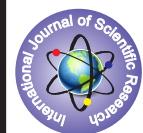


PROTECTIVE EFFECT OF TAURINE AGAINST PROPANIL- INDUCED NEPHROTOXICITY IN MICE



Zoology

KEYWORDS: Propanil, taurine, mice, kidney, antioxidants, free radical.

Arti Chauhan

Department of zoology, Holkar Science College, Devi Ahilya University, Indore-452001, Madhya Pradesh, India.

Usha Gaur

Department of zoology, Holkar Science College, Devi Ahilya University, Indore-452001, Madhya Pradesh, India.

ABSTRACT

Objective: The aim of this study was to investigate the protective effects of taurine on propanil-induced alterations in biochemical indices in serum and kidney of swiss albino mice. **Methods:** In an experimental study 24 albino mice were distributed in six equal groups of six each as follows: Control group, 100mg propanil/kg, 100mg taurine/kg, 200mg taurine/kg, propanil (100 mg/kg) + taurine (100 mg/kg), propanil (100mg/kg) + taurine 200mg/kg. Treatment was via oral route and was fed once daily for 90 days. The levels of serum aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) were measured to assess the nephrotoxicity and its amelioration by taurine. **Results:** compared to both the control groups, propanil leads to increase AST, ALT and ALP, decreased protein level and high density lipoprotein cholesterol (HDL). Treatment by taurine caused a significant reduction in serum levels of AST, ALT and ALP. Administration of propanil to mice significantly ($p < 0.05$) increased lipid peroxidation levels. Glutathione (GSH), Glutathione peroxidase (GPx), Catalase (CAT) Superoxide dismutase (SOD) decreased in the kidney tissue. The results indicate a protective effect for taurine against propanil induced hepatotoxicity. **Conclusion:** the present study suggests that taurine could be an important dietary component based on its ability to attenuate propanil induced hepatotoxicity.

INTRODUCTION

The environment is a topic of concern to entire humanity. With the gradual onset of scientific thoughts man realized the role of pest in damaging his crops. This leads to discovery of suitable biocidal agricultural chemicals collectively known as pesticides, which include insecticide, rodenticide, herbicide etc. Propanil (3,4 dichloropropionanilide) is an acetanilide which is used to control broadleaf weeds and most extensively used as post emergent herbicides for rice wheat and potato production worldwide. (Moore MT, Farris JL, 1997).

World Health Organization (WHO) has considered Propanil slightly hazardous in terms of human risk. (McMillan DC, Freeman JP, 1990). According to one study propanil can alter histopathology of liver and kidney by changing dose of it in tissue of exposed mice (Bluhm RE, Adidoyin A, Mc carver DG 1999).

Chloracne observed in industrial workers during propanil manufacturing which was primarily due to other chemical contaminants present in it (Morse and Baker, 1979; Kimbrough, 1980).

When given intraperitoneally to mice, it hinders the function of nervous system and many reflex activities (Singleton and Murphy, 1973). Male rats were fed 1600 mg/kg propanil over a period of 2-years and mortality was observed in them (Ambrose et al., 1972). Hemolytic anemia and methemoglobinemia, is found primarily in self poisoned humans who ultimately led to their death (Kimbrough, 1980; De Silva and Bodinayake, 1997). In cases of human propanil poisoning nephrotoxicity and hepatotoxicity have been reported (Wijekoon et al., 1974; De Silva and Bodinayake, 1997) and animal studies (Stevens and Sumner, 1991; Santillo et al., 1995).

Antioxidants play important role in regulation of physiological and pathological processes antioxidants protect the cells and tissues against deleterious effects of reactive oxygen species and other free radicals. Taurine (2-aminoethanesulphonic acid) is actually an amino acid which is present in many animal tissues like kidney, liver and brain majorly but in very minute quantity. (Wright et al; 1986). Taurine acts as antioxidant because it can stabilize the biomembranes and scavenge reactive oxygen species. (Wright et al; 1985) To the best of our knowledge, there are no studies concerning the nephroprotective effect of taurine against propanil intoxication. Therefore, the present study was carried out to investigate (a) the adverse effect of subchronic propanil intoxication on the kidney

based on serum biochemical parameters, oxidative stress. (b) the probable ameliorating effect of taurine against propanil intoxication in mice.

