Original article

The effect of different doses of silymarin on gentamicin-induced kidney damage in rats

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Summary:

Background: *Many in vivo and in vitro studies performed mainly in liver have been shown silymarin to be a potent anti-oxidant, and one of the most potent scavengers of hydroxyl radicals. Therefore, it is plausible to expect that it may produce these effects against oxidative stress consequences induced by gentamicin in the kidney.*

Objective: *Evaluation of the protective effect of different doses of silymarin given orally in protecting rats against gentamicin-induced nephrotoxicity.*

Materials and Methods: *Groups of rats (6 rats each) were pre-treated for 7 days with 250, 500, and lOOOmg/kg silymarin and vehicle orally before induction of renal toxicity with gentamicin, and another 6 rats were utilized as controls. The parameters of oxidative stress, malondialdehyde (MDA), and glutathione (GSH) were measured in the serum and kidney tissue homogenate, in addition to serum levels of urea and creatinine. Histopathological examination of stained tissue sections from the kidney was performed; in addition to silymarin level in the kidney tissue homogenate was evaluated using HPLC method.*

Results: *Analysis of data revealed significant amelioration of oxidative stress, experimentally induced in the kidney through lowering MDA and elevation of GSH levels, both in serum and tissue homogenate, associated with significant reduction of serum levels of urea and creatinine, and with positive histological evidences for the protective effect of silymarin which is found to be related to the increase in its renal tissue availability when the oral dose was increased.*

Conclusion: *These findings suggest that, silymarin is effective in preventing gentamicin-induced renal toxicity, which makes it a good candidate for clinical use in this respect.*

key words: *Silymarin, Gentamicin, nephrotoxicity.*

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Introduction

Gentamicin, an aminoglycoside antibiotic used for the treatment of Gram (-) bacterial infection , shows profound nephrotoxicity as a major side effect, and thought to account for 10-15% of all cases of acute renal failure*[2]. The specificity of gentamicin-induced kidney damage is apparently related to its preferential accumulation in the proximal convoluted tubules (PCT), causing direct tubular toxicity [3,4].

Although gentamicin's effect on biological membranes appears to be critical in the pathogenic events of its toxicity[5], the exact mechanisms are thought to be related to oxidative stress induced by reactive oxygen species, which produce renal tissue injury and necrosis via peroxidation of membrane lipids, denaturation of proteins and DNA damage[6]

Silymarin, a mixture of flavonolignans derived from milk thistle (Silybum marianum), comprised mainly of silybinin (SBN) A and B, isosilybinin (ISBN), Silychristin (SCN), silydianin (SDN), and taxifolin (TXF)[7]. It has documented hepatoprotective effects [8,9,10] attributed to its anti-oxidant and free-radical scavenging activities [12] ., plasma membrane stabilization and permeability regulating activities *[14]. Meanwhile , it promotes mRNA synthesis , and shows anti-fibrotic effects *[15], anti-inflammatory and immuno modulatory activities[17].

This study was designed to evaluate the possible relationship between dose, tissue availability and nephroprotective effects of silymarin against gentamicin-induced renal toxicity in rats.

Methods:.

Thirty rats *(Rattus norvigicus)* of both sexes, weighing (180-220 gm), allocated into 5 groups of 6 animal each, housed in the animal house-college of pharmacy under standard laboratory conditions and had free access to water and fed standard chow *adjibitum.* Animal groups are treated as follows:

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Group I: Six rats treated orally with corn oil (vehicle) and injected I.P. with normal saline, served as controls.

Group II: Six rats treated twice daily with 250mg/kg silymarin in corn oil, given orally 7-days before induction of renal toxicity by S.C. injection of 40mg/kg gentamicin twice daily for 5 days[19].

Group III and IV: Six rats in each group treated with 500 and lOOOmg/kg silymarin respectively, and continue the same procedure as in group II. Group V: Six rats treated with corn oil (P.O.) for 7 days, and then renal toxicity was induced by S.C. injection of 40mg/kg gentamicin twice daily for 5 days.

All animals were sacrificed on day 6 after gentamicin administration.

Serum and renal tissue homogenate were prepared by standard procedures, and the levels of MDA and GSH were analyzed in both samples[20> 21]; in addition to measurement of serum urea and creatinine levels [22,23]. Small pieces of kidney tissue were prepared for histopathological examination according to standard procedure [24] and evaluated by ordinary microscope after staining with hematoxylin and eosin. Finally, silymarin levels in the kidney homogenate were measured by HPLC according to the method of Zhao and Agarwil 2000,[25]compared with authentic standard purchased for this purpose.

