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Original Research Article

Reproductive toxicity in thiram induced protein deficient male Wistar (Albino) rat

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ABSTRACT
Thiram is a fungicide which has been extensively used in agriculture to increase food production and improve the quality of agricultural crops. It is used to prevent crop damage in the field and to protect harvested crops from deterioration in godowns. Thiram undergoes biodegradation which plays an important role in its toxicity. It has also been reported that reproductive toxicity of thiram in laboratory induced diabetic rats. The present study revealed that thiram causes reproductive toxicity in normal as well as laboratory induced protein deficient male Wistar (Albino) rats.

1. Introduction

The widespread use of pesticides has resulted in the occurrence of residues in crops, meat, milk, eggs and daily products. Contamination of livestock may be the result of direct contact with these when used as the ectoparasites controlling agents. Generally, the pesticides get accumulated in the body fat. These get mobilized and redistributed due to weight loss during starvation or illness. With the circulating blood these pesticides reach the target organs like brain, liver, kidney and reproductive organs. Thiram undergoes metabolism to produce CS_2 which plays an important role in its toxicity [1].

Various types of pesticides are there. One of the important groups of pesticides is of dithiocarbamates. The worldwide consumption of dithiocarbamates is between 25,000 and metric tons [2]. Dithiocarbamates are used as fungicides being effective against a broad spectrum of fungi and plant diseases caused by fungi. Dithiocarbamates have also been used to control various dermatophytes. These are also extensively used as an agricultural fungicide, insecticide as well as repellent for rodents and certain large animals that caused damage to field crops. The biodegradation of thiram probably plays an important role in its toxicity.

Nitsche et al. [3] reported that the fungicides ferbam, ziram and thiram are not stable under environmental conditions and they can be mobilized to toxic products such as CS_2 and hydrogen sulfide. Carbon disulfide was detected in the expired air after oral administration of thiram to man. One unique effect reported for the organophosphorus compound is premature ovarian failure [4]. During the first few days of life, male steroid enzyme levels are "imprinted" in the liver of male rats by the action of other androgens and chemicals [5]. Imprinting is the process by which neonatal harmone exposure permanently determines or alters the normal pattern of sex-related hepatic function.

Oral doses of endosulfan at a concentration of 5 mg Kg^{-1} and above on day 6 to 14 of gestation increased the mortality of rat and increased the rates of resorption and skeletal abnormalities in their foetus [6]. Oral doses of 10 mg Kg^{-1} Day^{-1} caused degeneration of seminiferus tubules and a significant increase in the weight of testes [7]. In a study where rats were treated with lindane at a dose of 60 mg Kg^{-1} increase in spontaneous abortion in a group of pregnant mice was recorded. Thiram treatment has also resulted in hematological parameter changes in diabetic male albino rats [8, 9].

Protein plays an important role in the development and physiology of the genital organs. Protein deficiency induced statistically significant reduction in the testicular weight, atrophy of seminiferous tubule was consistently observed. Testicular atrophy with diminished spermatogenesis is a feature of prolonged protein deficiency. Low dietary protein levels during the growth period delays sexual maturity and reduces weight of body, ovaries and oviducts.

2. Materials and Method

In the present study the following investigations are undertaken:

Thiram induced reproductive toxicological parameters like lactate dehydrogenase (LDH), succinate dehydrogenase (SDH), pyruvate dehydrogenase (PDH) and adenosine triphosphatase (ATPase) in protein deficient rats.

Thiram toxicity studies with rats

Male Albino rats were collected from experimental animal facility of All India Institute of Medical Sciences, New Delhi. The rats normally weighed 150-200 gms at the start of the experiment and were maintained in plastic cages. Before the start of experiment, they were provided powder-diet (prepared by HAFED plant, Rohtak). All the rats were free accessed to water and were acclimatized to laboratory conditions for at least one week before the onset of any experiment. The normal temperature for maintenance of rats was 20±2°C.

Induction of Protein Deficiency in rats

The protein deficient diet was prepared by HAFAD cattle feed plant, Rohtak. The condition of protein deficiency was induced in rats weighing 60-100 gm by feeding a low protein diet (12% protein) up to two months. Induction of the condition was tested by changes in body weight, blood protein concentration, blood glucose concentration and blood urea. The composition of the diet is given in the Table 1.

Table 1: Diet Composition per kg diet

Ingredients	Protein level % (g/kg)	
	12% (Low Protein Diet)	30% (Normal Diet)
Soybean meal, 48% protein	92	418
Ground Corn	780	478
Glucose monohydrate	25	08
Alfalfa meal	50	17
Dicalcium phosphate	33	33
Limestone	09	09
Vitamin mix a	05	05
Mineral mix. B	01	01
Selenium mix. C	01	01
Iodized salt	04	04
Soybean oil	-	27

Treatment of rats with thiram

In the present study two different routes of thiram administration were selected. This included intraperitoneal (IP) injection of a single as well as repeated doses of thiram. The dose level for intraperitoneal treatment was 60 mg kg^{-1} body weight of rats. Thiram suspension was made in refined ground nut oil and 0.5 ml of this suspension was injected into each rat. The result was taken after 5 hours, 24 hours and 7 days of treatment. The rats injected with the vehicle alone in a similar way served as their respective controls. In the repeated dose experiment, rats of each group were given a daily Ip dose of thiram (60 mg kg^{-1} body weight) up to 7 days. The vehicle alone was injected in some rats which served as controls.