2.METHODOLOGY

2.1 chemicals

Herbicide propanil PESTANAL #, analytical standard was purchased from Sigma-Aldrich Co.Ltd.St. Louis, USA and taurine was purchased from LOBA chemie. All other chemicals were of technical grade and purchased from Loba Chemie, Mumbai India.

2.2 animals

Colony bred Swiss albino mice weighing 18-20gm obtained from Institute of Animal health and Veterinary and Biological Products, Rasalpur, Mhow, Madhya Pradesh were used for this study. The animals were maintained at $22 \pm 3^\circ\text{C}$ with 50-70% relative humidity and 12:12 hrs of light and dark cycles and were kept in well ventilated cages. The animals were fed with calculated amount of laboratory pellet diet procured from government agricultural college, Indore, India, and water *ad libitum*. Animals were maintained as per the guidelines laid down by (CPCSEA).

2.3 Experimental protocol

Mice were divided into six groups of six each and were allowed free access to feed and water for 20 days before the commencement of the experiment. As both the drugs were given in pellet diet, so mice were closely studied for a period of 20 days to evaluate the consumption of food according to already studied equation. Daily dose was calculated on the basis of following equation: $DD = (SD \times BW) / F1$ (Research Diet)

DD=diet dose (mg compd/kg Diet), SD= Single Daily Dose (mg compd /kg BW/day) BW= Body Weight(gm BW/animal), F1= Daily Food Intake(gm Diet/day) and the group were as follows

- Group 1- Control animals (no treatment)
- Group 2- Propanil treatment)
- Group 3- Taurine treatment(100mg/kg BW)
- Group 4-Taurine treatment (200mg/kg/body weight a double dose treatment)
- Group 5- Propanil (100mg/kgBW) + Taurine(100mg/kg BW)
- Group 6-propanil (100mg/kg BW) + Taurine (200mg/kg BW)

The dose of nephroprotectant drug was increased as it was observed that the protection was improved by increasing the dose to twice. All the above groups except group 1 were treated daily for the period of

90 days. After the administration of the last dose animals were given rest overnight and were killed next day by exposing them to mild ether anesthesia. Blood from each animal was collected and serum was isolated for the estimation of different biochemical parameters.

2.4 Biochemical analysis in serum

For estimation of AST and ALT activities in serum samples, commercially available enzymatic kits, based on the reaction of 2, 4 dinitro phenyl hydrazine with oxaloacetate to form brown colored complex in alkaline medium were used (Reitman and Frankel, 1957). ALP, Triglyceride and cholesterol were measured by spectrophotometry in serum using randox commercial kits.

Biochemical estimation of LPO and GSH content

kidney lipid peroxidation was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS) with malondialdehyde, one of the major product of lipid peroxidation. Amount of MDA was measured by taking absorbance at 532nm, using shimadzu UV-1700 spectrophotometer according to method of Utley et. Al.(1967). Tissue reduced glutathione content was measure by taking the absorbance of the product at 412 nm following the method of Ellman(1959).

Estimation of CAT and SOD activities and protein content

SOD was estimated as per method of Marklund and Marklund(1974), which involves generation of superoxide by pyrogallol autoxidation and inhibition of superoxide- dependent reduction of tetrazolium dye MTT(3-(4-5 dimethyl thiazol 2- xl) 2,5 diphenyl tetrazolium bromide) to its formazan, measured at 570 nm. The reaction was terminated by addition of dimethyl sulfoxide (DMSO), which help to solublize the formazan formed. Catalase activity was estimated by considering the method of L.Groth (1991), based on thee estimation of amount of hydrogen peroxide decomposed. Tissue protein estimation was done by the routine method of Lowery et al. (1951) using bovine serum albumin as standard.

