RENOPROTECTIVE EFFECT OF SILYMARIN IN WISTAR RATS AGAINSTGENTAMICIN INDUCED RENAL DAMAGE

Dr. K. Balamurugan[1*], Dr. Joan Vijetha R[2]

Article History: Received: 23.03.2023 **Revised:** 08.05.2023 **Accepted:** 23.06.2023

Abstract

Silymarin (SN) is a lipophilic polyphenol flavonoid, isolated from the Silybum marianum L. Geartn (milk thistle) which belongs to the family Aster of Asteraceae or Compositae. Silymarin has anti-oxidative, antidiabetic, antifibrotic, anti-inflammatory and cytoprotective, hepatoprotector, immunomodulator properties. In the current research the renoprotective effect of Silymarin (SN) in Wistar rats against gentamicin induced kidney damage was studied. The results revealed that the Gentamicin (80 mg/kg, i.p.) treated rats body weight of was significantly lower than the CMC suspension treated rats. In rats the SN (100 mg/kg, p.o.) treated has shows increased in the body weight, in the biochemical estimation, gentamicin alone treated rats, showed significant decrease in total protein content and an increase in serum creatinine, serum urea, serum uric acid, and blood urea nitrogen (BUN) as compared to control rats, SN along with gentamicin treated rats resulted in a significant increase in total protein content and a decrease in serum creatinine, urea, uricacid, and BUN. In histopathological examination, compared to gentamicin-treated rats, SN decreased the severity of the gentamicin-induced renal necrotic damage by reducing the histopathological damages.

Keywords: Silymarin, gentamicin, renoprotective effect, antidiabetic, antifibrotic, kidney

[1*]Associate Professor, Department of Pharmacy, FEAT, Annamalai University, Annamalai Nagar,Chidambaram – 608002, Tamil Nadu, India.
[2]Associate Professor,School of Pharmacy, Sathyabama Institute of Science and Technology, Chennai-600119,Tamil Nadu, India.

***Corresponding author:**
Dr. K. Balamurugan[1*]
[1*]Associate Professor, Department of Pharmacy, FEAT, Annamalai University, Annamalai Nagar,Chidambaram – 608002, Tamil Nadu, India.
Email: [1*]placementbala@yahoo.co.in

DOI: 10.31838/ecb/2023.12.s3.538

1. INTRODUCTION

Herbal medicine has become a popular form of healthcare, herbs contain complicated mixtures of organic chemicals, the levels of which may vary substantially depending upon many factors related to the growth, production, and processing of the herbal product. (Bent 2004) Numerous surveys have shown that a large percentage of the population in the United States uses herbs to treat medical illness or improve health. Herbs have been used forcenturies to treat illness and improve health, and still account for approximately 80% of medical treatments in the developing world. Specific herbal extracts have been demonstrated to be efficacious for specific conditions. The medicinal plants serve humankind as the dawnof numbers of biologically active principles with definitive pharmacological and therapeutic actions in the field of pharmacotherapy. Below are several examples of active plant ingredients that provide medicinal plant uses for humans. **Alkaloids -** Morphine, caffeine, berberin, codeine; **Flavonoids** - Quercetin, reservaratrol, kaempferol, rutin, naringin, hesperidin ; **Saponins -** Diosgenin and hecogenin; **Terpenes** - Artemisinin, α-carotene, β- carotene, lycopene, lutein and zeaxanthin; **Phenolic acids-** Chlorogenic acid, tannic acid, gallic acid and ellagic acid and **Tannins** - Catechol, gallotannins and ellagitannins. (Shakya 2016)

