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Effect of Silymarin on Gentamicin Induced Nephrotoxicity in Rats

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Abstract

Background: As oxidative stress is an important factor in producing nephrotoxicity by a variety of drugs and chemicals; so, it may be assumed that agents having antioxidant property may protect the kidney from oxidative damage and can improve renal function. **Objective:** In the present study the ameliorative effect of silymarin was determined in a gentamicin-induced nephrotoxic model of rat. **Methodology:** This experimental animal study was carried out in the Department of Pharmacology at Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh from January 2014 to January 2015 for a period of one year. In this study nephrotoxicity was induced by administering gentamicin (80 mg/kg/day for 7 days) intraperitoneally. Silymarin was administered (500 mg/kg/day for 14 days) orally concomitantly with gentamicin (7 days) and sacrificed on 15th day. To determine nephrotoxicity and amelioration of nephrotoxicity as well as the status of oxidative stress and lipid peroxidation; serum creatinine, serum urea, renal cortical reduced glutathione and malondialdehyde levels were estimated. Changes in renal architecture were estimated by histopathology of renal tissues. **Results:** Group (II) rats were injected gentamicin intraperitoneally for 7 days and sacrificed on 15th day showed significant ($P< 0.001$) increase of serum creatinine and urea level while there was significant ($P< 0.001$) reduction of renal cortical glutathione and increase ($P< 0.001$) in malondialdehyde concentration when compared to the control group (group I). This was supported by histological observations of H&E and PAS stained transverse section of renal cortex which suggested significant ($P< 0.001$) level of structural damage of renal cortex as evidenced from glomerular atrophy, tubular degeneration, presence of desquamated cellular debries and cast in the tubular lumen, mononuclear inflammatory cell infiltration. Statistically significant amelioration was observed in all the biochemical parameters which were supported by histology of renal cortex in silymarin treated group. **Conclusion:** The results from biochemical and histological observations of the present study indicate that silymarin was probably effective to ameliorate the signs of toxic damage to the renal cortex. [*Bangladesh Journal of Infectious Diseases, December 2023;10(2):71-76*]

Keywords: Silymarin; gentamicin; nephrotoxicity

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Introduction

Kidneys, the pair of major excretory organs of vital importance of the human body and endowed with the natural filtration system. Renal disorder indicates impaired functions of the kidney which if untreated may lead to renal failure which can be mediated by nephrotoxic drugs or chemicals to which the kidneys may be exposed. Drugs constitute common mechanisms to exert their toxic effects. These include altered intraglomerular hemodynamics, tubular cell toxicity, inflammation, crystal nephropathy and thrombocytic microangiopathy. Successful management or prevention of acute kidney injury needs appropriate knowledge of pathogenic mechanisms responsible for nephrotoxicity[1]. Gentamicin (GM) is an aminoglycoside antibiotic which is used in clinical practice to treat severe gram-negative infections and is a well-known research tool for the induction of nephrotoxicity[3]. Gentamicin induced nephrotoxicity is associated with the production of reactive oxygen species; leading to macrophage infiltration, protein oxidation and lipid peroxidation which causes tubulointerstitial nephritis, renal tubular necrosis, decrease glomerular filtration rate and may culminate in renal failure but still they are preferred for their potent bactericidal activity, post antibiotic effect and low cost[4-5].

Silymarin (SM) is extracted from the seeds of *Silybum marinum,* commonly known as milk thistle and this plant is native to the Mediterranean region in Europe, in North Africa and in the Middle East[6]. Silymarin has powerful antioxidant property which can protect biological systems against oxidative stress by scavenging of free radicals, decreasing formation of reactive oxygen species and inhibition of fatty acid per oxidation[7]. Silymarin is used mostly in liver and gallbladder disorders and is well recognized hepatoprotective herbal medicine. It has been suggested that silymarin may be used in preventing free radical-related diseases as a dietary natural antioxidant supplement[8-9]. As oxidative stress is an important factor in producing gentamicin induced nephrotoxicity and silymarin possesses strong antioxidant property; it may be assumed that any drug or disease which produces acute tubular necrosis involving oxidants could be prevented by silymarin.

