weight spontaneous motor activity motor coordination

INTRODUCTION

The cyclodiene organochlorine insecticide, endosulfan is used widely against crop pests. Health hazards due to its occupational exposure have been reported by workers (1). Considerable effort has, therefore, been made to evaluate its toxic potential in animals. The reported findings dealt chiefly with its short term (15-30 days) effects in adult animals (2, 3). The present study was aimed to test the response of immature rats to repeated administration of a tolerated dose of endosulfan during the period of growth to maturity. Food intake and body weight gain of these animals were measured at regular intervals. In order to determine whether endosulfan-induced changes in food intake and body growth were accompanied by motor impairment, Spontaneous Motor Activity (SMA) and Muscle Coordination (MC) were tested at the appropriate interval. The changes in body tissues were determined by measuring their weight and protein concentrations 24 h after the last administration. The response of these animals to the convulsive action of picrotoxin was tested in order to evaluate whether endosulfan was effective centrally too.

METHODS

Immature (2 weeks after weaning), Wistar strain male rats weighing 60-70 g were used. The test and control groups (n = 10-12) were randomly selected,

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housed in groups (2 or 5) in plastic cages and were maintained at room temperature (30-34°C). The animals had free access to a balanced diet (Gold Mohur, Calcutta) and drinking water. Three days after acclimatization, experimental procedures were started.

Endosulfan (95%) containing α and β isomers in a 2 : 1 ratio, obtained from EXCEL Industries, Bombay was used. The dose (2 mg/kg/day) that was tolerated for 90 days by immature male rats in our previous study (4) was employed. A suspension of endosulfan was made in distilled water with an equivalent amount of tragacanth powder, so as to administer 0.2 ml/100 g body weight. A suspension containing only tragacanth was administered to control animals. The test and control groups received the respective suspension by oral intubation every morning between 10.30 and 11.30 and then feed was supplied.

Food consumption and body weight were determined before starting treatment in group 1 which consisted of 12 animals. Two rats were caged together so that there were 6 food intake and 12 body weight values in test and control groups. Food intake was measured daily for 15 days and after recording body weight of these animals, endosulfan treatment was started. Then food consumed every 15 days of the treatment period was measured. The body weight was measured on every 15th day. The percent changes from predrug values were calculated for both test and control groups and the data were compared. Twenty four h after the last endosulfan or tragacanth administration, the animals were sacrificed and brain, heart, liver, spleen, kidneys and adrenals were dissected out and weighed. Protein concentrations were estimated in the serum, brain, liver heart and skeletal muscle (gastrocnemius) of 6 animals in this group as described previously (5). The activities of Glutamic Oxaloacetic Transaminase (GOT) and Glutamic Pyruvic Transminase (GPT) were determined in the serum and liver of the rest of the animals (n = 6) using a previously described method (6).

Motor activity was determined in group 2 (n = 10) on every 15th day of the treatment, using a pretested vibration sensor cage devised by the authors. It monitored the vibrations caused by the movements of the animal placed in the chamber. The apparatus consisted of an acrylic (black) cabinet (40 × 40 × 40 cm) with a transparent perforated lid. The floor was a laminate plate fitted with an array of vibration sensors made up of piezo-electric crystals. The vibrations picked up by the sensors were converted to electrical signals. These signals activated the counter which recorded adding one digit for every activity pulse received during the test period (10 min). While recording activity, the instrument was placed on a cushion pad in a quiet room in order to eliminate externally-induced vibrations.

Picrotoxin (4 mg/kg, i.p.)-induced convulsive responses were tested in group 2, 24 h after the last endosulfan administration. Myoclonuc latency (the time between picrotoxin injection and the appearance of the first clonic movement which was invariably head clonus), the number of animals exhibiting tonus and mortality during 1 h test period were recorded. The intensity of myoclonic convulsions was scored using a 0-4 scale described previously (7).

A rota-rod apparatus (8) was used to assess muscle coordination of group 3 (n = 10) on every 15th day of the treatment. Prior to endosulfan administration, the animals were placed on the moving rod facing into the direction of the rotation to get accustomed and those which stayed for 1 min were chosen for this study. The animals were given 1 min exposure prior to each test. A test period of 2 min was then allowed to each animal and the endurance time was determined by measuring the elapsed time between the rat was placed on the rod and the moment it fell down.

Student's t-test was used to analyze time data. Scorings were analyzed using Mann-Whitney rank order test. Chi-square method was used to analyze the number of animals exhibiting tonus and mortality rate.