An extensive survey of literature revealed that the active phytocompound of Silybum marianum is a mixed composition of seven flavanolignans with silibinin or Silibin as the chief bioactive principle. Silibinin comprises of two diastereomers, Silibin A and Silibin B in 1:1 proportion. The other flavonolignans of Silymarin includes isosilibin, dehydrosilibin, silichristin, isosilichristin, silydianin and taxifolin, a flavonoid with antioxidant potential. The evidence suggested that Silymarin has anti-oxidative, antidiabetic, antifibrotic, anti- inflammatory and cytoprotective, hepatoprotector, Parkinson's disease, Alzheimer's disease, cerebral ischemia immunomodulator properties.(Sabir 2014) The bioactive phytocompounds from indigenous uses are regarded as therapeutic replacement to overcome the unwanted effects of synthetic medicines with their considerable potency and less side effects. The findings of the research may provide significant information regarding the pharmacotherapeutic efficacy of Silymarin (SN) by exploring their hidden pharmacological potential like renoprotective effect of diosgenin against gentamicin induced kidney damage on wistar rats.

2. RESULTS OF RENOPROTECTIVE ACTIVITY

Results of estimation of body weight in renoprotective activity of SN

Table No: 2 Results of estimation of body weight in renoprotective activity of SN

Group/Treatment	**al body weight(0 day) (g)**	**Final body weight (8^{th} day) (g)**
Group – I (Control - CMC 0.5 % suspension - 1 ml/kg, p.o.)	186.82±4.64	192.34±6.12
Group – II (Negative control –Gentamicin - 80 mg/kg, i.p.)	184.80±5.04	156.82±5.44**

Group – III (SN - 100 mg/Kg, p.o. + Gentamicin - 80 mg/kg, i.p.)	186.24±7.63	182.38±6.94**

Values are expressed as the mean ± SEM from 6 animals in each group; differences inmeans were estimated by using one-way ANOVA followed by Dunnet's post hoc test. The values of Group II were compared with Group I and Groups III, was compared with Group II.
**P<0.01 = moderately significant.

Table No: 2 represented there was significant (P<0.01) decrease in body weight (156.82±5.44 g) of group II rats treated with gentamicin (80 mg/kg, i.p.) alone on 8th day when compared to treated control group I rats (192.34±6.12 g). The initial body weight of gentamicin alone and CMC suspension treated group II and group I rats on 0 day was found as 184.80±5.04 g and 186.82±4.64 g, respectively. The group III rats treated with dose of SN (100 mg/kg, p.o.) along with gentamicin (80 mg/kg, i.p.) showed significant (P<0.05) decrease in body weight (182.38±6.94 g) on 8th day, compared to disease control group IIrats. There were no significant differences found in body weights between the treatment groups II – III and the control group I rats on initial day (0 day) of study.

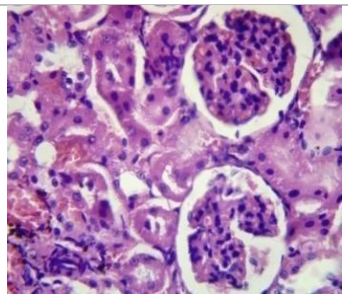
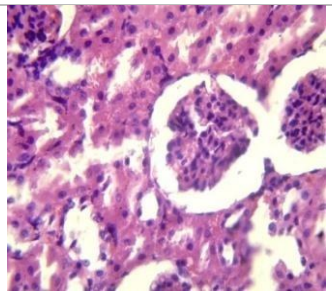
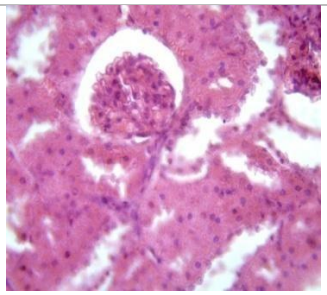
Results of biochemical estimation in renoprotective activity of SN

Table No: 3 Results of biochemical estimation in renoprotective activity of SN

Group/Treatment	al Protein (g/dl)	reatinine (mg/dl)	Urea (mg/dl)	Jric Acid (mg/dl)	Blood Urea Nitrogen-BUN (mg/dl)
Group – I (Control - CMC 0.5 % suspension - 1 ml/kg, p.o.)	8.46±0.08	0.47±0.20	48.03±1.90	2.13±0.23	22.44±0.40
Group – II (Negative control –Gentamicin - 80 mg/kg, i.p.)	4.86±0.20**	1.82±0.13**	98.27±3.77**	4.12±0.32**	45.92±1.65**
Group – III (SN - 100 mg/Kg, o. + Gentamicin - 80 mg/kg, i.p.)	6.83±0.14*	0.53±0.02**	52.67±1.96**	3.04±0.20*	24.61±0.77**

Values are expressed as the mean ± SEM from 6 animals in each group; differences inmeans were estimated by using one-way ANOVA followed by Dunnet's post hoc test. The values of Group II were compared with Group I and Groups III, was compared with Group II.
*P<0.05 = significant, **P<0.01 = moderately significant.

