ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: https://ijrps.com

Hepato & nephro protective effects of naringenin-loaded tpgs polymeric nanosuspension against cisplatin-induced toxicity

Sumathi Rajamani[1], Gobinath Kalyanasundaram[1], Tamizharasi Sengodan[1], Sivakumar Thangavelu[1], Nikhitha K Shanmukhan[2], Arun Radhakrishnan[*2]

[1]Department of Pharmaceutical Biotechnology, Nandha College of Pharmacy and Research Institute, Erode, Tamil Nadu, India
[2]Department of Pharmaceutics, JSS College of Pharmacy, JSS Academy of Higher Education & Research, Ooty-643001, Tamil Nadu, India

Article History:

Received on: 04.04.2019
Revised on: 19.07.2019
Accepted on: 23.07.2019

Keywords:

Cisplatin,
Cancer,
Naringenin,
Hepatotoxicity,
Nephrotoxicity

ABSTRACT

Cisplatin (*Cis*-Diammineplatinum (II) dichloride/CIS) is one of the most potent chemotherapeutic agents widely used in treatment of various cancers. Naringenin (NAR), a natural *flavonoid,* protect against *CIS*-induced injury in rats without hampering CIS beneficial cytotoxic *activity* . Even though NAR exhibits therapeutic potency, clinical evolution of the molecule is embarrassed because of very less aqueous solubility which corresponds to low availability at the site of the tumor. In our former analysis, nanosuspension of naringenin (NARNS) was developed by the method of high-pressure homogenization. The study had been continued to evaluate the protective role of D-α-Tocopheryl polyethylene glycol succinate (TPGS) coated NARNS, against oxidative stress-induced hepato and nephrotoxicity in male Wistar rats upon CIS treatment. Induction of acute hepato and neprotoxicity was done by intraperitoneal injection (i.p) injection of CIS (7 mg/kg of body weight) and administration of NAR and NARNS. Administration of NARNS virtually suppressed CIS-induced and liver injury evidenced by a reduction of lipid peroxidation level, blood urea nitrogen, serum uric acid, creatinine and elevated enzymatic antioxidant activities of superoxide dismutase, catalase, and glutathione peroxidase in rats liver tissue. Histological studies substantiated the biochemical parameters. The study suggests that NARNS has strong hepato and nephroprotective effect compared to NAR.

*Corresponding Author

Name: Arun Radhakrishnan
Phone: 7402222019
Email: arun.r@jssuni.edu.in

ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v10i4.1544

Production and Hosted by

IJRPS | https://ijrps.com

INTRODUCTION

Even though CIS platin is an extensively used chemotherapeutic agent, it uses causes a wide range of side effects since they effect normal and cancerous tissues. Ototoxicity, neurotoxicity, nephrotoxicity, and hepatotoxicity are the major adverse events occurs due to the drug administration. CIS induced cytotoxicity is a contribution to reduce its application in therapy. Reactive oxygen species generation in cells caused by the CIS oxidative harm to mitochondria and inhibition of the antioxidant enzymes and arresting their activity leads to the release of free radicals. These mechanisms result in tissue damage and the toxic effect caused by

CIS. Naringenin a flavanoid with its proved protective mechanism in inflammation, antioxidant, neuroprotective, immunomodulator, anticarcinogenic agent, antiatherogenic and antiapoptotic activity were proved by the previous studies. Naringenin nanosuspension was formulated to overcome the bioavailability and solubility problems associated with the drug and studied further for their anti-inflammatory activity. This formulation with a significantly high anti-inflammatory activity compared to naringenin was studied further in the current study to evaluate the hepatoprotective and nephroprotective activity against CIS platin induced toxicity (Yen *et al.*, 2009; Sumathi *et al.*, 2017).

MATERIALS AND METHODS

Chemicals

TPGS was procured from commercial supplier Ludwigshafen, Germany. Cis was purchased from Sigma-Aldrich, Germany. NAR was bought from Zim laboratories Limited, Nagpur. Soya lecithin was obtained from Glenmark generics limited, Mumbai, India. And Chemicals purchased and used were of analytical grade.

Animals

Male albino Wistar rats weighing 130-140g were purchased for the study. Animals were exposed to controlled environmental conditions with constant temperature (25 ±2 °C) and humidity (55 ± 5%) and fed with laboratory feed and water. They were under a dark/light cycle of 14/10 h. Animals were in the specified laboratory conditions prior a week from the start of the study to ensure the animals were acclimatized to it. Institutional animal ethics committee (NCP/IAEC/2015-2016-04) was there for the performance of the study.

Hepatoprotective activity

30 Albino Wistar strain rats were equally distributed to five groups, each containing six animals. Intraperitonial injection of (7 mg/kg b.w.) was administered to induce CIS-toxicity. Group I (Control group): Animals were considered as normal control throughout the study procedure. Group II was treated with a single dose of cisplatin i.p on the fifth day of the experiment at a dose of 7 mg/kg (Koyuncu *et al.*, 2017). Group III, rats of this group were administrated with silymarin (10 mg/kg i.p; once daily) for 9 days, and on 5th day CIS (7 mg/kg b w) was injected by i.p route (Singh *et al.*, 2014). Group IV (CIS-NAR) rats of this group were administered with NAR (50 mg/kg) for 9 days, and on the 5th day, CIS was injected by i.p at a dose of 7 mg/kg. Group V**: (**CIS-NARNS) rats of this group were administered with NARNS (50 mg/kgi. P; once daily) for 9 days and CIS at a dose of 7 mg/kg was injected by i.p.on the fifth day of the treatment (Kapoor and Kakkar, 2014).