Statistical analysis of data was performed utilizing student's t-test and ANOVA. 95% confidence of data was considered for significance.

Results:

The data presented in tables 1 and 2 showed that gentamicin produces a highly significant increase in serum and tissue levels of MDA and highly significant decrease in GSH levels in both compartments, compared to controls. Pre-treatment of animals with various doses of silymarin (250, 500, and lOOOmg/kg) for seven days produced significant reduction in MDA and elevation in GSH levels, both in serum and tissue homogenates, compared to vehicle-treated group.

In table 3, treatment with gentamicin resulted in a highly significant increase in serum levels of urea and creatinine compared to saline injected group (control). Pre-treatment with various doses of silymarine (250, 500, and lOOOmg/kg) for seven days resulted in significant reduction of serum levels of urea and creatinine compared to vehicle-treated group.

Kidney tissue histology showed necrotic changes in the PCT as a result of treatment with gentamicin; in addition to tubular epithelial loss, there were hyaline casts and blood vessels congestion was observed. (Figures 1 and 2).

Increasing doses of silymarin reduced the nephrotoxic changes induced by gentamicin as evidenced by the decreased severity of tubular necrosis. However, hyaline casts were still evidenced in some sections with mild blood vessels congestion (figures 3, 4 and 5).

In figure 6, The HPLC profile of standard silymarin was founed to contain six major constituents which are supposed to be taxifolin, Silychristin, silydianin, silybinin A, silybinin B and isosilybinin, sequenced according to their appearance in the chromatogram, compared with previous data reported by others[26], with the retention times of 1.5, 2, 2.5, 3,4.8, and 6 minutes, respectively.

The renal tissue distribution of different doses of silymarin, clearly demonstrated that, silymarin is well absorbed after oral administration, and had good tissue availability in the kidney.

The renal tissue distribution of orally administered silymarin in doses of 250, and 500mg/kg showed the appearance of two peaks which may represent taxifolin and Silychristin in concentration of 573.8 and 497.5 ug/g tissue, respectively (Figures 7 and 8) compared to standard; while kidney tissue availability of the dose l000mg/kg represented by the appearance of 3 peaks expected to be taxifolin,. Silychristin and silydianin, in concentrations of 574.6, 507.9, and 77.8 ug/g tissue, respectively compared to standard. (Figure 9).

Discussion:

A relationship between oxidative stress and nephrotoxicity has been well documented in many experimental animal models [27]. In gentamicin-treated, the anti-oxidant defense mechanisms are found to be severely impaired in association with drastic depletion of the endogenous anti-oxidants. Gentamicin enhances the production of hydrogen peroxide (H2O2) in isolated renal mitochondria[1] , and pre-treatment with various types of anti-oxidants is found to be effective in ameliorating the oxidative stress induced renal damage [29>30,31].

The biochemical and histological evidences presented in this study, confirm the mechanism of gentamicin-induced nephrotoxicity, which is thought to be attributed to the induction of oxidative stress.

The nephroprotective effect of silymarin observed in this study is found to be comparable with the observations repoted by others in that administration of one of the active constituents of silymarin (silybinin) before the treatment with cisplatin, significantly reduces tubular toxicity and prevent the impairment in renal function [32,33].

The protective effects observed for silymarin against gentamicin-induced nephrotoxicity may be attributed to its powerful anti-oxidant activity, and may be related to the observed enhancement in the intracellular anti-oxidant enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in kidney tissue[34,35]. Additionally, silymarin may attenuate tissue lipid peroxidation either by scavenging and inactivating peroxides and hydroxyl radicals directly

or by binding free iron as a chelating agent[6].The critical role of excessive superoxide anion generation in gentamicin-induced renal damage was establishes through the observation that the treatment with SOD and dimethyl thiourea have protective effect in this respect *[37].Furthermore, silymarin and silybinin, by interacting with the lipid component of the cell membrane, can influence their chemical and physical properties; and studies in erythrocytes, mast cells, macrophages and hepatocytes have shown that silymarin and silybinin renders cell membrane more resistant to lesions [14,38].

Numerous studies on the biochemical mechanism of action of silymarin, confirmed that, the transcription process in the hepatic tissue of rats and mice *in vivo* is accelerated under the influence of silymarin and purified silybinin. *[39] *In vitro* experiments with isolated nuclei and nucleoli, the enzymatic activity of DNA dependent RNA polymerase I is stimulated by silymarin[40], which subsequently accelerates the synthesis of 28S and 18S ribosomal RNA's and also promotes the formation of complete ribosome and intensifying protein synthesis indirectly. In view of this biochemical mechanism, silymarin was expected to exhibit similar effect on other cell types, like the cells of renal tissues.