RESULTS

The test animals were consuming lesser food than the control group during the treatment period and the body weight gain of these animals decreased correspondingly (Figure 1 A, B). The SMA of control animals decreased gradually after repeated exposures to the chamber due to habituation (Figure 1 C). The test animals also exhibited habituation in the first 3

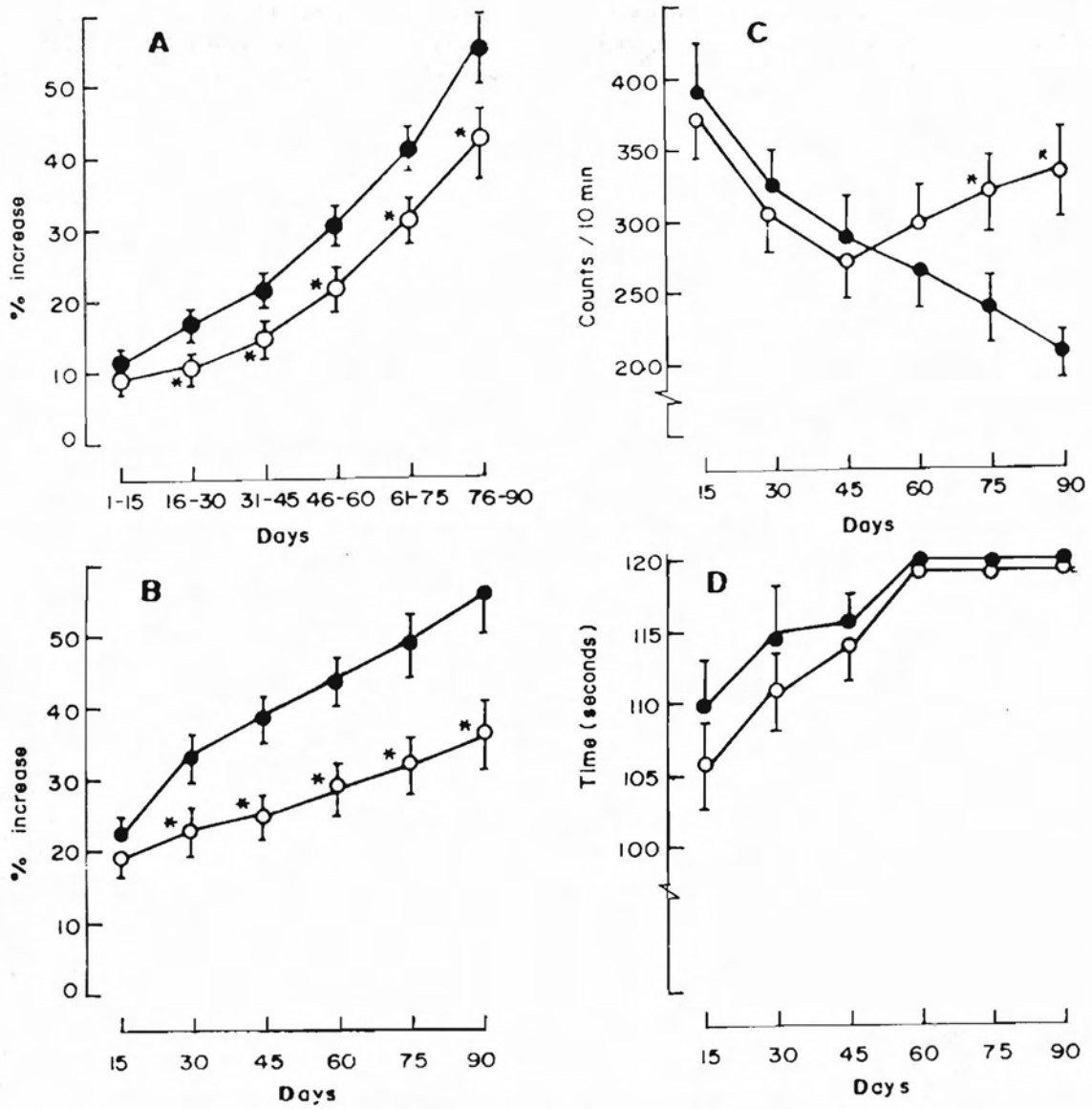

Fig. 1 : Food intake (A, n = 6), body weight gain (B, n = 12), SMA (C, n = 10) and MC (D, n = 10) of endosulfan (o) and tragacanth (•)-treated rats. Animals received endosulfan (2 mg/kg/day) orally for 90 days. Each point represents mean±SEM of number tested.

*P < 0.05 (t-test).

sessions (15th, 30th and 45th day), but from the 75 to 90 days of the treatment, the activity of these animals increased significantly. There was no change in the MC of these animals (Figure 1 D). The general behaviour was not interrupted by tremors or convulsions.

Weight and protein concentrations of tissues, except that of liver, were unaffected (Table I). Endosulfan increased liver weight and its protein content. The concentrations of both GOT and GPT were increased in the liver. An increased GPT was found in the serum too.