Animals were euthanized at the end of the experiment, under light anesthesia with diethyl ether; rats were euthanized by cervical decapitation. For biochemical assays, blood samples were collected, and serum was separated by centrifugation at 2500 rpm for 15 min. The samples were analyzed according to the prescribed procedure for required biochemical parameters. Total bilirubin and direct bilirubin was evaluated by Jendrassik & Grof's method (Garber, 1981). Triglycerides activity of serum estimated using Glycrokinase peroxidase method. CHOD-PAP assay was preferred for the study of total cholesterol activity. Albumin and total protein were evaluated by the biuret method (Gornall *et al.*, 1949). AST, ALT and ALP levels were assessed from the collected serum with commercially available kits by using an autoanalyzer (Garba *et al.*, 2011). Livers were excised, rinsed, cleaned in saline and preserved in 10% formalin for histopathological study (Upreti *et al.*, 1991).

Nephroprotective activity

Albino Wistar strain rats were divided into five groups of six each. Single-dose i.p. of CIS (7 mg/kg b.w.) was used to induce nephrotoxicity. Group I, the Control group animals, were selected as normal control. Group II-CIS treated group rats were administered with an intraperitoneal CIS i.p dose of 7 mg/kg on the fifth day from the start day of the experiment. Group III CIS-Cystone, rats of this group were administrated with Cystone (5ml/kg; p.o; once a day) for 9 days and on the 5th day CIS (7 mg/kg b w) was injected by i.p route (Rao and Rao, 1998; Sahoo *et al.*, 2011). Group IV-CIS-NAR, rats of this group, were administered with NAR (50 mg/kg i.p; once daily) for 9 days and on the 5th day, CIS was injected by i.p at a dose of 7 mg/kg. Group V-CIS-NARNS rats of this group were administered with NARNS (50 mg/kg i.p; once daily) for 9 days, and CIS at a dose of 7 mg/kg was injected by i.p. On the fifth day of the treatment (Rajappa *et al.*, 2017). Once the experiment was completed, rats were euthanized under light anesthesia with diethyl ether. Rats were euthanized by cervical decapitation. For biochemical assays, blood samples were collected and serum was separated by centrifugation at 2500 rpm for 15min and investigated for various biochemical constraints such as Blood Urea Nitrogen by DAM method (Allston, 1993), Serum Creatinine by Jaffe's Alkaline picrate method (Vasiliades, 1976; Zaveri *et al.*, 2011), and serum uric acid

were estimated using commercial kits (Hooper *et al.*, 2000; Zare *et al.*, 2006; Hooper *et al.*, 1998). Rats were euthanized, kidneys were excised, cleaned by rinsing with saline and10% formalin was used to preserve for histopathological study.

Tissue preparation

Dissected tissues were rinsed with cold PBS buffered solution (pH 7.4) and preserved at -80^0 C until the analysis. Tissue homogenate was prepared by homogenizing the tissue in a ratio of 1:3 (w/v; 1 g tissue with 3 ml PBS, pH 7.4) at 10000 rpm for 15min at 4^{0}C. The Clear supernatant was used for the determine malondialdehyde (MDA) contents, reduced glutathione (GSH) and antioxidant enzyme activities.

Biochemical estimation of markers of oxidative stress in liver and kidneys

Estimation of lipid peroxidation levels

The process of lipid peroxidation is fixed in the supernatant collected after the homogenization of hepatic tissue by the thiobarbuturic acid (TBA) method that figures the MDA formation. 1ml of 1% TBA and 50μl of tissue homogenates were added with 500μl of 70% alcohol. The prepared samples in test tubes were subjected to boiling water for 20min. 50μl of acetone was added to all the samples once they were cooled and absorbance was determined at 535nm in a spectrophotometer (Niehaus and Samuelsson, 1968).

Estimation of reduced glutathione levels

sulphydryl group-containing compound gives a yellow color with 5,5-dithiobis-2- nitrobenzoic acid. To 3ml of reaction mixture 50μl of tissue, the homogenate was added [1mg of β -NADPH + 1 mm glutathione reduced]. The sample was mixed by inversion and kept for equilibrated to 25^{0}C. Samples were then analyzed spectrometrically 340 nm. The sample containing 50μl of phosphate buffer (pH 7) and dithiothreitol was taken as blank. 50μl of 0.042% of hydrogen peroxide was equally added to the samples and instantly mixed by downturn and measured the reduction in absorbance at 340nm for (Ellman, 1959).

Estimation of antioxidant enzyme activities

The activity of Glutathione peroxidase (GPx) was calculated as an alteration of the calorimetric method (Flohé and Günzler, 1984), using hydrogen peroxide as a substrate in the existence of GSH. The absorbance was observed at 420nm. Glutathione-s-transferase (GST) was determined by the ensuing the conjugation of glutathione with 1-chloro-2,4-dinitrobenzene (CDNB) at 340nm (Habig *et al.*, 1974). Superoxide dismutase (SOD) activity was determined based on the capacity of SOD to inhibit the autoxidation of pyrogallol (Saggu *et al.*, 1989). Catalase (CAT) activity was determined from the rate of decomposition of H_2O_2 by the described method (Bergmeyer and Gawehn, 1974).