STATISTICAL ANALYSIS

All values have been expressed as mean \pm standard deviation (SD) of six observations. Data were analyzed using one way analysis of variance (ANOVA) followed by Dunnett's posttest for analysis of biochemical data. Statistical analysis was performed using SPSS statistical version 8 software package. Values were considered statistically significant at $P < 0.05$.

RESULT

To study the effects of Propanil on mouse kidney, we treated mice for 90 days and euthanized them after administration of the final dose. All the animals were alive at the end of experiment but there was not any significant clinical sign of disease during the experimental period and at the end of the assay.

Animal's water and food consumption.

There was statistically significant difference in water consumption during the experimental period, although throughout the experiment, Food and water intake was lower in the propanil-treated group, however, only at the 9th week this became statistically evident ($p=0.045$) compared to the control group. The mean weight of the animals' kidney was taken.

Activities of AST, ALT and ALP

On evaluating plasma marker indices in mice, propanil group showed significant renal toxicity which were ameliorated by taurine. Propanil intoxication caused increase in serum ALT (U/L), AST (U/L) and ALP(U/L) values in comparison to control group ($P < 0.05$). However, taurine reduced ALT and AST activities.

Estimation of lipid peroxidation (MDA), SOD, GSH, CATALASE

Lipid peroxidation is complex process which disrupt the structure and function of membranes, because of free radical as these radicals attacks membrane lipids. Moreover propanil also inhibits free radical inhibiting enzymes such as catalase, glutathione peroxidase,

and superoxide dismutase.

A significant increase in GSH level was observed with taurine treatment. Propanil resulted in an increase in level of MDA ($P < 0.05$) in kidney tissue homogenate of mice (table 2) however taurine brought significant reduction in the MDA. The GSH concentration was significantly reduced with administration of Propanil as compared to normal control group. CAT and SOD was greatly reduced but on dosing taurine there was significant increase in activities of SOD, CAT. However, at higher double dose taurine did not show any significant efficacy in normalizing the value of these antioxidant enzymes.

DISCUSSION

Propanil is a crystalline solid, and it is applied after rice has grown at 3.5- 5.6 kg ha⁻¹ to control unwanted grass weeds, as farmers are very careless at application protocol, like at wearing protective clothes when they are spraying in field and they wash their spray pumps in any near water body of daily use for animals including humans,(self observation. Propanil is very efficient herbicide comes in acetanilide group; it is sold in at least 20 different brand names, mostly as 36% solutions. The lethal dose in man is as little as 10ml of undiluted compound and on taking more than 200 ml of propanil shows severe poisoning. (Ohashi N, Ishizawa J et al., 1996). Even though the world health organization identified propanil as slightly toxic in terms of human risk (World Health Organization, 2004), Propanil caused multi organ damage by increasing LPO of tissues (Santillo M, Rippa C et al., 1995). In this study protective effect of taurine is studied. Biochemical and hematological profile are two such an important parameters which gives crucial information about the internal environment of the organism. Taurine is an antioxidant (Trachtman H, Futterweit S 1994) and the most important role of taurine as antioxidant is probably systemic and even local scavenging of reactive oxygen species. Taurine chloramines which is formed after reaction with hypochlorous acid act as an oxidant reservoir, (Ogino T, Than TA 2009). Increase in alkaline phosphatase activity can be due to cellular necrosis or increase in permeability of plasma membrane, which indicate that animal is under stress. Taurine reduced the enzyme ALT, ALP, AST concentration. Oxidative injury, is an outcome of imbalance between antioxidant defense systems and oxidative forces. Increases in MDA level causes oxidative damage to cell membrane and present study suggests that increased lipid peroxidation contribute to propanil toxicity as it may form covalent linkage between protein and carbonyl group of MDA. The susceptibility of specific tissue could be attributed to ability of herbicide to cause tissue damage in case of propanil (Sefi M, Bouaziz H, Soudani N et al., 1994); and as in our study MDA level increased to a significant extent in mice kidney and liver.. GSH is the most important biomolecule against chemically induced toxicity and GSH participates in removing reactive intermediates formed, by reducing hydroperoxides in presence of GSH dependent enzymes. This functions as a free radical scavenger, a generator of α -tocopherol and also an important role in maintaining protein sulfhydryl group. GSH level depleted with increase in oxidative stress which goes with our findings, and treatment with Taurine increased the level of GSH. Catalase, an oxidant enzyme that protect cell from oxidative stress of hydrogen peroxide, is actually induced on the generation of free radical in the cell. This antioxidation plays important role in protection against deleterious effect of lipid peroxidation. Catalases protect superoxide dismutase against inactivation against hydrogen peroxide and in a reciprocal manner SOD protect CAT from inhibition by superoxide radicals. In present study Taurine enhanced the enzyme activity.

