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**Research Article**

# AMELIORATING EFFECT OF SILYMARIN AGAINST PROPANIL INDUCED HEPATOTOXICITY AND OXIDATIVE STRESS IN SWISS ALBINO MICE

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**ABSTRACT**

Propanil a selective post emergent herbicide, used extensively on paddy crops, find their way to enter into mammalian system and causes toxicity. In this study protective effect of herb silymarin was investigated against propanil herbicide induced alteration in biochemical indices in blood and hepatotoxicity caused in swiss albino mice. In an experimental study albino mice were distributed in four equal groups of six in each: Control group, 20mg propanil/kg, 100mg silymarin/kg, propanil (20 mg/kg) + silymarin(100 mg/kg). Treatment was via oral route and was fed once daily for 90 days. Propanil administration elicited significant ($P \leq 0.05$) increase in plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphtase (ALP) activities compared to control. In present study level of malondialdehyde were found to be elevated significantly ($P<0.001$) but the activities of catalase (CAT), reduced glutathione (GSH), and superoxide dismutase(SOD) were decreased. Suplementation of silymarin attenuated the adverse effect of propanil intoxication by reducing lipid peroxidation level and was able to reduce serum enzymes and reduced glutathione.The present study suggests that silymarin could be treatment herb based on its ability to attenuate propanil induced hepatotoxicity.



## INTRODUCTION

Propanil (dichlopropinailide) is a very selective post emergent contact herbicide (Bartha and Pramer, 1970). Propanil is known as one of the most commonly used herbicide used in wheat and rice crops production throughout the world and because of this massive usage, Propanil is kept in top 20 pesticides applied in agriculture. As rice and wheat are most extensively used staple crops of India, which is used at our homes on daily basis, so there are bright chances that this toxic compound or its metabolite could get entry into mammalian food chain which can be possible by various means as follows through the food products we eat, from water resources, by biomagnifications at each tropic level and lastly enter into mammalian system, occupational exposure etc.

The foremost toxicity study on human poisoning from propanil was methemoglobinemia, which has resulted in fatalities (Kimbrough, 1980; Desilva and Bodinayake, 1997). Propanil administration also resulted in hemolytic anemia and also led to methemoglobinemia as shown clearly by (Ambrose *et al*., 1972; Singleton and Murphy, 1975; Mc Millan *et al*.; 1990b, 1991). The active metabolite (3,4 dichloroanilide) of Propanil was found toxic to rat and this toxic metabolite primarilly attacked liver, urinary bladder and kidney, this study was conducted by Zhang B,Sen L (2009). Rankin and Racine in 2008 have demonstrated that Propanil has potential to induce nephrotoxicity and cytotoxxicity. Morse and Baker (1979) have demonstrated that occupational exposure in Propanil manufacure led to chloracne in industrial workers.

There are several studies conducted that suggested the beneficial effect of various antioxidants on many a toxicants. Silymarin is one of the popular herbs in studies these days. Silymarin is a polyphenolic flavonoid isolated from milk thistle plant. Silymarin has four main isomeric compounds which contains flavonolignans and that is: silychristin, silydianin and two groups of disaster eoisomeric flavonolignans, silibinin and isosilibinin. The most active and major component of silymarin is silibinin was shown by Saller R, Meirer *et al* 2001.

Luper S.(1998) showed that silymarin has protected in toxic hepatitis, fatty liver cirrhosis and radiation toxicity. He has also shown the regenerating effect of silymarin on liver and also lipid peroxidative effect. Schriewer H (1976) demonstrated that silymarin protected rat and mouse liver against liver toxicity caused by thioacetamide.

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## METHODOLOGY

### *Chemicals*

Herbicide propanil PESTANAL #, analytical standard was purchased from Sigma-Aldrich Co.Ltd.St. Louis, USA and taurine was purchased from LOBA chemie, (EDTA) hydrogen peroxide, sulphuric acid, diethyl triamine penta acetic acid, sodium dodecyl sulphate, TBA and pyrogallol, were purchased from E Merks Ltd. Mumbai India. All other chemicals were of technical grade and purchased from Loba Chemie, Mumbai India.

### *Animals*

Colony bred Swiss albino mice weighing 18-20gm obtained from Institute of Animal health and Veterinary and Biological Products, Rasalpura, Mhow, Madhya Pradesh were used for this study. The animals were maintained at 22±3$^{o}$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).