Histopathological examination

Rats were sedated under light anesthesia with diethyl ether. The kidneys and liver of the animal were dissected out. The liver and kidney were further perfused with buffer saline to remove excess blood. Kidney was isolated and stored at -20°C. The kidney was stabilized with 10% neutralized buffered formalin. Fixed materials were implanted in paraffin wax for the sectioning of the sample in a thickness of 5μm thickness. Slides were stained with hematoxylin and eosin for histological study.

Statistical analysis

The value represents as mean $\pm$ SEM. The results were studied using a one-way ANOVA followed by Dunnet test. * *p < 0.01, significant difference compared with the control group, * p < 0.05, significant difference compared with control group (n = 6).

RESULTS AND DISCUSSION

Parameters Assessed For Liver Functions

CIS administration resulted in significant elevation of bilirubin, triglycerides, total cholesterol, AST, ALT and ALP and bilirubin levels. Protein levels were significantly decreased in the test group compared to the normal control group indicating liver damage. Pretreatment with silymarin, NAR and NARNS remarkably prevented the biological changes induced by CIS. The effect of NAR and NARNS on liver parameters are given in Table 2, Figure 1 A & Figure 1 B. Administration of NAR and NARNS at a dose of 50mg/kg notably (* p < 0.05; * *p < 0.01) prevented hepatotoxicity induced by CIS. Data obtained are summarized into Table 1.

Estimation of lipid peroxidation levels

Degenerative properties of free radicals are well indicated with the lipid peroxidation of biomembranes. Levels of MDA in the above groups were observed. MDA level was markedly better in the CIS treated animals compare to the control group. Silymarin, NAR and NARNS significantly cause reduction of CIS-elevated MDA level compared to those of the CIS group.

Effects of NARNS on GSH contents

GSH is a sulfhydryl-having compound which is responsible for holding homeosis of cellular

Table 1: Effect of *NAR and NARNS on liver* function of CIS–induced liver injury in rats

Animal Group	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)	Triglycerides (mg/dl)	Total cholesterol (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	AST (U/L)	ALT (U/L)	ALP (U/L)
I	1.63± 0.02	0.50± 0.01	152± 3.24	206.5± 3.41	8.5± 0.20	5.26± 0.06	103.83± .98	40.35± 1.88	102.66± 0.55
II	3.82± 0.05	1.45± 0.03	238± 4.39	378.66± 5.02	4.75± 0.40	2.75± 0.33	166.5± 1.2	273.15± 6.05	215.0± 1.36
III	2.2± 0.01	0.70± 0.01	166± 3.92	249.0± 5.01	7.23± 0.04	4.87± 0.02	119.±1 .07	102.2± 3.71	161.0± 1.75
IV	3.61± 0.03**	1.31± 0.02**	202± 4.96**	307.16± 3.5**	6.33± 0.06*	4.09± 0.09**	134.16± 1.07**	190.1± 3.21	194.66± 1.11**
V	3.14± 0.02**	0.99± 0.04**	182± 5.04*	279± 4.42**	5.9± 0.12**	3.75± 0.03**	128.5± 1.05**	170.4± 6.12	179.66± 1.54**

Value represents as mean ± S.EM. * *p <0.01, significant difference compared with the control group, * p < 0.05,significant difference compared with control group (n = 6)

