Original Article

Protective Effect of L-Arginine on Diclofenac Mediated Renal Toxicity in Adult Albino Rats

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ABSTRACT

Objective: The objective of this study is to observe the protective effect of L-arginine in the Diclofenac sodium in experimental animal study.

Study Design: An experimental study

Place and Duration of Study: This study was conducted at the Institute of Bio Medical Sciences (IBMS) with the cooperation of Dow Diagnostic Research and Reference Laboratory (DDRRL) in Dow University of Health Sciences (DUHS), Ojha campus from December, 2013 to January 2014.

Materials and Methods: Forty adult albino rats weighing 200 ± 20 gms were used and divided into four groups. The rats in the control group A,(n=10) each were given intra-muscular injection, one cc of physiologic saline. The second group B,(n=10), was given Diclofenac sodium 2 mg/kg body weight, intra-muscular. The rats in the third group C,(n=10) were given Diclofenac sodium 2mg/kg by intra-muscular injection and L-arginine, 1mg/kg of body weight, by feeding tube. The fourth group D,(n=10) was given L-arginine, with the ratio of 1mg/kg of body weight, by feeding tube orally. Gross and histological changes were observed in the proximal convoluted tubules (PCT) of the kidney of albino rats, after completion of the two weeks treatment.

Results: The result of present study revealed that diclofenac sodium significant decrease in body weight in adult albino rats was after 2 weeks treatment with in a single daily dose of 2mg/kg intra muscular. There were changes in absolute kidney weight, cortical thickness of kidney and diameter of proximal convoluted tubules. Treatment with L-arginine in a daily dose of 1mg/kg body weight, partially prevented the Diclofenac sodium induced damage of proximal convoluted tubules. L-arginine alone in same daily dose had no adverse effect on kidney in young albino rats.

Conclusion: Diclofenac sodium, an NSAID induces nephrotoxicity and L-arginine when given simultaneously partially protects the kidneys from its harmful effects.

Key Words: Diclofenac sodium, L-arginine, proximal convoluted tubules

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INTRODUCTION

Diclofenac sodium, belongs to NSAIDs family, manifests its analgesic and anti-inflammatory activities by inhibiting cyclooxygenase pathway of prostaglandin synthesis.[1]

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As a consequence of its anti-inflammatory and analgesic activities, it has been recommended for pain management in various inflammatory disorders like ankylosing spondylitis, rheumatoid arthritis, degenerative joint disease etc.[2] It is available universally, so its side-effects are found frequently in the general population. Its unusual adverse effects are hypersensitivity, renal disease, gastrointestinal disorders, and cardiovascular disease.[3,4]

Diclofenac sodium decreases the formation of prostaglandin, by inhibiting an enzyme called cyclooxygenase in the cells. Prostaglandin ranges from 1.20 to 1.81 ng /ml in the plasma. Prostaglandin is produced and consumed by kidneys, which helps in renal physiology like glomerular filtration rate, control of renal blood stream, renin secretion, relocation of ion and water metabolism in the tubules.[5]It was postulated that prostaglandins, also participate in the self-regulation of renal blood supply, metabolism of renin and tubular exchange mechanisms.[6]

L-arginine an α-amino acid, it is levo-rotatory form of Arginine.[6] In humans, arginine commonly originates as

a semi essential or essential amino acid, relying on the growing phase and fitness of the person. Arginine is not synthesized by preterm infants, needs it from animal or plant sources.[7] L-arginine woks by producing Nitrous oxide (NO) is produced through L-arginine by nitrous oxide synthetase.[8] It is also postulated that arginases are group of enzymes made up of arginine, responsible for the healing of different tissues are made up of arginine.[8,9] However, in persistent renal illnesses, ureteral obstruction, diabetic nephropathy, high blood pressure and in aging process, valuable properties of L-arginine have been verified.[10]

The freedom to buy pharmaceutical drugs that have not been prescribed by registered health practitioners, is raising serious concerns in Pakistan. Unlike developed countries where the management of medicines is under effective regulations and over the counter medicines are well categorized. In Pakistan buying almost any type of medicine without prescription is easy.[11]

Self -medication is one of the causes of the renal injury. Diclofenac sodium is one of the commonest over- the-counter used drug. It is used commonly to manage pain and fever in pre and post-operative patients. Therefore, this study was conducted to assess the role of L-arginine in the renal tubular damage caused by the Diclofenac sodium in the albino rat.

MATERIALS AND METHODS

It was an experimental study. It was conducted after getting approval from Institutional Review Board (IRB). The experiment was accomplished in the Institute of Bio Medical Sciences (IBMS), with the cooperation of Dow Diagnostic Research and Reference Laboratory (DDRRL) in Dow University of Health Sciences (DUHS), Ojha Campus, Karachi in 2013. Non probability, purposive, sampling technique was used. Adult albino rats of both sexes, active and healthy, were included in the study. They were weighing 180-200 grams, and taking food and water normally. However, lethargic, inactive and constantly weight declining animals were considered unfit for the experiment. Sample size was forty albino rats of 180-200 grams of both sexes.

