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POTENTIAL NEPHROPROTECTIVE EFFECT OF SILYMARIN AGAINST IFOSFAMIDE INDUCED TOXICITY IN RATS

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ABSTRACT

The present study was undertaken to investigate the Nephroprotective effect of silymarin against ifosfamide (IFO) induced nephrotoxicity in rats. In IFO model rats of either sex (n=6) were pre-treated with silymarin (50mg/kg, p.o.) for 5 days by oral route, IFO toxicity was induced by administering IFO (50mg/kg) on third day by intra-peritoneal route. The influence of prophylactic treatment was analyzed by quantification of Serum/Urinary biomarkers and antioxidants and histopathological observations. Silymarin treatment in presence of IFO was responsible for significant reduction in Serum; Urea, Creatinine, Aspartate transaminase (AST), Alanine transaminase (ALT) and Urinary; Total Protein, Sodium and Potassium compared to IFO control group. Silymarin treatment was also responsible for significant increase in Serum Albumin and antioxidants such as superoxide dismutase (SOD), Glutathione (GSH) and Catalase activities in kidney tissue homogenate compared to IFO control group. Similarly, there was an increase in the urine volume and decrease in the kidney weight in the silymarin treated groups compared to the IFO control group. Results were further supported by histopathological studies. Investigation witnessed the administration of silymarin 50mg/kg dose was effective in normalizing the abnormal conditions of kidney induced by IFO. Thus investigational finding conclude that silymarin possess potential benefits in treating animals with nephrotoxicity.

INTRODUCTION

The kidney is an essential organ required by the body to perform several important functions including the maintenance of homeostasis, regulation of the extracellular environment, such as detoxification, and excretion of toxic metabolites and drugs. Therefore, the kidney can be considered as a major target organ for exogenous toxicants.¹ Nephrotoxicity is a kidney-specific feature in which excretion does not go smoothly owing to toxic chemicals or drugs. Drug-induced nephrotoxicity is an important cause of renal failure. Kidney disease is the ninth leading cause of death. Approximately, 19 million adults have chronic kidney disease and an estimated 80,000 persons have chronic kidney failure diagnosed annually in India. Recent literature, have shown a prevalence of chronic renal failure of 0.16% and 0.79% in India.²

Herbal remedies have been recorded to cure all kinds of diseases as a critical therapeutic method in many cultures. The use of plants, parts of plants and isolated photochemical for the prevention and treatment of various health ailments has been in practice from time immemorial. It is estimated that about 25% of the drugs prescribed worldwide are derived from plants and 121 such active compounds are in use.³

Silybum marianum, commonly known as Milk thistle, Holy thistle, Marian thistle, Mary thistle, belongs to the family Asteraceae. Silymarin being a potent antioxidant protects the liver from several hepatotoxins, including Amanita mushrooms, paracetamol and alcohol. It acts by antioxidative, anti-lipid peroxidative, antifibrotic, anti-inflammatory, membrane stabilizing, immunomodulatory and liver regenerating mechanisms⁴. Till now there is no study reporting the effect of Silymarin against IFO induced nephrotoxicity in rats. Hence, the present study is designed to demonstrate the nephroprotective effect of silymarin against IFO induced nephrotoxicity by using rat as an experimental animal.

MATERIALS AND METHODS

Experimental animals

Rats of either sex weighing 175-250 g were housed in standard polypropylene cages and maintained under controlled room temperature ($25^{\circ} \pm 5^{\circ}\text{C}$) and humidity ($55 \pm 5\%$) in a well-ventilated animal house under 12:12 h light and dark cycle. All the rats were provided with commercially available standard pellet diet, water *ad libitum*. Prior to each study, the animals were made to fast for 12–14 h but had free access to water.

The guidelines of committee for the purpose of control and supervision of experiments on animals (CPSCEA), Govt. of India were followed and prior permission was sought from the institutional animal ethics committee for conducting the study.

Procurement of silymarin and its dose selection:

Pure sample of silymarin was procured from Sigma Chemicals, USA. Silymarin was dissolved in distilled water then administered orally to the animals by gastric intubation using a force feeding needle. Based on earlier literature review therapeutic dose of silymarin in rat was found to be 50mg/kg the same dose was selected for the present study.⁵

EXPERIMENTAL MODELS

Ifosfamide (IFO) induced nephrotoxicity:⁶

Experimental rats were divided into four groups of six animals each. Group I and II were treated with saline and served as normal control and IFO control respectively. For Group III and IV Silymarin treatment was given with a dose of 50mg/kg. All treatments were given for five days and by oral route. On third day apart from normal control group all other treatment groups were treated with IFO (50 mg/kg)⁶ by intra peritoneal route to induce the nephro-toxicity.