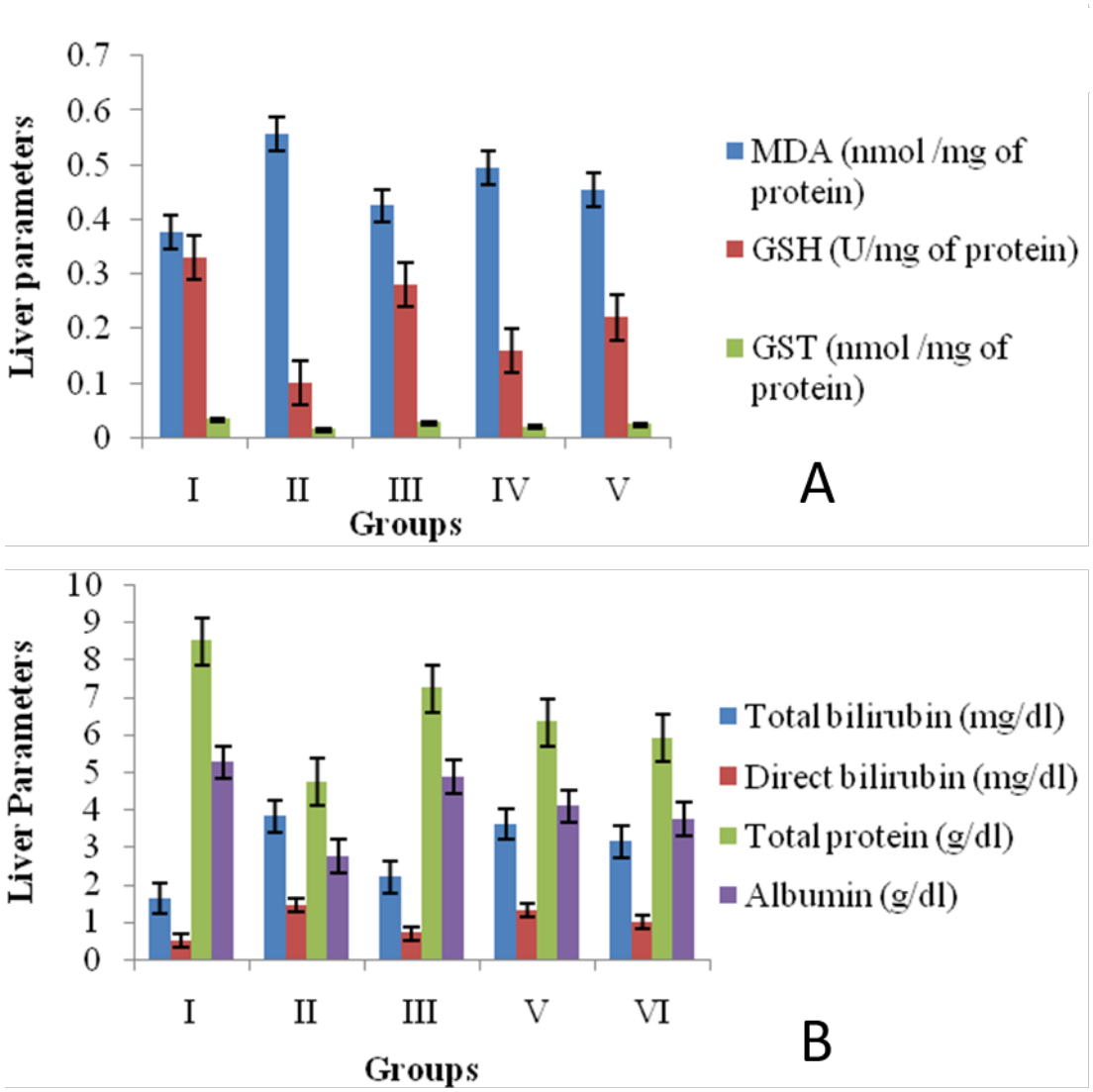

Figure 1: A) Effect of NAR & NARNS on antioxidant activity. B) Effect of NAR &NARNS on liver function

Table 2: Effect of *NAR* on liver tissue MDA, GSH levels and antioxidant enzyme activities of control and treated rats

Animal Group	MDA (nmol /mg of protein)	GSH (U/mg of protein)	GPX (U/mg of protein)	GST (nmol /mg of protein)	SOD (U/mg of protein)	CAT (U/mg of protein)
I	0.376±0.006	0.33±0.01	24.45±0.20	0.033±0.001	21.251±0.24	55.45±0.23
II	0.558±0.006	0.10±0.007	10.65±0.09	0.015±0.0008	10.48±0.17	30.48± 0.27
III	0.425±0.007	0.28±0.01	21.51±0.17	0.027 ± 0.003 0.027±0.0009	19.31± 0.27	50.26±0.23
IV	0.496±0.006**	0.16±0.006**	16.51±0.09**	0.027 ± 0.003 0.020±0.0008**	14.45±0.19**	37.56±0.24**
V	0.453±0.005*	0.22±0.007**	19.48 ±0.11**	0.024±0.0004*	17.75±0.18**	45.51±0.34**

Value represents as mean ± S.EM. * p< 0.05, significant difference compared with control group, ** p < 0.01, significant difference compared with 0.34 group (n = 6)

Table 3: Effect of *NAR and NARNS on* kidney function of CIS–induced Nephro injury in rats

Animal Group	BUN (mg/dl)	Serum uric acid (mg/dl)	Serum Creatinine (mg/dl)
I	14.52±0.03	2.43±0.02	0.92±0.01
II	42.74±0.03	6.53±0.03	2.65±0.07
IV	20.07±0.01	2.38±0.05	1.44±0.01
V	32.13±0.02**	2.71±0.05**	2.13±0.007**
VI	25.51±0.12**	2.55±0.01*	1.92±0.01**

Value represents as mean ± S.D. * *p < 0.01, significant difference compared with control group, * p < 0.05, significant difference compared with CIS group (n = 6)

oxidation-reduction. Alterations in GSH may be reflected as a representation of functional cell damage. Levels of GSH in the above groups were noted. The toxicity of CIS appreciably reduced the levels of hepatic GSH in the CIS treated animals while comparison with the control group. Administration of silymarin, NAR and NARNS ameliorated the GSH levels in when compared with the CIS-treated group.

Effects of NARNS on antioxidant enzyme activities

The activities of GPx, GST, SOD and CAT of the studied liver tissue were shown in Table 2, Figure 2 A & Figure 2 B. Administration of CIS lead to a lower GPx, GST, SOD and CAT activities while comparison with the control animals. But animals treated with silymarin expressed obviously increase in antioxidant enzyme level followed by NARNS as compared to CIS group.

Histopathological examination of liver tissue

The examination of liver tissues of the control group exhibited normal liver cell with a nucleus, central veins and preserved cytoplasm (Figure 3 A). While the CIS treated rats where observed with liver sections where severe structural damage identified with necrosis around the central vein, degeneration and sinusoidal dilatation and inflammatory cell infiltration (Figure 3 B). Histological section of the liver of the rat treated with CIS and silymarin (10 mg/kg) showed (Figure 3 C) marked development in contrast with CIS control group, and it reverted the histological appearance seen in the latter group to normal in examined tissue fields. However, the histopathological studies of liver sections from NAR (50mg/ kg) treated animals, were observed with a moderate degree of liver damage. Lesser inflammation, reduced hepatocyte degradation and centrilobular necrosis were observed with NAR treated group (Figure 3 D). The NARNS treated group was found with normal hepatocytes with least prominent inflammation.