In the experimental room of animal house, animals were kept on well-adjusted food and water with 12 hours day and night cycle. Every animal was weighed at the start of experiment and before sacrifice. Animals were divided into four groups: A, B, C and D groups comprising of ten rats in each group.

Group –A (control): The animals of this group received normal saline 1 cc intra-muscular for 2 weeks.

Group-B (Diclofenac sodium treated group): Diclofenac sodium 2mg/kg body weight intramuscularly daily was given for 2 weeks.

Group-C (Diclofenac sodium + L-arginine treated group): Diclofenac sodium 2mg/kg body weight intra-muscular and L-arginine was given orally at a dose of 1mg/kg body weight, daily for 2 weeks.

Group-D (L-arginine treated group): L-arginine 1mg/kg body weight was given, daily for 2 weeks. (L-arginine was administered through feeding tube half an hour prior to the administration of Diclofenac sodium)

Each animal was anaesthetized in ether before sacrificing. A sharp scalpel was used for incision, starting from xiphoid process up to the pubic symphysis. A magnifying glass was used to recognized and examine the kidneys, for any noticeable variations in size, shape, color, outline and uniformity. The kidneys were isolated and "Sartorius balance" (Sartorius Precision Balance, Model No.MSE 1203S, Sartorius Lab Instruments, GmbH & Co.KG) was used to measure the absolute and relative weight of each kidney.

Tissue section was made by using a rotary microtome of "Bright instrument co. model OTF 5000 and UK" longitudinal sections of 5µm thick were cut, floated on a hot water bath of "Thermo- Fisher Scientific, USA" on a 370c, fixed on gelatinized glass slides. Histological sections of kidney tissue of approximately 10x10 mm in size from each group-A, B, C & D, were fixed in10% formalin and stained with heamatoxylin and eosin.

General morphology and architecture of the kidneys was observed and after screening, ten observations per animal were recorded. However, five observations for each of the parameters were recorded. Parameters noted in this study were, weight of animals (initial and after two weeks). However, microscopic variables were cortical thickness of kidney, in which 10X optical and 4X objective lens with the support of counting reticule were used to calculate the cortical thickness of kidney. The diameter of proximal convoluted tubules was calculated along their long and short axis, under 10X ophthalmic and 10X objective lens with the aid of counting reticule in randomly chosen five spots within the juxtamedullary regions in all animals.

Then micrometry is done by calibration of the ocular micro meter and the counting reticule and synchronized with stagemicro- meter. Right eye was used for ocular micro meter and left eye for reticule by using the3B Scientific Binocular course Microscope Model 300, Hamburg, Germany. Each and every observation and measurement was verified by the supervisor.

Statistical analysis: Statistical analysis was done by using SPSS version 16. To assess the significance between various groups studied, one-way analysis of variance (ANOVA) was applied. Multiple comparisons to a control are also referred to as many-to-one comparisons. The outcomes were stated as mean ± standard deviation and $p < 0.05$ was measured statistically significant at confidence interval of 95%.

RESULTS

During experimental period all the animals remained alive. The animals of group A (control), group-C (Diclofenac sodium and L-Arginine treated) and group D (L-Arginine treated) appeared healthy, reacted quickly to outer stimuli and increased weight steadily. However, the animals of group B (Diclofenac sodium treated) looked lethargic and revealed a weaker response to the outer stimuli gradually from 3rd to 14th day; their appetite and body weight was reduced.

Initial body weights of Albino rats were compared in the study. In group A(control) initial body weight was (204.20 ± 6.08 gm) compared to other group B (diclofenac treated, wt:199.90 ± 8.76 gm), group C (diclofenac and arginine, 193.50 ± 31.88 gm) and group D (L-arginine, 193.50 ± 31.88 gm) was insignificantly decreased (P-Value obtained at C.I of 95 % was 0.546).

However, after two weeks of administration of Diclofenac sodium and L-Arginine, final body weight of albino rats was reducing significantly (P- Value at C.I of 95 % obtained was < 0.001) where as insignificant decrease was observed when compared to group D (the P-Value at C.I of 95 % obtained was < 0.012) shown in Figure No.1.