TABLE I: Tissue weight and biochemical parameters in endosulfan (2 mg/kg for 90 days) and tragacanth (control)-treated rats. Values are mean±SEM of the number tested.

	Serum	*Liver*	*Brain*	*Heart*	*Kidneys*	*Adrenals*	*Spleen*	*Skeletal muscle*
Weight (g/100 g)								
tragacanth	—	4.42	1.49	0.38	0.94	0.032	0.32	—
(n = 12)		±-.12	±0.16	±0.01	±0.06	±0.002	±0.02	
Endosulfan	—	4.85*	1.51	0.41	0.98	0.038	0.32	—
(n =12)		±0.16	±0.07	±0.03	±0.03	±0.004	±0.03	
Protein (mg/g in g/100 ml tissues)								
Tragacanth	4.81	47.20	8.45	22.86	—	—	—	26.2
(n = 6)	±0.19	±0.63	±0.30	±3.04				±2.85
Endosulfan	5.29	54.06	8.62	24.40	—	—	—	30.46
(n = 6)	±0.32	±2.18	±0.20	±1.10				±2.52
GOT (µmol) pyruvate liberated)	(ml/min)	(g/min)	—	—	—	—	—	—
Tragacanth	18.95	0.60	—	—	—	—	—	—
(n = 6)	±212	±0.39						
Endosulfan	14.22	1.68*	—	—	—	—	—	—
(n = 6)	±2.90	±0.01						
GPT (µmol pyruvate liberated)								
Tragacanth	17.59	1.72	—	—	—	—	—	—
(n = 6)	±4.90	±0.09						
Endosulfan	28.68*	3.08*						
(n = 6)	±3.40	±0.11						

*P < 0.05 (t-test).

The endosulfan-treated animals responded greatly than control animals to all convulsive elements of picrotoxin (Table II). Myoclonus latency was shortened. Tonus and death rate were greater in this group when compared with control group. These animals scored a greater clonic convulsions than the control animals 1-10 min after picrotoxin injection.

TABLE II: Tissue weight and biochemical parameters in endosulfan (2 mg/kg for 90 days) and tragacanth (control)-treated rats. Values are mean±SEM of the number tested.

	Myo-clonus Latency (min) ±SEM	*Number showing*		*Myoclonus scorings±SEM after (min) picrotoxin*					
		Tonus	*Death*	*1-10*	*11-20*	*21-30*	*31-40*	*41-50*	*51-60*
Tragacanth	7.47 ±0.31	3	2	1.82 ±0.09	2.48 ±0.12	2.74 ±0.13	2.28 ±0.12	1.82 ±0.06	0.62 ±0.04
Endosulfan	6.38* ±0.47	9**	9**	2.75# ±0.12	2.55 ±0.14	2.82 ±0.15	2.42 ±0.11	1.94 ±0.08	0.84 ±0.02

*P < 0.05 (test); **P < 0.05 (Chi-squre); #P < 0.05 (Mann-Whitney rank order test)

DISCUSSION

The data presented here clearly indicated that endosulfan-induced inhibitation of body weight gain was resultant of its anorexic action. Oral administration of endosulfan was found to produce functional changes in the gastrointestinal mucosa (9). This action was accounted to the anorexic action of endosulfan. Although body weight gain decreased gradually, the tissue weight and protein concentration data indicated that these animals were not undernourished. The results of SMA and MC tests showed that the motor system of these animals was not affected adversely. However, several days after commencing treatment, SMA increased significantly.

In order to dertermine whether endosulfan-induced hypermotor activity was a centrally mediated action, these animals were injected with a convulsive dose of picrotoxin which was a well documented antagonist of the inhibitory synaptic action of γ-aminobutyric acid (GABA) (10). The results indicated that endosulfan increased the convulsive potency of picrotoxin suggesting that, like picrotoxin, endosulfan stimulated central nervous system. Endosulfan appeared to mediate this action by inhibiting GABA mechanism, since the site of its action was found to be indistinguishable from that of picrotoxin (11, 12). However, endosulfan failed to produce convulsions in this study. This finding indicated that the dose employed here might have blocked GABA activity not to the extent of inducing convulsions but sufficiently to facilitate the action of picrotoxin.

Endosulfan increased selectively liver weight and its protein content. Its hepatic action was further studied by conducting biochemical investigations pertaining to liver toxicity and the results provided evidence for the hepatotoxic action of endosulfan by showing an increased GPT and GOT levels in the liver.

Thus, the findings of this study provided evidence that immature rats responded readily to the anorexic action of endosulfan and that a significant functional changes were likely to occur in the liver and central nervous system too.

ACKNOWLEDGEMENTS

Financial assistanc by ICMR, New Delhi is greatefully acknowledged.

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