In conclusion, the results of this study clearly demonstrated that, tissue availability of silymarin constituents is dose dependent and explain the relationship between the dose and the cytoprotective effects. They produce against gentamicin-induced nephrotoxicity; and this may provided a new area of investigations for the clinical use of silymarin in this respect.

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Table (1): mean ± standard deviation of different doses of silymarin on MDA contents and GSH levels in rat kidney homogenate Compared to GN- treated and control groups:

Treatment	MDA contents nmol / g tissue	GSH level µg / g tissue
Group I N=6	(134.29 ± 2.07) a	(11.35 ± 0.75) a
Group II N=6	(161.966 ± 8.05) a	(2.891 ± 0.05) c
Group III N=6	(119.44 ± 3.75) a	(3.48 ± 0.058) c
Group IV N=6	(118.27 ± 3.17) a	(2.965 ± 0.018) c
Group V N=6	(240.81 ± 3.17) b	(1.299 ± 0.042) b

Values with non-identical subscripts (a,b,c) within each parameter are significantly different (P < 0.05).
N = no. of animals.

Table (2): mean ± standard deviation of the effect of different doses of silymarin on serum MDA contents and GSH levels, compared to GN-treated and control groups:

Treatment	MDA contents mmol / L	Serum GSH Levels µg / L
Group I N=6	(0.573 ± 0.012) a	(3.298 ± 0.23)a
Group II N=6	(0.599 ± 0.114) a	(4.635 ± 1.72)a
Group III N=6	(0.657 ± 0.17) a	(5.65 ± 0.346) c
Group IV N=6	(0.203 ± 0.47) c	(5.5 ± 0.391) c
Group V N=6	(3.518 ± 1.01) b	(0.273 ± 0.016) b

Values with non- identical subscripts (a,b,c) within each Parameter is significantly different (P < 0.05).
N = no. of animals

Table (3): Mean ± standard deviation of the effect of different doses of silymarin on serum urea and serum creatinine , compared to GN- treated and control groups:

Treatment	Serum urea g / dl	Serum creatinine mg / dl
Group I N=6	(21.43 ± 6.66) a	(0.5 ± 0.248) a
Group II N=6	(17.53 ± 3.58) a	(0.626 ± 0.126)a
Group III N=6	(26.02 ± 9.52) a	(0.633 ± 0.195)a
Group IV N=6	(17.23 ± 2.4) a	(0.61 ± 0.15) a
Group V N=6	(96.5 ± 9.9) b	(2.95 ± 0.66) b

Values with non- identical subscripts (a,b) within each parameter are significantly different (P < 0.05).
N = no. of animals.

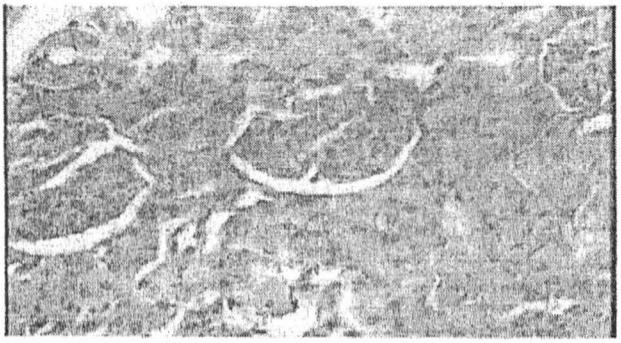

Fig.1:Section showing normal rat's kidney.

Fig.2: Section showing morphological alteration of kidneys from gentamicin-treated rats.

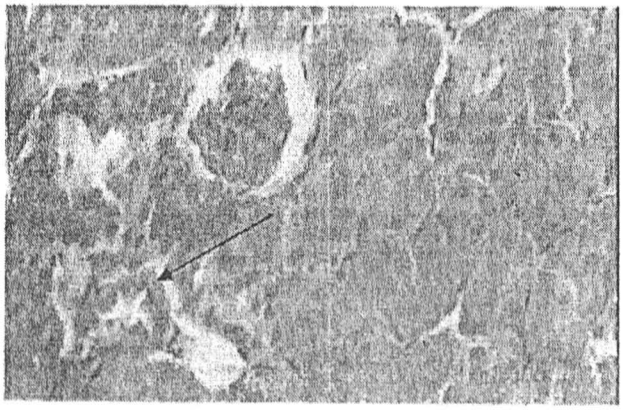

Fig.3: Section showing silymarin administered in dose of 250mg/kg improved gentamicin-induced renal damage. Hyaline casts still present.