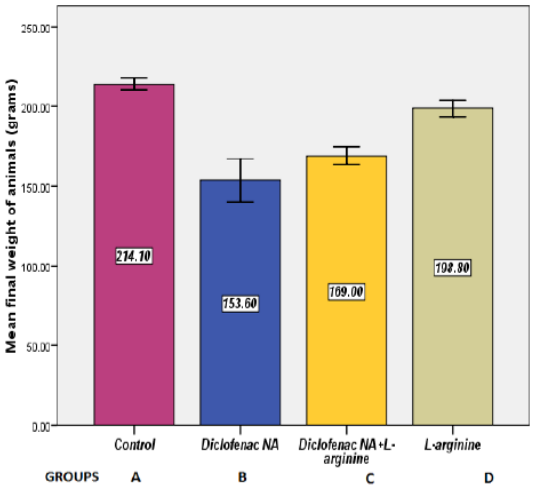

Figure No. 1: Mean final weight of animals (gram)

Table No. 1: Multiple comparison Dunnet test for cortical thickness (µm) among groups

Groups		Mean Difference	Std. Error	Sig.
Group (B) Diclofenac Na	Control Group (A)	8.000	6.109	0.426
Group (C) Diclofenac Na+ L-arginine	Control Group (A)	10.480	6.109	0.223
Group (D) L-arginine	Control Group (A)	7.740	6.109	0.452

In H & E stained sections, diameter of proximal convoluted tubules of kidneys was measured and compared between the control and the treated groupsshown in Figure No.: 2-5. Mean diameter of proximal tubule in group A was found to be 50.68 ± 11.86 µm. When compared with proximal tubule diameter in group B (113.52 ± 25.68 µm), a highly significant increase (P- Value at C.I of 95 % obtained was < 0.001) was observed. However, in histological sections ingroup C(67.94 ± 10.54 µm) diameter decreases insignificantly withP-Value at C.I of 95 % obtained was 0.051. Andgroup D (56.79 ± 9.40 µm) also reveal an insignificant decrease in diameter (P-Value at C.I of 95 % obtained was 0.722).

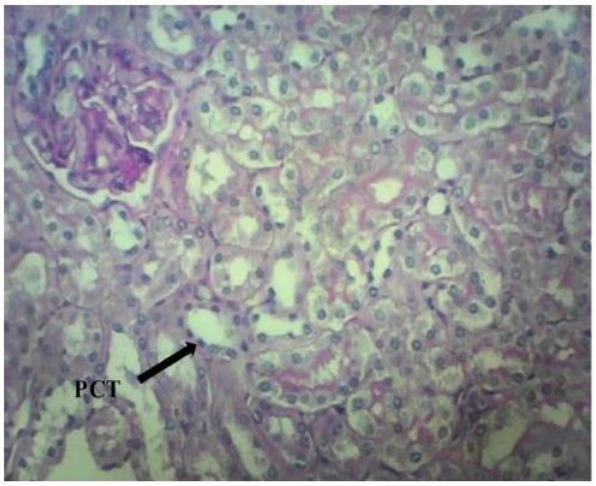

Figure No. 2: H&E stained, 5µm thick longitudinal section of kidney from group A (control) showing normal proximal convoluted tubule (PCT) 10x10 X

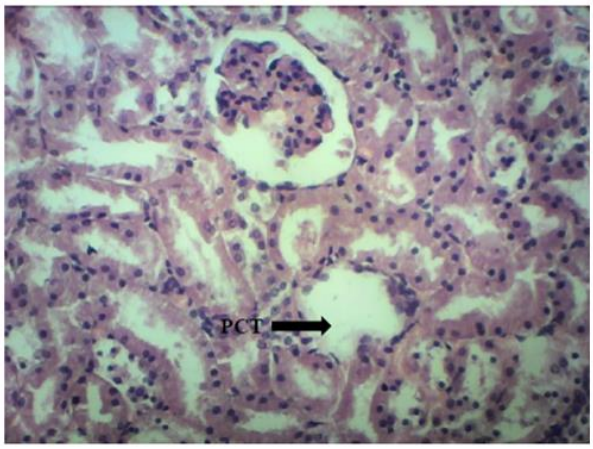

Figure No.3: H&E stained, 5µm thick longitudinal section of kidney from group B (Diclofenac sodium) showing dilated proximal convoluted tubule (PCT) 10x10 X

Microscopically, when assessment of cortical thickness of kidneys within the control group A (163.28 ± 16.84 µm) and the treated groups (group B: 171.28 ± 14.16µm, group C: 152.80 ±7.27 µm, groupD: 155.54

±14.45 µm) was compared. A significant reduction in the cortical thickness in kidneys was observed in all treated groups, except group B, in which cortical thickness was increased insignificantly shown in Table No.1.