Methodology

This was an experimental animal study conducted in the Department of Pharmacology at Bangabandhu Sheikh Mujib Medical University from January 2014 to January 2015 for a period of one year.

Chemicals and Reagents: Kit for estimation of serum creatinine and urea were obtained from Human (Germany). Reduced glutathione was obtained from Loba Cheme (India) for estimation of GSH level. Thiobarbituric acid, trichloroacetic acid and 1,1,3,3 Tetraethoxy Propane (MDA standard) was obtained from Sigma Aldrich Chemie Gmbh (Germany) for estimation of MDA. Gentamicin (80mg/ml) was obtained from Opsonin Pharma Ltd. Bangladesh. 50 gm of silymarin was obtained from Square Herbal & Nutraceuticals Ltd. Bangladesh.

Silymarin: Based on detailed studies a non-toxic concentration of 500 mg/kg/day was selected for oral administration to the rats[7, 10].

Animals: Adult Long Evans Norwegian rats aged between 8 to 12 weeks and weighed between 150-250g were obtained from the animal house of Bangabandhu Sheikh Mujib Medical University. The rats were housed in standard size metallic cages (3 rats/ cage) and were allowed to live at room temperature with 12 hours of light and 12 hours of dark schedule in a well ventilated room. They were fed normal rat diet and given water *ad libitum*. For the purpose of identification, rats were marked with permanent ink daily on their body surface.

Experimental Design: Each group contains eight rats and groupings were done as follows. Group I (C) Distilled water was injected (1 ml/rat/day i.p for seven days) and sacrificed on eighth day. This served as the Control group. Group II (GM) rats received intraperitoneal (i.p) injection of Gentamicin at a dose of 80 mg/kg/day for seven days and were sacrificed on the fifteenth day. Group III (GM+SM) rats received concomitant treatment with intraperitoneal (i.p) injection of Gentamicin at a dose of 80 mg/kg/day for seven days and Silymarin at a dose of 500 mg/kg/day orally through Ryle's tube for fourteen days and were sacrificed on the fifteenth day. Group IV (SM) rats received Silymarin (500 mg/kg/day) orally for fourteen days and sacrificed on the fifteenth day.

Sample Collection Procedure: Two samples were collected. One was serum and another was kidneys.

Collection of Blood and Serum: Approximately 3-4 ml blood from each rat was collected from the carotid artery in separate clean and dry test tubes with proper identification numbers. Separated serum was collected into separately labeled

Eppendorf's tubes with the help of micropipette after centrifugation at 4000 rmp and stored at -20^{0}C for biochemical analysis.

Collection and Preservation of Kidneys: Both kidneys were dissected out after opening the abdomen by median incision of the rats. One kidney was immersed immediately into Tyrode's solution which was placed in an ice bath and kept at -20^{0}C until homogenized. Another kidney was preserved in 10% formalin for subsequent histological processing.

Biochemical Measurements: Serum creatinine concentration by alkaline picrate method[11], Serum urea concentration by enzymatic method[12], Renal cortical GSH concentration of renal cortex by Ellman's method[13], Renal cortical MDA concentration[14].

Histological Procedure: Sections from three levels per kidney of each rat of different groups were taken transversely and placed in the fixing fluid (10% formalin), dehydrate in graded alcohol then embedded in paraffin wax. They were sectioned at 5µm thickness and stained with Haematoxiline and Eosin (H&E) stain and Periodic acid Schiff (PAS) stain for light microscopic examination. Level of damages were evaluated by the indices such as glomerular atrophy, tubular degeneration and necrosis, accumulation of desquamated cellular debris and cast into tubular lumen, mononuclear inflammatory cell infiltrations. Arbitrary scoring was done as follows[15]: 0 for no detectable lesion, 1 for less than 25% examined field revealed degenerative change (mild), 2 for Less than 50% examined field revealed degenerative change (moderate), 3 for less than 75% examined field revealed degenerative change (severe), 4 for more than 75% examined field revealed degenerative change (devastating).