Results of histopathological examination of kidney in renoprotective activity of SN
Photomicrograph of sections (H&E staining, magnificationX40)

 FIG:1 CMC treatedkidney cells of rat	 G: 2 Gentamicin treatedkidney cells of rat	 FIG: 3 SN + Gentamicin treated kidney cells of rat

From **Table No: 3** it was observed there was a significant ($P<0.05$) decrease in serum total protein content, and increase in serum creatinine, urea, uric acid and blood urea nitrogen in gentamicin treated group II rats when compared to control group I. On administration of SN in group III rats showed significantly altered in the serum total protein content, and increase in Serum creatinine, urea, uric acid and blood urea nitrogen contents when compared to control groups.

The results revealed there was a significant decrease in body weight of gentamicin (80 mg/kg, i.p.) treated rats compared to normal control, CMC suspension treated group. The independent dose of SN (100 mg/kg, p.o.) treated rats increase the body weight significantly with respect to disease control group against gentamicin injection.

The histopathological results of kidney cells of CMC suspension treated control group of rats showed normal cellular architecture of renal glomerular and tubular cells (**FIG: 1**). Kidney cells of gentamicin treated group showed histological changes including interstitial nephritis with inflammatory cell infiltration, tubular necrosis, glomeruli congestion and dilatation of tubules with degeneration of epithelial cells (**FIG: 2**). The histology of kidney cells of SN with gentamicin treated rats showed moderate degree of interstitial inflammation with periglomerular and peritubular fibrosis (**FIG: 3**).

Gentamicin induced renal damage has manifested to be an exceptional working animal model for exploring the activity of many number of renoprotective drugs. Renal injury caused by gentamicin includes an elevated plasma creatinine and urea levels with critical proximal renal tubular necrosis, with progressive deterioration and renal failure (Mishra 2021) Nephrotoxicity may develop as a result of improper functioning of kidney-specific detoxification and excretion because of the damage or destruction of kidney function by toxicants of both exogenous and endogenous origin. Drug-induced nephrotoxicity or renal damage abides a serious complication as uses of nephrotoxic drugs are unpreventable in clinical therapy.(Aulbach 2023) Among all other nephrotoxic drugs, the aminoglycosidal antibiotic gentamicin is the widely used one because of its extensive broad spectrum of activity against infections, particularly aerobic gram-negative bacteria. Renal toxicity and ototoxicity are predominant toxic effects of gentamicin.

Gentamicin nephrotoxicity also engage with mesangial and vascular contraction and donates to inflammation, which is recognized by the infiltration of numerous

inflammatory cells like monocytes and macrophages. These immune cells release various pro-inflammatory cytokines including IL-1 and TNF-α, and activate the nuclear factor kappa B (NF-κB) pathway (Goldminz 2013).

Nephrotoxicity of gentamicin demonstrates a nonoliguric acute renal failure with declining renal blood flow (RBF) and disorders of urinary concentration and dilution. Therefore, it causes hypo-osmolar urinary output and increases in plasma creatinine after several days of treatment. Creatinine is produced from the metabolism of protein in muscles, with most creatinine being filtered out of the blood by the kidney and excreted in urine. The increases in urea and creatinine levels may designate reduction in the glomerular filtration rate due to gentamicin intoxication, as the serum concentration of these two variables depends predominantly on the glomerular filtration. (Arshney 2011)

The results revealed there was a significant decrease in body weight of gentamicin (80 mg/kg, i.p.) treated rats compared to normal control, CMC suspension treated group. The SN (100 mg/kg, p.o.) treated rats increase the body weight significantly with respect to disease control group against gentamicin injection. There was a significant decrease in total protein content and increase in serum creatinine, serum urea, serum uric acid and blood urea nitrogen (BUN) seen in biochemical estimation of gentamicin alone treated rats as comparable to normal control rats and the histopathological results of kidney cells supported with above results.