(Figure 3 A) Normal sinusoidal space with normal morphology of hepatocytes with fine nuclear chromatin pattern. No perivenular inflammatory cell infiltration. (Figure 3 B) The histopathological observation in CIS treated rats liver shows sinusoidal space dilation. Mild dilation and congestion

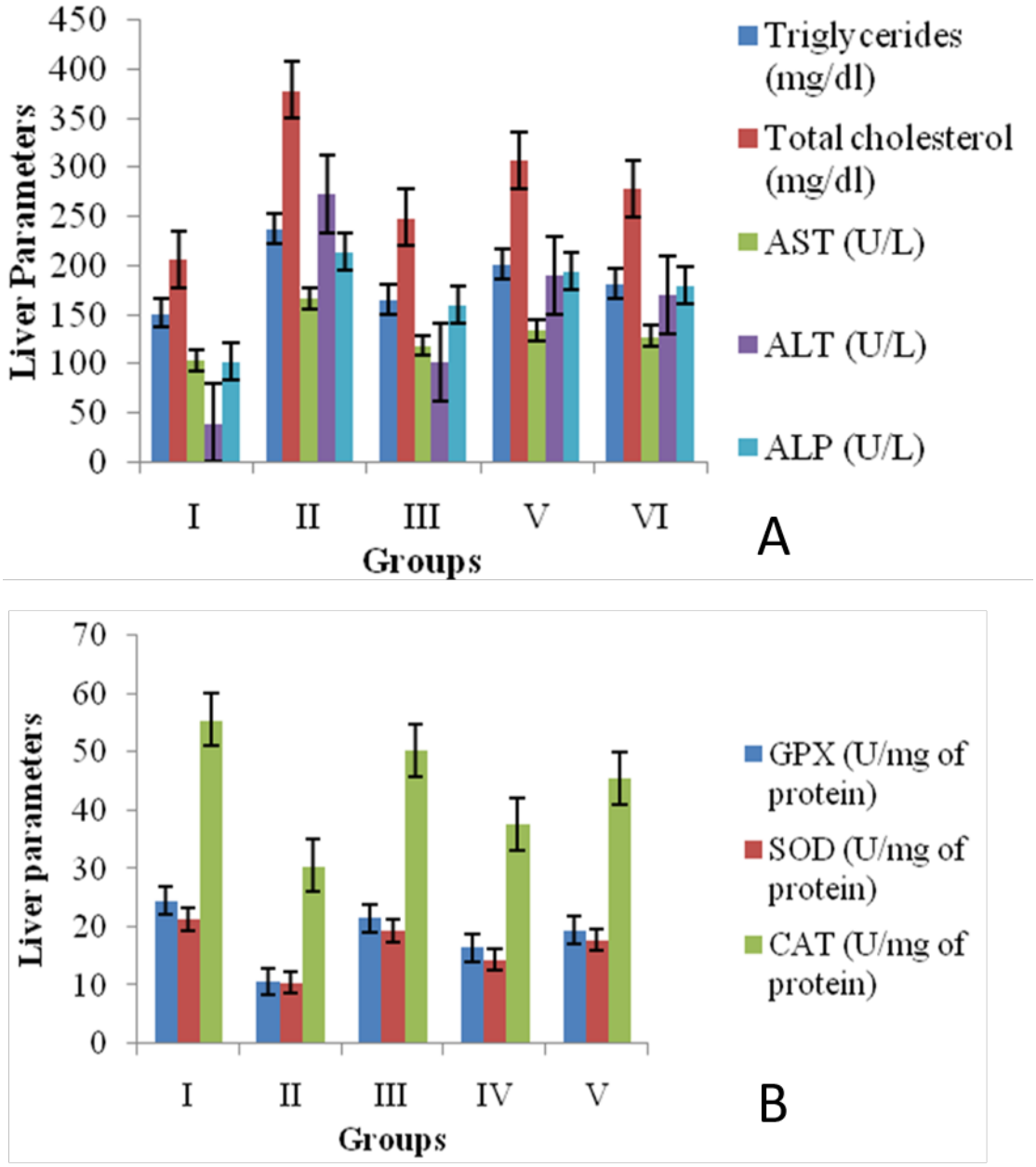

Figure 2: A) Effect of NAR & NARNS on liver function. B) Effect of NAR & NARNS on antioxidant activity

of sinusoidal space seen with hemorrhage Degeneration of hepatic cells with glycogen depletion and coarse nuclear chromatin condensation. Focal perivenular inflammatory cell infiltration. (Figure 3 C) The treatment with silymarin group observed minimal sinusoidal space dilation. Mild dilation and congestion of sinusoidal space seen without hemorrhage. Regeneration of hepatic cells with glycogen depletion and fine nuclear chromatin. Minimal perivenular inflammatory cell infiltration. (Figure 3 D) NAR treatment on rat liver shows mild glomerular atrophy, less disarrangement of hepatocytes, minimal proximal tubular necrosis, as well as marked regeneration activity. (Figure 3 E) Almost normal sinusoidal space of the liver. Regeneration of hepatic cells seen with normal glycogen content and fine nuclear chromatin. Minimal perivenular inflammatory cell infiltration.