Statistical Analysis: The results obtained from biochemical findings were expressed as mean ± SD. Data were analyzed by one-way ANOVA followed by Students unpaired 't' test to determine significance between different groups. The difference between groups were considered highly significant at $p<0.001$, moderately significant at $p<0.01$ and significant at $p<0.05$.

Results

Group (II) rats were injected gentamicin intraperitoneally for 7 days and sacrificed on 15th day showed significant ($P< 0.001$) increase of serum creatinine and urea level while there was significant ($P< 0.001$) reduction of renal cortical glutathione and increase ($P< 0.001$) in malondialdehyde concentration (Fig. I) when compared to the control group (group I). This suggested that these rats were made model for nephrotoxicity. This was supported by histological observations of H&E and PAS stained transverse section of renal cortex which suggested significant ($P< 0.001$) level of structural damage of renal cortex as evidenced from glomerular atrophy, tubular degeneration, presence of desquamated cellular debries and cast in the tubular lumen, mononuclear inflammatory cell infiltration (Table 1; Fig. II: Photographic plate II). The renal cortical sections of control group of rats showed normal appearance of glomeruli, renal tubules and interstitium (Table 1; Fig. II: Photographic plate I).

Silymarin treated nephrotoxic rat groups (group III) showed significant ($P< 0.001$) reduction of serum creatinine, urea and significant ($P< 0.001$) elevation of renal cortical glutathione and reduction of ($P< 0.001$) MDA level when compared to the corresponding gentamicin treated nephrotoxic group (II) of rats (Fig. I). Histopathological observations of renal cortical architecture of silymarin supplemented nephrotoxic rat groups had also shown significant level of recovery when compared to corresponding gentamicin treated groups (Table I; Fig. II: Photographic plate III).

Table 1. Histology of Renal Tissues with Qualitative Changes and Arbitrary Score

Histopathological changes	Groups			
	I (C)	II (GM)	III (GM+SM)	IV (SM)
Glomerular Atrophy	0.0 ± 0.0	1.4 ± 0.4^a	1.2 ± 0.3^{ad}	0.0 ± 0.0^b
Tubular Degeneration	0.0 ± 0.0	2.8 ± 0.5^a	2.1 ± 0.3^{ac}	0.0 ± 0.0^b
Debris in Tubular Lumen	0.0 ± 0.0	1.6 ± 0.5^a	1.1 ± 0.3 ^{ac}	0.0 ± 0.0^b
Mononuclear Inflammatory Cell Infiltration	0.0 ± 0.0	1.1 ± 0.3^a	0.4 ± 0.5 ^{ac}	0.0 ± 0.0^b
Presence of Cast in Tubular Lumen	0.0 ± 0.0	1.2 ± 0.4^a	0.7 ± 0.2 ^{ac}	0.0 ± 0.0^b

^{a}P< 0.001 and ^{b}p >0.05 when compared to control group (I); ^{c}P< 0.001 and ^{d}p> 0.05 when compared to the group (II)