3. CONCLUSION

It is observed from the above results that administration of SN concurrently with gentamicin significantly inhibited the amelioration of kidney injury markers, like total protein, urea, uric acid, Blood Urea Nitrogen- BUN and creatinine. The study providesscientific evidence for renoprotective effects of orally administered bioactive compounds SN against gentamicin induced renal damage.

4. MATERIALS AND METHODS

a. Materials

i. Drug profile

Silymarin is (2R,3R)-3,5,7-trihydroxy-2-[(2R,3R)-3-(4-hydroxy-3-methoxyphenyl)- 2-(hydroxymethyl) -2,3- dihydrobenzo [b] [1,4] dioxin-6-yl] chroman-4-one. It is an anhydrous subststance, MP 158°. Silymarin induces apoptosis primarily through a p53- dependent pathway involving Bcl-2/Bax, cytochrome c release and caspase activation. It inhibits PGE2 -induced cell migration through inhibition of EP2 signaling pathways (G protein dependent PKA-CREB and G protein-independent Src-STAT3). (Katiyar 2005)

ii. Experimental animal

Healthy albino Wistar rats of either sex, weighing 180-220 g were procured from animal house of Sankaralingam Bhuvaneshwari College of Pharmacy, Sivakasi, Tamil Nadu were used for this study. All experiments were performed in accordance with CPCSEA guidelines and approved by Institutional Animal Ethics Committee (SBCP/2019-20/CPCSEA/IAEC/I(4)/F16/69).

Statistical results were expressed as mean ± SEM; differences in means were estimated by means of one way ANOVA followed by Dunnet's multiple comparison tests using Graphpad prism software.

b. Method

i. Study of renoprotective activity of SN

The screening of renoprotective

activity of SN were done in 3 different groups (n=6/group) of rats. The following **Table No: 1** shows the grouping pattern of rats and drug treatment used for the evaluating renoprotective activity by gentamicin-induced nephrotoxicity.

Study of renoprotective activity of SN in rats by gentamicin-induced nephrotoxicity

The renoprotective effect of SN against gentamicin-induced nephrotoxicity was studied in Wistar rats by treating them for 7 days as per the above treatment schedule. Group I rats received CMC 0.5 % suspension (1 mg/kg, p.o.) once daily for 7 days. Group II rats were treated only with gentamicin (80 mg/kg,i.p.) for 7 days and Group III, rats wereingested with SN (100 mg/kg, p.o.) 7 days. In order to induce nephrotoxicity, Group III rats was administered with gentamicin (80 mg/kg. i.p.) along with test drugs for 7 successive days.(Karadeniz 2008)

Table No: 1 grouping of animals for renoprotective activity of SN

S. No.	Treatment groups	Rats treated with
1	Group I (Control)	CMC 0.5 % suspension (1 ml/kg, p.o.)
2	Group II (Negative Control)	Gentamicin (80 mg/kg, i.p.)
3	Group III	SN (100 mg/kg, p.o.) + gentamicin (80 mg/kg, i.p.)

Estimation of body weight (Chinnala 2017)

The changes in the body weights were recorded initially on 0 day and finally on 8th day using electronic balance and the percentage change in body weights were calculatedusing formula:

$$\text{Percentage change in weight (g)} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

Sample collection and biochemical estimation

The blood was collected via retro-orbital sinus puncture under mild ether anesthesia 24 hrs after last injection for biochemical estimation. The collected samples were centrifuged for 10 min at 4500 rpm and the serum was separated rapidly. The separated serum samples were then processed for determining total protein, serum creatinine, serum urea, serum uric acid and blood urea nitrogen as an indicator of kidney damage (Chinnala 2017)**.** The results are given in Table No: 2. At the end of the study period, after determining biochemical parameters, the rats were sacrificed and both kidneys was dissected out, after washing in tap water further processing of histopathological studies were carried out as per the standard procedure, the results of images of histopathological studies were shown in

FIG: 1 to **3.**

Acknowledgements
The author wish to thank Ms.B.Sathya who helped in the preliminary work in the research.