Parameters Assessed For Kidney Functions

Biochemical parameters such as blood urea nitrogen, Serum urea, uric acid and creatinine concentrations were significantly improved in the group treated with only CIS as matched with the normal control, on the other hand, groups treated with Cystone, NAR and NARNS showed significantly low values when equaled with the positive control group. NARNS was observed as predominant nephropro-

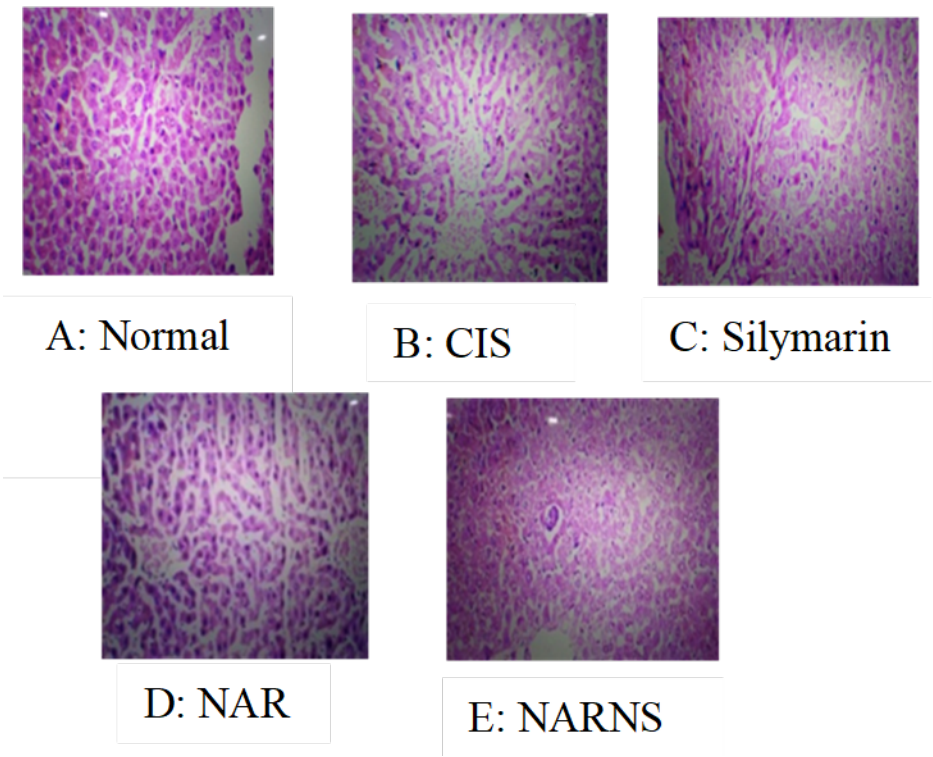

Figure 3: Photomicrographs of liver sections stained with hematoxylin–eosin below the light microscope