3. Results

Impact of thiram treatment on testicular LDH activity

The level in normal rats fed ad libitum was 25.3 µg formazan min^{-1} mg^{-1} protein and in protein deficient rats, the respective value was 25.31 µg formazan min^{-1} mg^{-1} protein.

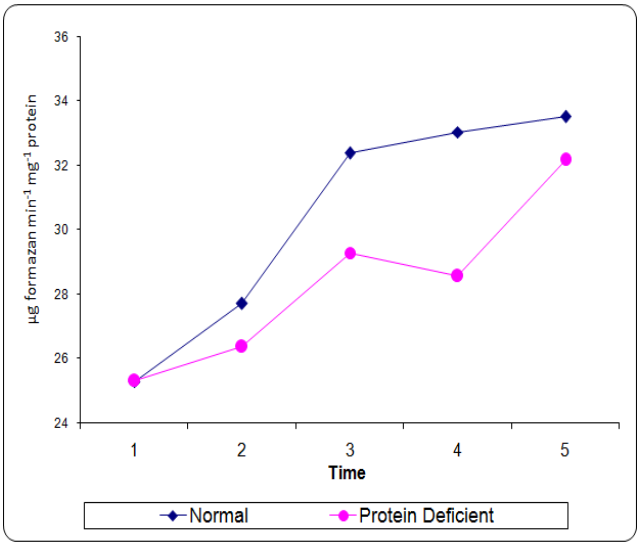

Figure 1: Changes in testis LDH activity following intraperitoneal exposure of thiram to rats (1 to 5 denote different treatments) (1) Ad libitum control; Intraperitoneal (Ip) treatments (60 mg kg^{-1} bw); (2) After 5 hrs. of a single Ip dose; (3) After 24 hrs. of single Ip dose; (4) After 7 days of a single Ip dose; (5) After 7 days of repeated Ip dose.

The exposure to single Ip dose of thiram caused increase of LDH activity. The LDH activity presented elevation of 9.5% after 5 hrs, which became 28.1% after 24 hrs and 30.7% after 7 days in normal rats. The corresponding values in protein deficient rats were 4.2%, 15.8% and 12.9% after 5 hours, 24 hours and 7 days respectively. The repeated exposure to thiram for 7 days resulted in 32.5% and 27.2% elevation in normal and in protein deficient rats respectively. These values show elevation of LDH activity with time (Figure 1).

Impact of thiram treatment on testicular ATPase activity

In normal rats the level of ATPase was 3.64 while in protein deficient rats the value was 3.641 µ moles of Pi min^{-1} mg^{-1} protein.

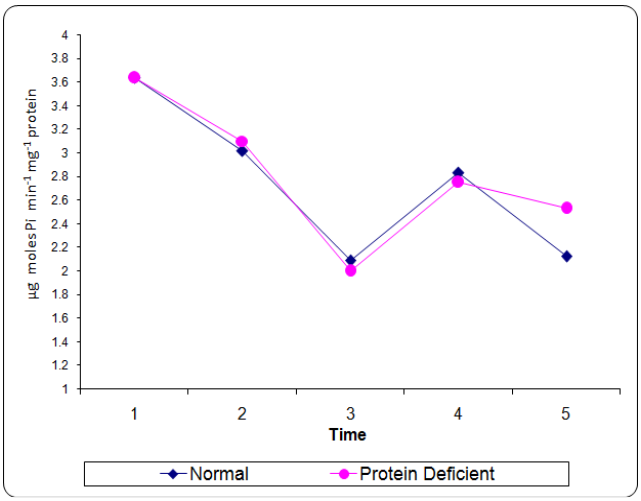

Figure 2: Changes in testis ATPase activity following intraperitoneal exposure of thiram to rats (1 to 5 denote different treatments as described in Figure 1)

The reduction was 17.3% after 5 hours, 42.5% and 22.1% after 24 hours and 7 days. The values were 14.7%, 44.9%, 24.3% in protein deficient rats after 5 hours, 24 hours and 7 days, respectively. The repeated exposure to thiram for 7 days caused a decrease of 41.6% and 43.6% in normal and protein deficient rats, respectively. The exposure to thiram caused a reeducation of ATPase activity (Figure 2).

Impact of thiram treatment on testicular SDH activity level

The levels were 5.535 and 5.539 µ formazan $min^{-1}mg^{-1}$ protein in normal and protein deficient rats, respectively.