CONCLUSION

Present study revealed that taurine protected against nephrotoxicity induced by propanil herbicide in mice. Taurine reduced the level of free radicals and antioxidant enzymes and GSH content increased, but the double dose treatment did not produce any significant results in our study. Serum biochemical enzymes AST, ALT, ALP enzymes remarkably decreased and lipid per oxidation marker MDA

alon
g with GSH and CAT suggest that taurine is a strong antioxidant. Hence dietary taurine play a pivotal role in reducing propanil toxicity in propanil intoxicated mice which might be as a result of multiple mechanisms. It is thought that taurine has beneficial effect on renal toxicity. So from this study it can be concluded that taurine is capable of reducing oxidative stress as it has ability to inhibit per oxidation of lipids and protect cell against reactive oxygen species, hence dietary taurine play very crucial role in reducing propanil toxicity.

TABLES

Table1. Effect of Propanil (100 mg/kg/day), Taurine (100 mg/kg/day), Taurine (200mg/kg/day) administration for 90 days on the activities of serum AST, ALT (U/L), ALP(U/L), TG(mg/dl), Total Protein in mice.

Paramet ers	Control	Propanil	Tau 1	Tau 2	Propanil +Tau 1	Propanil+ Tau2
AST U/L	17.48±0.11	38.4±1.02 [#]	19.07±0.04	24.95±0.13	24.3±2.02 [*]	21.6±1.24 [*]
ALT U/L	43.03±0.42	114.4±2.36 [#]	44.21±0.43	53.87±2.01	52.4±1.32 [*]	57.47±2.13 [*]
ALP U/L	22.79±1.01	34.9±1.04 [#]	23.03±0.17	19.6±1.02	24.4±1.19 [*]	23.67±1.32 [*]
TG(mg/dl)	54.6±1.13	44.3±2.89 [#]	49.05±1.32	48.63±2.01	49.12±1.34	52.7±1.16
Total Protein	8.22±1.13	9.48±2.01	7.47±2.03	7.32±0.81	6.76±0.08	5.38±1.58

Values are mean ±SD of six mice in each group. Significance at P < 0.05
* compared against propanil
compared against control

Table 2: Effect of Propanil (100 mg/kg/day), Taurine (100 mg/kg/day), Taurine (200mg/kg/day) administration for 90 days on the activities of kidney GSH content (GSH mol/min/g protein), SOD (U/g protein) and CAT (μM H2O2 decomposed/min/ mg protein) and on control in mice.

Paramet er	Control	Propanil	Tau 1	Tau 2	Propanil +Tau 1	Propani l +Tau2
GSH	6.50±0.31	3.07±0.13 [#]	5.02±0.7 [*]	6.11±1.4 [*]	4.80±0.7 [*]	5.13±0.7 [*]
SOD	6.56±1.21	3.19±1.21 [#]	7.93±1.47 [*]	8.28±1.23 [*]	5.08±0.23 [*]	5.24±0.81 [*]
CAT	47.74±3.01	29.62±2.48 [#]	59.06±3.02 [*]	57.36±1.31 [*]	38.39±2.3 [#]	42.16±1.29 [*]

Values are mean ±SD of six mice in each group. Significance at P < 0.05
* compared against propanil
compared against control

Conclusion

This study concluded that exposure to propanil leads to formation of free radicals, enzymatic activities and biochemical parameters had marked alterations and Taurine showed significant ameliorating affect to bring it to normal level.

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