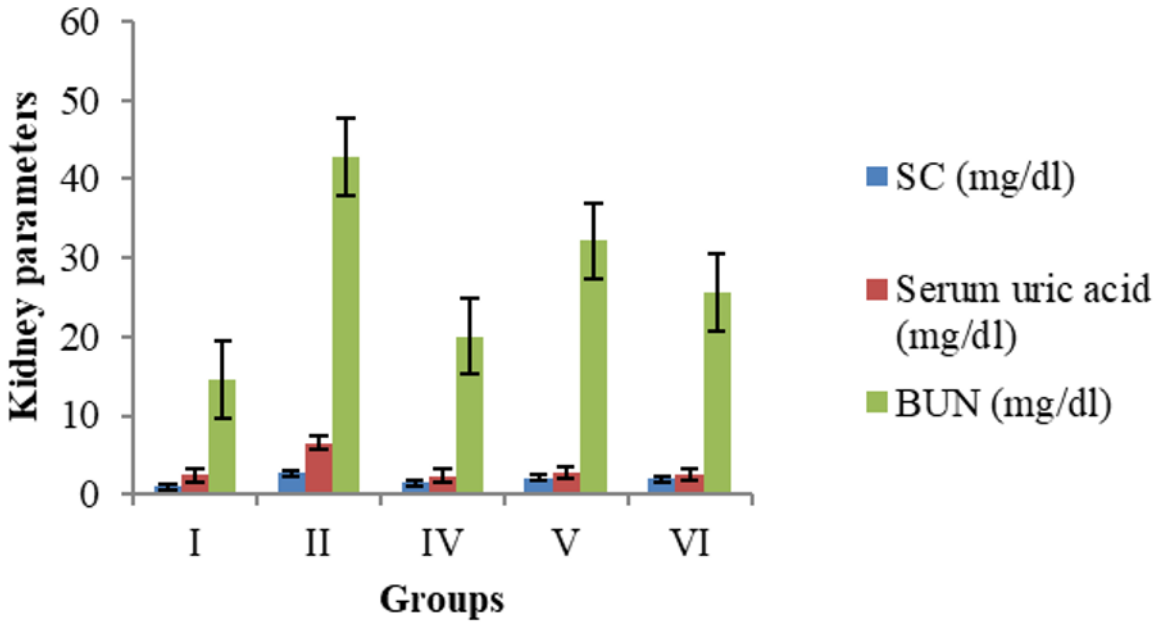

Figure 4: Effect of NAR & NARNS on kidney function

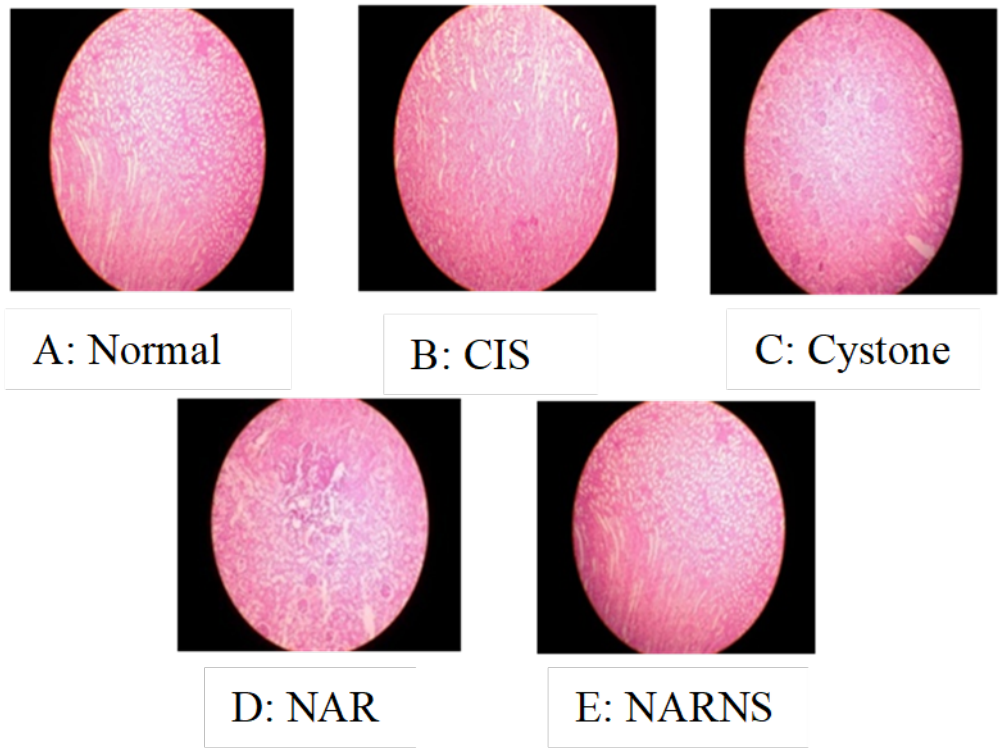

Figure 5: Photomicrographs of kidney sections stained with hematoxylin–eosin under the light microscope

tective effect compared to NAR (* $p < 0.05$; * *$p < 0.01$) was represented in Table 3 and Figure 4.

Histopathological examination of Kidney tissue

The sections of the kidney of normal control rats exhibited normal renal tubules and renal corpuscles. The glomeruli and Bowman's capsule appeared to be normal and prominent (Figure 5 A). In the CIS control group, the kidney demonstrated discriminating tubular necrosis, dilated proximal convoluted tubules, plodding of epithelium due to desquamation and atrophic glomeruli, cellular debris in the tubular lumen and better tissue in the interstitium, were an indication of CIS-induced necrosis of kidney, which were signs of nephrotoxicity (Figure 5 B). A Histological section of the kidney of the rat treated with CIS and Cystone (5 ml/kg) showed an improvement while comparing the CIS control group, and it reverted the histological characteristics observed in the latter group to normal in examining tissue fields. There were areas of tubular injury with tubular atrophy, tubular necrosis, and interstitial fibrosis, but these factors had a lesser intensity than in CIS control group (Figure 5 C). Kidney histological sections of rats treated with CIS + NARNS (50 mg/kg) showed marked improvement, showing normal kidney histology and architecture, with interstitial fibrosis. There were some foci viewing tubular injury with, tubular atrophy, tubular necrosis and interstitial fibrosis, which were less intense than those seen in CIS control group and CIS + NAR (50 mg/kg) group (Figure 5 D).

(Figure 5 A) Normal glomeruli and tubules were observed in normal rat kidney. It shows a normal glomerulus, proximal and distal tubules. (Figure 5 B) The histopathological observation in CIS treated rats showed glomerular atrophy, significant proximal tubular necrosis, tubular edema, hydropic degeneration of tubular epithelial cells and edematous intertubular spaces. (Figure 5 C) The treatment with Cystone group observed normal glomeruli, edematous proximal tubules without necrosis, normal tubular epithelial cells. (Figure 5 D) NAR treatment on rat kidneys shows mild glomerular atrophy, minimal proximal tubular necrosis, tubular edema, normal tubular epithelial cells and edematous intratubular spaces. (Figure 5 E) Almost normal appearance of kidney glomeruli and tubules were regenerated in NARNS.

CONCLUSION

The study results clearly suggested that oxidative stress, inflammation and apoptosis/necrosis play a critical role in the pathogenesis of CIS induced hepato and nephrotoxicity. Pretreatment with NARNS significantly attenuated CIS-induced functional and histological liver and renal deterioration compared

to NAR. One of the potential reason behind the NARNS-mediated protection of the cells were maybe the antioxidant activity. Before CIS administration, pretreatment with NARNS could inhibit the origin of free radicals produced by CIS prior to accomplish DNA and causing damage. The study delivers a solid proof for the use of the NARNS as hepato and nephroprotective activity against CIS induced damage in the liver and kidney. Therefore, NARNS may act as a chemoprotective agent to protect the cells from CIS-mediated toxicity.

REFERENCES

Allston, C. A. 1993. Non protein nitrogenous compounds and renal function. *Clinical Chemistry: Concepts and Application*, pages 369–369.

Bergmeyer, H. U., Gawehn, K. 1974. Methods of Enzymatic Analysis. volume 2, pages 438–439. Academic press. Weinheim Verlag Chemine.