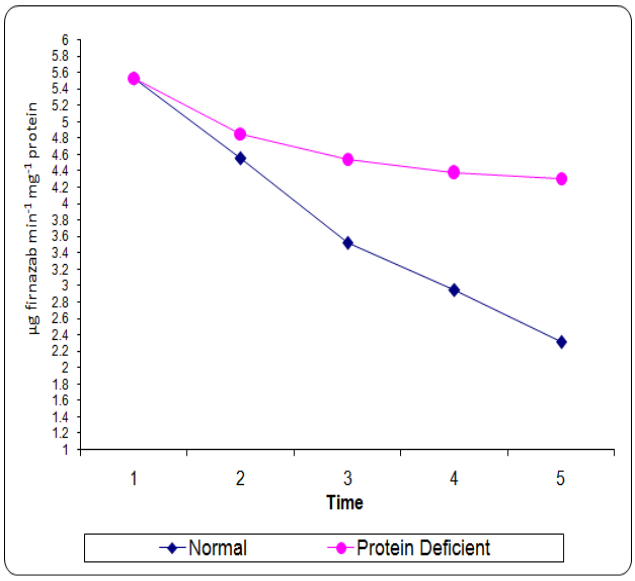

Figure 3: Changes in testis SDH activity following intraperitoneal exposure of thiram to rats (1 to 5 denote different treatments as described in Figure 1).

The exposure to single Ip dose of thiram caused inhibition of SDH activity, After 5 hours, the percent reduction was 18.3 and 36.3, 46.7 after 5 hours, 24 hours and 7 days in normal rats and the percent reduction was 12.2, 17.8, 20.8 after 5 hours, 24 hours and 7 days respectively in protein deficient rats. The repeated exposure to thiram caused 58.1 and 22.8 percent inhibition of testicular SDH activity in normal and protein deficient rats, respectively. The exposure to thiram caused a reduction of SDH activity (Figure 3).

Impact of thiram treatment on testicular PDH activity level

The control values for normal and protein deficient rats were 4.132and 4.133µg of formazan formed min^{-1} mg $^{-1}$ protein respectively.

The exposure to a single Ip dose of thriam caused inhibition of PDH activity. The inhibition after 5 hours was 7.6% and 12.2% and 11.9% after 24 hours and 7 days, respectively in normal rats and 9.12%, 15.6% and 6.6% in protein deficient rats after 5 hrs, 24 hrs and 7 days respectively. The repeated exposure to thiram for 7 days causes an inhibition of 14.5% and 19.2% in normal and protein deficient rats respectively. The exposure to thiram caused a reduction of PDH activity (Figure 4).

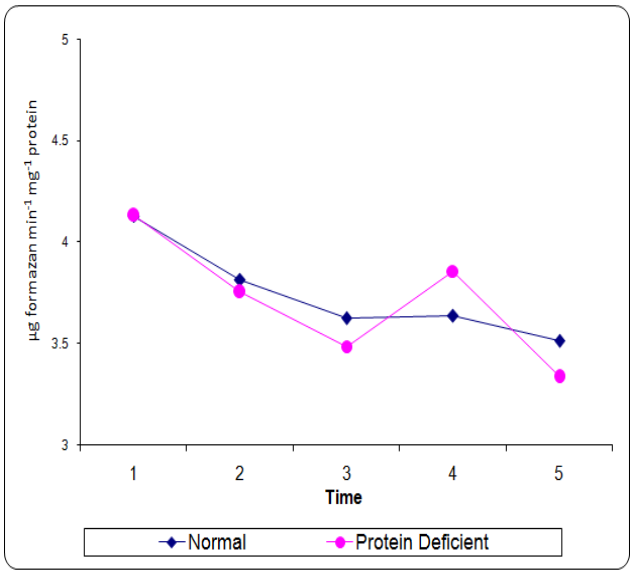

Figure 4: Changes in testis PDH activity following intraperitoneal exposure of thiram to rats (1 to 5 denotes different treatments as described in Figure 1).

4. Discussion

Thiram caused adverse effects on the testes which was again dependent upon the pathological status. The lysosomal enzymes are very important in normal spermatogenesis and are used to interpret testicular growth and development. A high concentration of LDH is present in the testis of newborn rats and its activity declines with the development of testis [10]. The thiram treatment resulted in increase of LDH activity. Increase in LDH after thiram exposure suggests a depletion of the germ cells in seminiferous tubules.

In buffaloes supermatozoa, the SDH has been reported to be bounded strongly to the mid-piece [11]. The level of SDH has been positively correlated to the initial motility, live sperm percentage and sperm concentration [12]. The inhibition of testicular SDH suggests the retarded metabolic status of the testes following exposure to thiram. The inhibition observed in SDH activity following exposure to thiram in the present study is the indicative of non-motile and abnormal spermatozoa along with a decrease in their count.

The ATPase provides energy for sperm motility [13]. ATPase is also involved in permeability and transport process of the spermatioza [14]. The decrease in ATPase is due to of testicular damage. The inhibition in ATPase, recorded in the present study is, thus, indicative of reduced motility and increased permeability of spermatozoa. The thiram treatment resulted in decrease of PDH activity which indicates the reduced flux of pyruvate into TCA cycle.

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