Twenty four hour after the last treatment different biochemical analysis were performed on collected serum and urine.

The different parameters estimated were:

- Serum: Albumin, Creatinine, Urea, Alanine aminotransferase (ALT) & Aspartate aminotransferase (AST).
- Urine: Urine volume, Na⁺, K⁺ ions & Total Protein.
- Kidney homogenate: Glutathione Peroxidase, Catalase & Super Oxide Dismutase.
- Physical parameters: Kidney weight.
- Histological analysis.

Statistical analysis

Results are expressed as mean±SE. Statistical significance was assessed using one-way Analysis of variance (ANOVA) followed by Tukey-karmer multiple comparison tests. P<0.05 was considered significant.

RESULT**Ifosfamide induced nephrotoxicity:****Effect on urine biomarkers;****Effect on urine volume**

IFO control demonstrated extremely significant decrease ($p < 0.001$) in urine volume compared to normal control.

Silymarin+IFO group showed moderately significant increase ($p < 0.01$) increase ($p < 0.001$) in urine volume compared to IFO control. (Table no 1) (Figure 1)

Effect on total protein

IFO control demonstrated extremely significant increase ($p < 0.001$) in total protein compared to normal control.

Silymarin+IFO group showed moderately significant decrease ($p < 0.01$) increase ($p < 0.001$) in total protein compared to IFO control. (Table no 1) (Figure 2)

Effect on sodium

IFO control documented extremely significant increase ($p < 0.001$) in sodium compared to normal control.

Silymarin+IFO group showed extremely significant decrease ($p < 0.001$) in sodium compared to IFO control. (Table no 1) (Figure 3)

Effect on potassium

IFO control documented extremely significant increase ($p < 0.001$) in potassium compared to normal control.

Silymarin+IFO group showed extremely significant decrease ($p < 0.001$) in potassium compared to IFO control. (Table no 1) (Figure 4)

Table 1: Effect on urine biomarkers against IFO induced nephrotoxicity

Treatment	Urine volume (ml)	Total protein (mg/dl)	Sodium (meq/l)	Potassium (meq/l)
Normal control	16.10±0.60	29.54±1.76	165.23±1.43	5.02±0.08
IFO control	6.10±0.80 ^{***}	49.32±1.54 ^{***}	256.65±1.90 ^{***}	7.11±0.03 ^{***}
Silymarin	14.10±0.70 ^{###}	30.32±1.76 ^{###}	165.45±1.87 ^{###}	2.90±0.21 ^{###}
Silymarin + IFO	10.50±0.60 ^{**##}	38.59±1.98 ^{**##}	189.23±1.72 ^{###}	3.43±0.17 ^{***###}

Values are expressed as MEAN±SEM. n=6. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ when compared to normal. ## $p < 0.01$, ### $p < 0.001$ when compared with IFO induced.

Effect on Serum Biomarkers;

Effect on serum albumin

IFO control demonstrated extremely significant decrease ($p < 0.001$) in albumin compared to normal control.

Silymarin+IFO group showed extremely significant increase ($p < 0.001$) in albumin compared to IFO control. (Table no 2) (Figure 5)

Effect on serum creatinine

IFO control showed extremely significant increase ($p < 0.001$) in creatinine compared to normal control.

Silymarin+IFO group showed extremely significant decrease ($p < 0.001$) in creatinine compared to IFO control. (Table no 2) (Figure 6)

Effect on serum urea

IFO control showed extremely significant increase ($p < 0.001$) in urea compared to normal control.

Silymarin+IFO group showed moderately significant decrease ($p < 0.01$) in urea compared to IFO control. (Table no 2) (Figure 7)

Effect on serum ALT

IFO control showed extremely significant increase ($p < 0.001$) in ALT compared to normal control.

Silymarin+IFO group showed extremely significant decrease ($p < 0.001$) in ALT compared to IFO control. (Table no 3) (Figure 8)

Effect on serum AST

IFO control documented extremely significant increase ($p < 0.001$) in AST compared to normal control.