Ellman, G. L. 1959. Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics*, 82(1):90090–90096.

Flohé, L., Günzler, W. A. 1984. Assays of glutathione peroxidase. *Oxygen Radicals in Biological Systems*, 12:114–120.

Garba, A., Mada, S. B., Ibrahim, G., Abarshi, M. M., Dauran, I. A., Hamza, A. B., Chopra, K. 2011. A convenient method for determining of Serum and bile phosphataseactivity. *American Journal of Biochemistry and Molecular Biology*, 4(1):342–346.

Garber, C. C. 1981. Jendrassik-Grof analysis for total and direct bilirubin in serum with a centrifugal analyzer. *Clinical Chemistry*, 27(8):1410–1416.

Gornall, A. G., Bardawill, C. J., David, M. M. 1949. Determination of serum proteins by means of the biuret reaction. *The Journal of Biological Chemistry*, 177(2):751–766.

Habig, W. H., Pabst, M. J., Jakoby, W. B. 1974. Glutathione S-transferases the first enzymatic step in mercapturic acid formation. *Journal of biological Chemistry*, 249(22):7130–7139.

Hooper, D. C., Scott, G. S., Zborek, A., Mikheeva, T., Kean, R. B. 2000. Uric acid, a peroxynitrite scavenger, inhibits CNS inflammation, blood-CNS barrier permeability changes, and tissue damage in a mouse model of multiple sclerosis. *The FASEB Journal*, 14(5):691–698.

Hooper, D. C., Spitsin, S., Kean, R. B., Champion, J. M., Dickson, G. M., Chaudhry, I., Koprowski, H. 1998. Uric acid, a natural scavenger of peroxynitrite, in experimental allergic encephalomyelitis and multiple sclerosis. *Proceedings of the National Academy of Sciences*, 95(2):675–680.

Kapoor, R., Kakkar, P. 2014. Naringenin accords hepatoprotection from streptozotocin induced diabetes in vivo by modulating mitochondrial dysfunction and apoptotic signaling cascade. *Toxicology Reports*, 1:569–581.

Koyuncu, I., Kocyigit, A., Gonel, A., Arslan, E., Durgun, M. 2017. The Protective Effect of Naringenin-Oxime on Cisplatin-Induced Toxicity in Rats. *Biochemistry Research International*, 2017:1–10.

Niehaus, W. G., Samuelsson, B. 1968. Formation of Malonaldehyde from Phospholipid Arachidonate during Microsomal Lipid Peroxidation. *European Journal of Biochemistry*, 6(1):126–130.

Rajappa, R., Magesh, B., Sarvajayakesavulu, S., Madhunapantula, V. 2017. Nephroprotective Effect of Naringenin against Multiple Low Dose Streptozotocin (MLDSTZ) Induced Renal Damage in Mice. *Biomedical and Pharmacology Journal*, 10(02):583–593.

Rao, M., Rao, M. N. 1998. Protective effects of cystone, a polyherbal ayurvedic preparation, on cisplatin-induced renal toxicity in rats. *Journal of Ethnopharmacology*, 62(1):3–8.

Saggu, H., Cooksey, J., Dexter, D., Wells, F. R., Lees, A., Jenner, P., Marsden, C. D. 1989. A Selective Increase in Particulate Superoxide Dismutase Activity in Parkinsonian Substantia Nigra. *Journal of Neurochemistry*, 53(3):692–697.

Sahoo, S., Panda, P., Pani, S., Mishra, S. 2011. Nephroprotective effect of Bauhinia variegata (linn.) whole stem extract against cisplatin-induced nephropathy in rats. *Indian Journal of Pharmacology*, 43(2).

Singh, S., Girotra, P., Gupta, S. 2014. Targeting silymarin for improved hepatoprotective activity through chitosan nanoparticles. *International Journal of Pharmaceutical Investigation*, 4(4):156–163.

Sumathi, R., Tamizharasi, S., Sivakumar, T. 2017. Formulation and evaluation of polymeric nanosuspension of naringenin. *International Journal of Applied Pharmaceutics*, 9(6):60–70.

Upreti, G. C., Davis, C., Oliver, J. 1991. Preparation of representative homogenates of biological tissues: Effect of salt on protein extraction. *Analytical Biochemistry*, 198(2):298–301.

Vasiliades, J. 1976. Reaction of alkaline sodium picrate with creatinine: I. Kinetics and mechanism of formation of the mono creatinine picric acid complex. *Clinical Chemistry*, 22(10):1664–1671.

Yen, F. L., Wu, T. H., Lin, L. T., Cham, T. M., Lin,

C. C. 2009. Naringenin-Loaded Nanoparticles Improve the Physicochemical Properties and the Hepatoprotective Effects of Naringenin in Orally-Administered Rats with CCl4-Induced Acute Liver Failure. *Pharmaceutical Research*, 26(4):893–902.

Zare, F., Magnusson, M., Bergström, T., Brisslert, M., Josefsson, E., Karlsson, A., Tarkowski, A. 2006. Uric acid, a nucleic acid degradation product, down-regulates dsRNA-triggered arthritis. *Journal of Leukocyte Biology*, 79(3):482–488.

Zaveri, M., Desai, N., Movaliya, V. 2011. Effect of Ocimum basilicum on cisplatin models of acute renal failure. *Adv Res Pharm boil*, 1:91–100.