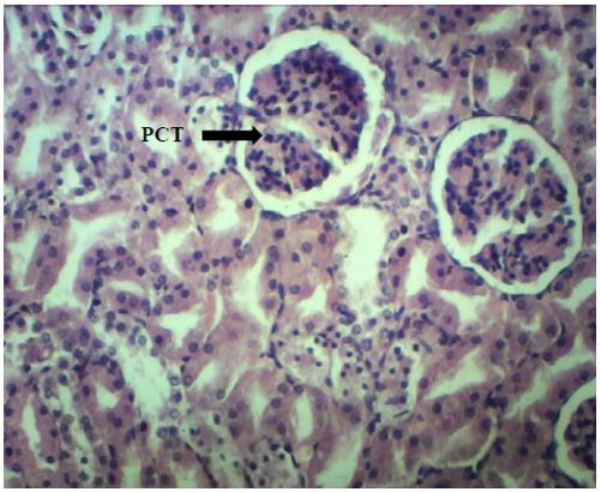

Figure No. 4: H&E stained, 5µm thick longitudinal section of kidney from group C (Diclofenac sodium + L arginine) showing near to normal proximal convoluted tubule (PCT) 10x10 X

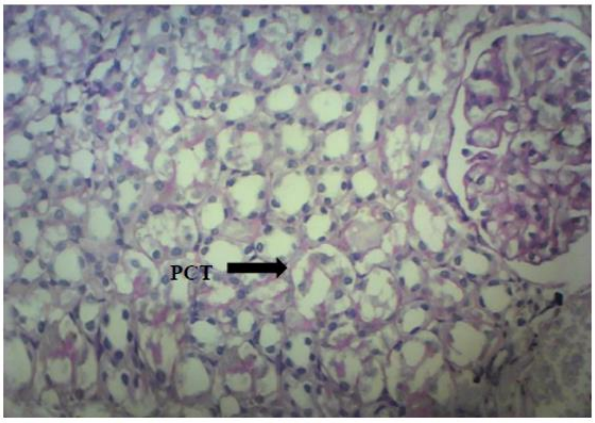

Figure No. 5: H&E stained, 5µm thick longitudinal section of kidney from group D (L-arginine) showing normal proximal convoluted tubule (PCT) 10x10 X

DISCUSSION

Non-steroidal anti-inflammatory drugs (NSAIDS) in prolong use causes nephrotoxicity, several researches have reported. Many attempts have been made to prevent the analgesic nephropathy with vitamins and anti-oxidants like ascorbic acid. However, a study reported that L-arginine produces a protective effect against drugs, radiations or nephrotoxicity induced by ischemia.[12]

In this study loss in body weight in albino rats was observed after treatment with diclofenac sodium. Reduction in the body weight was probably because of lack of hunger as one of the side effects of Diclofenac sodium on gastrointestinal tract. Our study observations are in agreement with Farag et al, who noted decrease in body weight in patients receiving Diclofenac sodiu.[13] However, insignicant decrease in body weight was seen in group D, which may be due to effects of L-arginine on proximal convoluted tubules, as it increases blood circulation causing vasodilatation. Our results are in agreement with Witting and Horwit, who also reported supportive effects of L-arginine on growth rate.[14]

Microscopically cortical thickness in group B was notably increased as compared to group A, which probably due to cell swelling, inflammation and dilatation of proximal convoluted tubules. This observation was in agreement with Lansa et al and Wua G et al.[15] However, significant reduction in cortical thickness was observed after treatment with L-arginine in groups C and group D, which may be attributed to protective effect of L-arginine that may have prevented the acute damage to kidney by ischemic release of free radicles. [16,17]

In this study microscopically, in renal cortex, diameter of proximal tubules was found tobe increased significantly in group B as compared to groups A, C and D, this might be due to cells destruction in proximal convoluted tubules and mild edema. Our findings are in complete agreement with Scott et al, who observed, after ingestion of NSAID there will be damage and shedding of renal tubular cells in urine.[18,19] However, group C treated with L-arginine prior to administration of Diclofenac sodium there was no significant change in the diameter of proximal tubules when compared with control. These results may be explained on the basis of stabilization of the blood flow of proximal tubules due to beneficial effects of L-arginine. Our findings are in conformity with study conducted by Rhoden, who described that due to pre-treatment with L-arginine, lipid per oxidation of renal cells and renal dysfunction induced by renal ischemia in rats were significantly reduced.[20]

CONCLUSION

It is concluded that diclofenac sodium induces renal toxicity, however, L-arginine produces protective effect on Diclofenac sodium induced nephrotoxicity and these results could be reflected promising enough to permit further studies.

Author's Contribution:

Concept & Design of Study:	Abdul Rehman Rajput
Drafting:	Maria Mohiuddin, Hemant Kumar
Data Analysis:	Amtul, Rosheena
Revisiting Critically:	Azmat, Maria Mohiuddin
Final Approval of version:	Abdul Rehman Rajput

Conflict of Interest: The study has no conflict of interest to declare by any author.

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