Silymarin+IFO group showed extremely significant decrease ($p < 0.001$) in AST compared to IFO control. (Table no 3) (Figure 9)

Table2: Effect on serum biomarkers against IFO induced nephrotoxicity

Treatment	ALBUMIN (mg/dl)	CREATININE (mg/dl)	UREA (mg/dl)
Normal control	38.65±1.43	0.78±0.07	31.54±1.98
IFO control	10.43±1.54 ^{***}	2.98±0.04 ^{***}	74.32±1.45 ^{***}
Silymarin	32.65±1.32 ^{###}	0.71±0.09 ^{###}	42.76±1.87 ^{###}
Silymarin+IFO	25.65±1.23 ^{***###}	1.21±0.04 ^{**###}	64.23±1.98 ^{***##}

Values are expressed

as MEAN±SEM. n=6. **p < 0.01,***p<0.001 when compared to normal. ##p < 0.01, ###p<0.001 when compared with IFO induced.

Table 3: Effect on serum biomarkers against IFO induced nephrotoxicity

Treatment	ALT (U/L)	AST (U/L)
Normal control	53.32±1.54	69.34±1.76
IFO control	154.00±2.11 ^{***}	165.66±1.32 ^{***}
Silymarin	79.22±1.67 ^{###}	83.54±1.43 ^{###}
Silymarin+IFO	84.65±1.21 ^{*###}	90.23±1.23 ^{**###}

Values are expressed as MEAN±SEM. n=6. **p < 0.01,***p<0.001 when compared to normal. ##p < 0.01, ###p<0.001 when compared with IFO induced.

Figure1: Effect on Urine volume

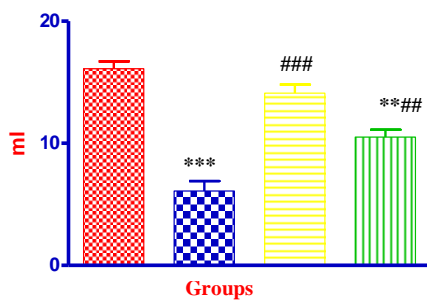


Figure2: Effect on Total protein

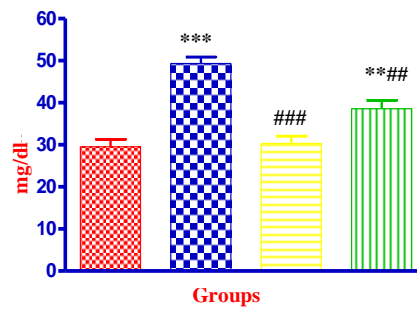


Figure3: Effect on Sodium

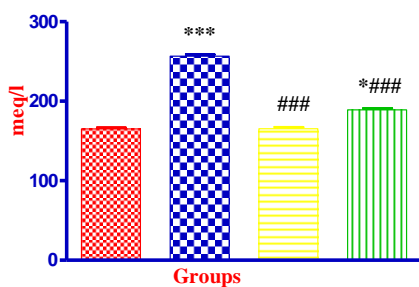


Figure4: Effect on Potassium

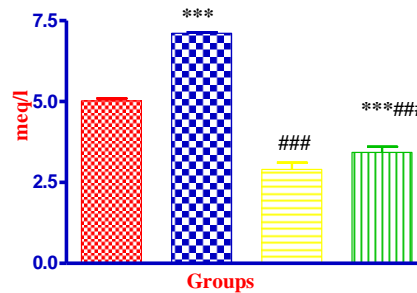


Figure5: Effect on albumin

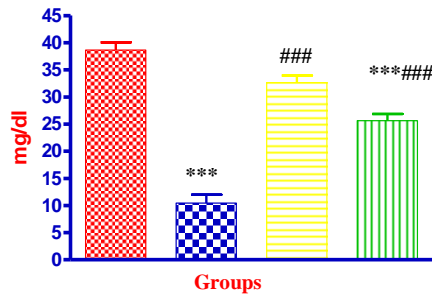
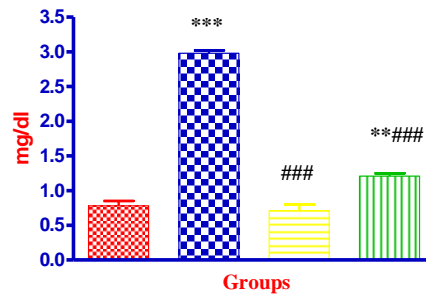


Figure6: Effect on creatinine



Values are expressed as MEAN±SEM. n=6. *p <0.05, **p < 0.01, ***p<0.001 when compared to normal. ##p < 0.01, ###p<0.001 when compared with IFO control.