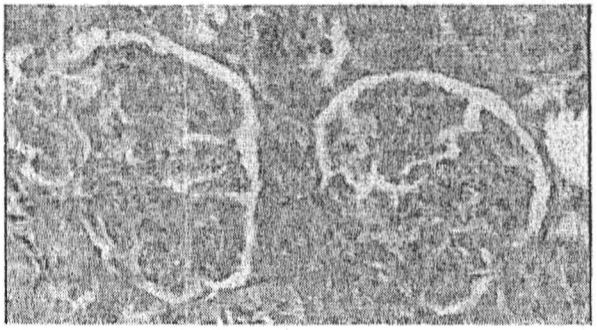

Fig.4; Section showing the normalization of 500mg/kg silymarin against gentamicin-induced renal damage.

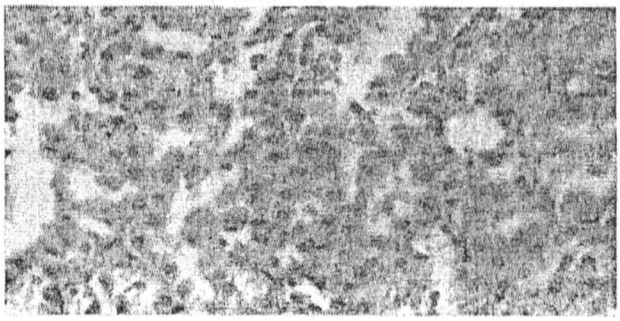

Fig.5; Section showing the normalization of 1000mg/kg silymarin against gentamicin-induced renal damage.

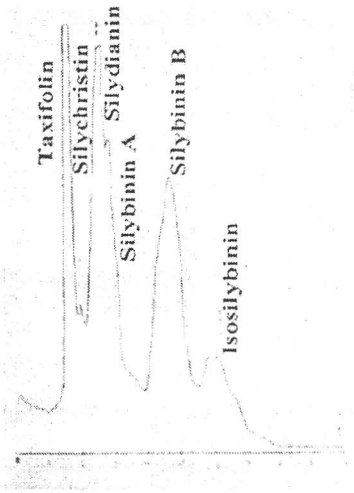

Fig.6: Separation of standard silymarin on reverse phase C_{18}, 5µm (4.5x150mm) mobile phase methanol: water (50:50 v/v) mixture, detection at 288nm.

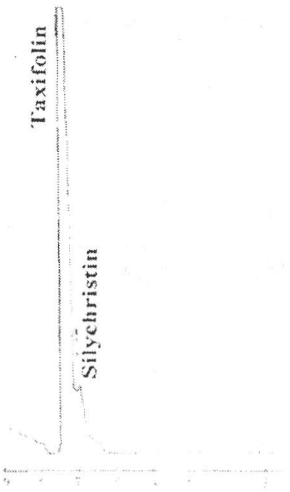

Fig.7: HPLC profile of kidney homogenate after silymarin treatment (250mg/kg) 7-days prior to and during treatment with gentamicin.

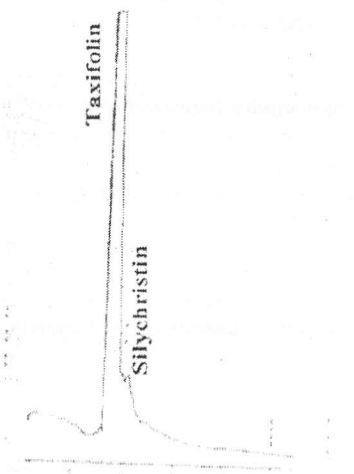

Fig.8: HPLC profile of kidney homogenate after silymarin treatment (500mg/kg) 7-days prior to and during treatment with gentamicin.

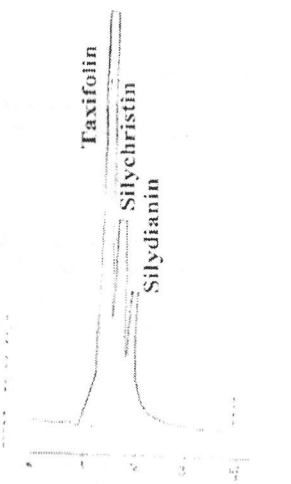

Fig.9: HPLC profile of kidney homogenate after silymarin treatment (1000mg/kg) 7-days prior to and during treatment with gentamicin.