Author contributions: Concept – S.Ö, B.Ü., A.K..; Design – S.Ö. A.K.; Supervision – S.Ö., A.K.; Resources – S.Ö, B.Ü., A.K.; Literature Search – S.Ö, B.Ü.; Writing S.Ö, B.Ü., A.K.; Critical Reviews – S.Ö, A.K.

Conflict of interest statement: The authors declared no conflict of interest.

5. References

1. Bent S, Ko R. Commonly used herbal medicines in the United States: a review. The American journal of medicine. 2004 Apr 1;116(7):478-85. https://doi.org/10.1016/j.amjmed.2003.10.036
2. Shakya AK. Medicinal plants: Future source of new drugs. International journal of herbal medicine. 2016;4(4):59-64.
3. Sabir S, Arsshad M, Asif S, Chaudhari SK. An insight into medicinal and therapeutic potential of Silybum marianum (L.) Gaertn. Int J Biosci. 2014;4(11):104-5. http://dx.doi.org/10.12692/ijb/4.11.104-115
4. Katiyar SK, Roy AM, Baliga MS. Silymarin induces apoptosis primarily through a p53-dependent pathway involving Bcl-2/Bax, cytochrome c release, and caspase activation. Molecular cancer therapeutics. 2005 Feb;4(2):207-16. https://doi.org/10.1158/1535-7163.207.4.2
5. Karadeniz A, Yildirim A, Simsek N, Kalkan Y, Celebi F. Spirulina platensis protects against gentamicin- induced nephrotoxicity in rats. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 2008 Nov;22(11):1506-10. https://doi.org/10.1002/ptr.2522
6. Chinnala KM, Achanta P, Vangala VL, Elsani MM. Evaluation for nephroprotective activity of ethanolic extract of Allium cepa linn. In gentamicin-induced nephrotoxicity in rats. EVALUATION 2017;10(3). http://dx.doi.org/10.22159/ajpcr.2017.v10i3.16271
7. Raju S, Kavimani S, Reddy KS, Kumar GV. Floral extract of Tecoma stans: A potent inhibitor of gentamicin-induced nephrotoxicity in vivo. Asian Pacific journal of tropical medicine. 2011 Sep 1;4(9):680-5. https://doi.org/10.1016/S1995-7645(11)60173-9.
8. Mishra P, Mandlik D, Arulmozhi S, Mahadik K. Nephroprotective role of diosgenin in gentamicin-induced renal toxicity: biochemical, antioxidant, immunological and histopathological approach. Future Journal of Pharmaceutical Sciences. 2021 Dec;7(1):1-3.
9. Aulbach AD, Ennulat D, Schultze AE. Interpretation of Clinical Pathology Results in Nonclinical Toxicity Testing. InHaschek and Rousseaux's Handbook of Toxicologic Pathology, Volume 2: Safety Assessment Environmental Toxicologic Pathology 2023 Jan 1 (pp. 505-566). Academic Press. https://doi.org/10.1016/B978-0-12-821047- 5.00027-0.
10. Goldminz AM, Au SC, Kim N, Gottlieb AB, Lizzul PF. NF-κB: an

essential transcription factor in psoriasis. Journal of dermatological science. 2013 Feb 1;69(2):89-94. https://doi.org/10.1016/j.jdermsci.2012.11.002.

11. Varshney A, Rehan M, Subbarao N, Rabbani G, Khan RH. Elimination of endogenous toxin, creatinine from blood plasma depends on albumin conformation: site specific uremic toxicity & impaired drug binding. PLoS One. 2011 Feb 28;6(2):e17230. https://doi.org/10.1371/journal.pone.0017230.