### *Experimental protocol*

Mice were divided into four 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 X BW) / F1 (Research Diet)

DD=diet dose (mg cmpd/kg Diet), SD= Single Daily Dose (mg cmpd /kg BW/day) BW= Body Weight (gm BW/animal), F1= Daily Food Intake (gm Diet/day) and the group were as follows
C- Control animals (no treatment)
T1 - Propanil treatment (20mg/kg BW)
T2- Silymarin treatment (100mg/kg BW)
T3-Propanil (20mg/kg BW) + (100mg/kg/body weight)

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

### *Analysis of AST, ALT and ALP in serum*

Serum was used to estimate the following liver enzymes, alnine transaminase (ALT; EC 2.6.1.2) and aspartate transaminase (AST; EC 2.6.1.1) was calculated according to (Reitman &Frankel 1957). Alkaline phosphtase (ALP; EC 3.1.3.1) was assessed by Randox commercial kit.

### *Biochemical estimation of Catalase, SOD, LPO and GSH content*

Total (Cu- Zn and Mn) superoxide dismutase (SOD; EC 1..15.1.1) was calculated by previous well described method of Marklund and Marklund (1974), which involves generation of superoxide by pyraogallol 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 570nm. The reaction was terminated by addition of dimethylsulfoxide DMSO), which help to solubilise the formazan formed. Catalase activity was estimated by considering the method of L.Groth (1991), based on the estimation of amount of hydrogen peroxide decomposed. Tissue reduced glutathione content was measured by taking the absorbance of product formed at 412 nm following the method of Ellman (1959). Liver lipid peroxdation was determined by measuring the formation of thiobarbituric acid reactive substances (TBRAS) with malondialdehyde, one of the major products of lipid peroxidation. Amont of MDA was measured by taking absorbance at 532nm, using Schimandzu UV-1700 spectrophotometer according to method of of Utely *et al* (1967)

## STATASTICAL ANANLYSIS

All values have been expressed as mean ±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 AND DISCUSSION

### *Animal's water and food consumption*

There was statistically significant difference in water consumption during the experimental period, although throughout the experiment, the Propanil-treated group manifested lower water consumption compared to the control group (data not shown). Food intake was also lower in the propanil-treated group, however, only at the 9$^{th}$ week this became statistically evident (p=0.045) compared to the control group. The mean weight of the animals' organs was taken.

***Oxidative stress:*** Biochemical and hematological profile are two such an important parameters which gives crucial information about the internal environment of the organism. silymarine is an antioxidant (Trachtman H, Futterweit S 1994) and the most important role of silymarine as antioxidant is probably systemic and even local scavenging of reactive oxygen species. The data summarised in table.1 and shown in graphically clearly indicates that AST, ALT and ALP significantly increased ($p \leq 0.05$) in mice group administerd (20mg/kg/BW/day) however the same indices in mice receiving silymarin extract alone group T2 were significantlly lower ($p \leq 0.05$) than the control group. Group T3 receiving combination of both propanil and silymarin extract demonstrated decreased level of serum level. Further propanil led to significant increase ($p \leq 0.05$) in creatinine and urea level while silymarin extract showed a significant recovery in these parameters ($p \leq 0.05$) by reducing their concentration in serum. Treatment with silymarin extract plus propanil significantly alleviated the undesirable increased level of urea and creatinine when compared to propanil treated animal. Increase in alkaline phosphtase activity can be due to cellular necrosis or increase in permeability of plasma membrane, which indicate that animal is under stress. silymarine reduced the enzyme ALT, ALP, AST concentration. Oxidative injury is an outcome of imbalance between antioxidant defence 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.

**Table1.** Effect of Propanil (20 mg/kg/day), silymarin (100 mg/kg/day), propanil(20mg/kg/day) + silymarin 100mg/kg/BW administration for 90 days on the activities of serum AST, ALT (U/L), ALP(U/L).

| GROUP | ALT U/L | ALP U/L | AST U/L |
|---|---|---|---|
| C | 43.03±0.42 | 22.9±2.12 | 17.4±.11 |
| T1 | 114.4±2.36 | 34.9±1.04 | 38.4±1.02 |
| T2 | 45.16±1.87 | 24.2±1.23 | 19.98±0.08 |
| T3 | 46.5±1.67 | 21.6±1.06 | 16.9±1.03 |