Figure7: Effect on urea

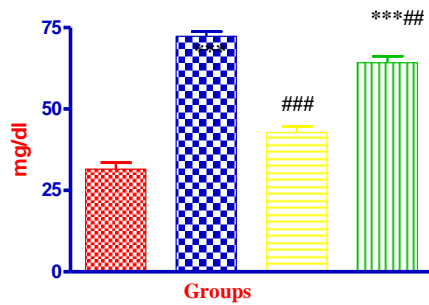


Figure8: Effect on ALT

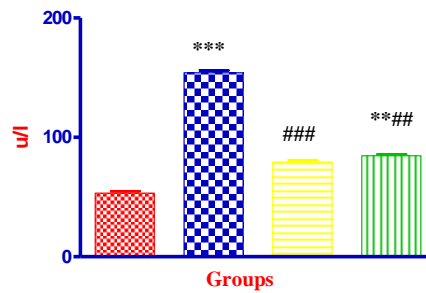
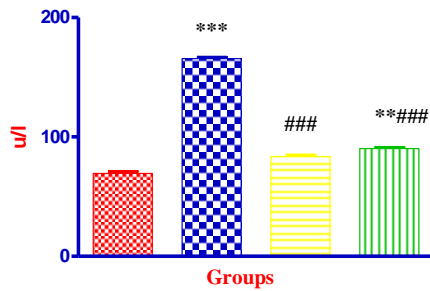


Figure9: Effect on AST



Values are expressed as MEAN±SEM. n=6. **p < 0.01, ***p<0.001 when compared to normal. ##p < 0.01, ###p<0.001 when compared with IFO control.



DISCUSSION

The aim of the present study was to investigate the nephroprotective activity of silymarin against ifosfamide (IFO) induced toxicity in rats. In IFO induced nephrotoxicity model, toxicity was induced by treating the experimental animals with dose of 50 mg/kg by intraperitoneal route. The metabolites of IFO are responsible for development of nephro toxicity.⁷

IFO is a pro drug that is hepatically metabolized by the cytochrome P450 enzymes 3A4, 3A5 and 2B6 to its active metabolite IFO mustard.⁸ There active toxic metabolite chloroacetaldehyde, which is produced by the side-chain oxidation of IFO in renal tubular cells, is believed to be responsible for the nephrotoxic effect.⁹

Observed results suggested that silymarin (50mg/kg, p.o.) showed beneficial results. silymarin indicated better results against IFO induced nephrotoxicity in rats.

IFO treated group has been demonstrated that significant increase in urine biomarker level such as total protein, sodium, potassium level. It was also revealed a significant decrease urine volume. In this this model it has been reported that significant increase in serum biomarkers level such as creatinine, urea, AST, ALT and significant decrease in albumin level was seen. Treatment with silymarin reversed the elevated levels of all the serum markers such as creatinine, urea, ALT, AST and decreased level of albumin, decreased antioxidant enzyme to the near normal levels in this model. Silymarin is a potent antioxidant. It neutralizes free radicals and restores SOD, GSH and catalase levels in tissue. This adaptogenic property helps to prevent kidney from free radical stress.

Silymarin maybe recommended as a nephro protective agent to attenuate toxicity of some IFO that currently have a high likelihood of inducing nephrotoxicity.

CONCLUSION

With the findings of the present study it can be concluded that the Silymarin has demonstrated significant nephroprotective effect against ifosfamide (IFO) induced nephrotoxicity in rats.

Treatment with silymarin reversed IFO induced elevated levels of all the urine biomarkers such as total protein, sodium, and potassium, decreased urine volume near to the normal levels. It is worth mentioning that silymarin efficiently trims down the elevated levels of serum biomarkers such as AST, ALT, creatinine and urea. It has also restored antioxidant parameters without producing any adverse effect. The results from the present study and histological analysis indicate the administration of silymarin has protective effects against IFO induced renal necrosis state.

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