**Table 2** Effect of Propanil (20 mg/kg/day), silymarin (100 mg/kg/day), propanil(20mg/kg/day) + silymarin (100mg/kg/BW) administration for 90 days on the activities of SOD (U/g protein), GSH (µmol/min/g protein), CAT (µM H2O2 decomposed/min/ mg protein) Total Protein in mice.

| | SOD U/g protein | CAT µM H2O2/min/mg protein | GSH µmol/min/g protein | Urea mg/dl | Creatinine mg/dl | MDAmol/mg protein |
|---|---|---|---|---|---|---|
| C | 6.25±0.13 | 52.14±2.8 | 5.5±0.13 | 24.48±0.16 | 0.58±0.02 | 33.42±1.26 |
| T1 | 2.27±0.14 | 31.62±2.3 | 3.07±0.22 | 32.18±0.46 | 0.98±0.03 | 92.43±1.21 |
| T2 | 8.84±0.12 | 53.7±1.5 | 6.06±0.7 | 22.84±0.54 | 0.57±0.02 | 32.13±0.22 |
| T3 | 5.59±0.11 | 56.26±3.2 | 4.8±0.012 | 28.83±0.32 | 0.74±0.03 | 44.32±1.03 |

Values are mean ±SD of six mice in each group. Significance at P< 0.05

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-tochopherol and also an important role in maintaining protein sulfhydril group. GSH level depleted with increase in oxidative stress which goes with our findings, and treatment with silymarine 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 silymarin enhanced the enzyme activity.

There was significant affect on hepatic oxidative stress parameters. Plasma total antioxidant enzyme concentration SOD, CAT, GSH activities significantly decreased ($p \leq 0.05$) in propanil treated group in comparison to control, however, the concentration and activity of total antioxidant enzymes, GSH, SOD and CAT were significantly increased 9 ($p \leq 0.05$) when silymarin extract was given in both groups as compared to T1.Administration of Propanil alone induced a significant increase in total antioxidant capacity, GSH, SOD and CAT compared to control group. Animal treated with Propanil led to significant increase ($p \leq 0.05$) in the level of MDA concentration as compared to control group. Simultaneous treatment with silymarin (T2 and T3) significantly abolished the enhancing effect of propanil on hepatic lipid peroxidation.

In this study, a sub lethal dose of propanil (20mg/kg/BW/day) was used for 90 days because propanil induced liver injury has been reported to be evident at this time in experimental studies. Significant increase in AST, ALT ALP were observed. In this study, increase in AST, ALT and ALP induced by propanil was significantly reduced by silymarin. Silymarin is quite a popular herb marketed to treat hepatic disorders as shown by Wu Jw, Lin L C *et al*;2009. The hepatoprotection provided by silymarin may be due to loss of functional integrity of the cell membrane in the liver. The result from the study clearly indicated that silymarin has good hepatoprotective and antioxidant potential against propanil induced damage caused. These findings goes in concordance with Natrajan SK, Thomas *et.al*; (2006) kaur M. (2007) has shown increasing interest in herbal product to cure cancer cells from proliferating in both invite and in vivo animal models of various epithelial cancers.

Mira L, Silva Manso C F. (1994) has demonstrated silybin as effective radical scavenger and antilipoperoxidant. Valenzuela A, Garrido A.(1994) studied pharmacological action of silymarin and have shown that silymarin preserve the functional and structural integrity of hepatocyte membranes by preventing alterations of this phospholipids' structure produced by toxicant and thus restoring alkaline phosphtase and GSH activities.

## CONCLUSION

Present study revealed that silymarin has protected against nephrotoxicity induced by propanil herbicide in mice. Silymarin reduced the level of free radicals and antioxidant enzymes and GSH content increased. Serum biochemical enzymes AST, ALT, ALP enzymes remarkably decreased and lipid per oxidation marker MDA along with GSH and CAT suggest that silymarine is a strong antioxidant. Hence dietary silymarin 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 silymarin has beneficial effect on renal toxicity. So from this study it can be concluded that it 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 silymarin play very crucial role in reducing Propanil toxicity.

***Ethical Considerations:*** Ethical issues (including animal model, plagiarism, misconduct, data fabrication double publication) have been completely taken into account by the authors.

***Conflict of interest:*** The authors declared no competing interest and both the authors participated in the study have read the paper and provided the final approval.

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