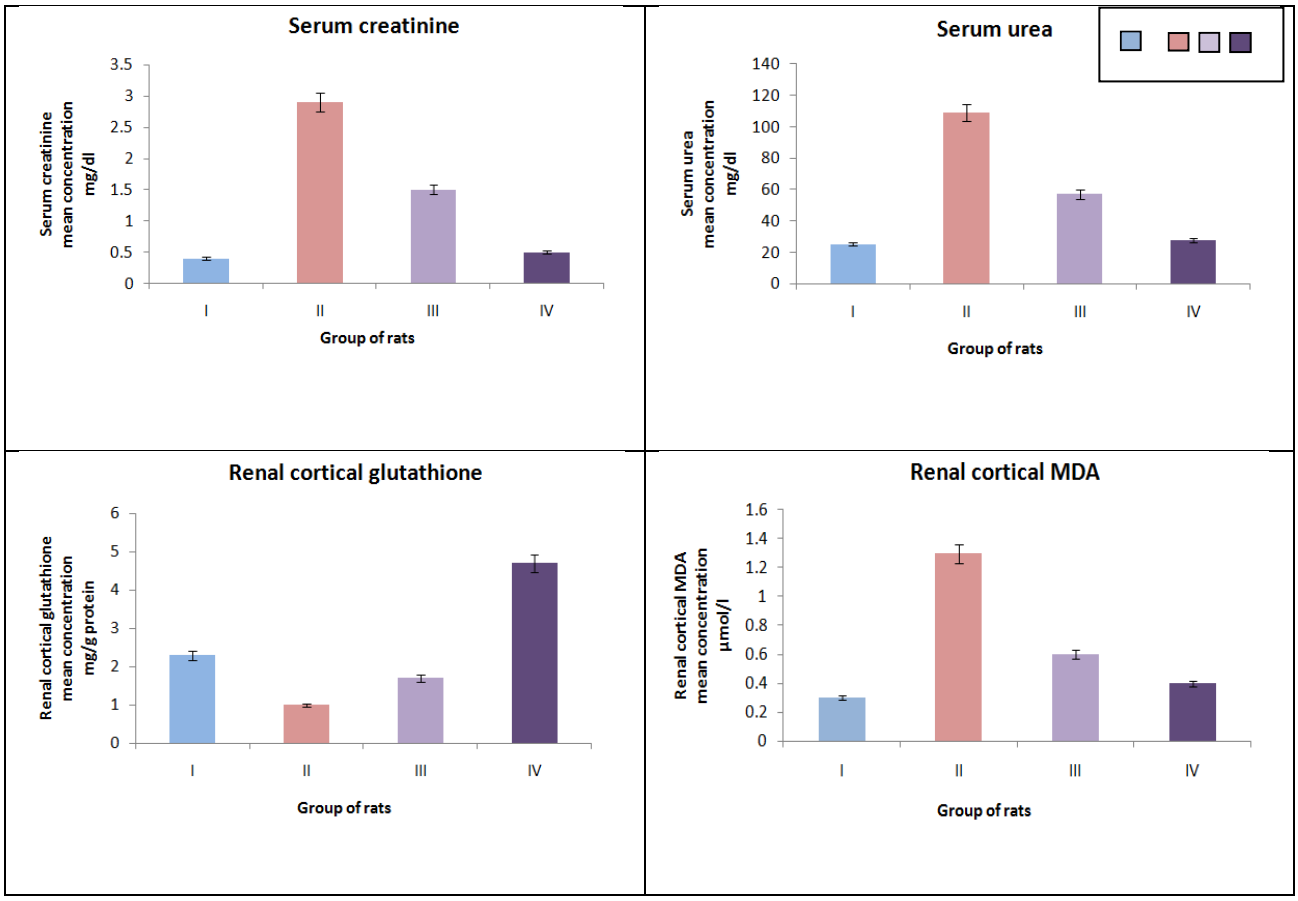

Figure I: Bar diagram showing the mean concentrations of serum creatinine (A), urea (B), renal cortical glutathione (C) and malondialdehyde (D) levels in different groups of rat [Control group I, Gentamicin (GM) treated group II, Concomitant treatment group III (GM + SM), Only silymarin treated group IV (SM)]

Rats treated with silymarin alone (group IV) showed significant increase in serum creatinine ($P< 0.05$), serum urea ($P<0.001$) and significant ($P<0.001$) elevation of renal cortical glutathione level, while there were no significant changes in renal cortical MDA levels (Figure I) when compared to control group (I) and the histological observations of renal cortex shows almost similar renal architecture to those of control group rats (Table I; Photographic plate IV).

Discussion

In this study, a widely used aminoglycoside antibacterial, gentamicin was administered intraperitoneally to rats to induce nephrotoxicity. Nephrotoxicity was evident by increase in serum creatinine, serum urea and renal cortical MDA level and depletion of renal cortical glutathione level which was supported by histology of renal cortex. These observations were similar to previous research work done by other researchers[6,17]. Silymarin is a well-known ready available powerful antioxidant medicine which can protect organs against oxidative damage[8-9] and used in the present study to antagonize the oxidative damage of the renal cortex by free radicals as a result of gentamicin administration.

The results of biochemical and histological observations showed significant alleviation of toxic effects in silymarin treated nephrotoxic group of rats when compared to those of corresponding gentamicin treated nephrotoxic groups. These observations appear similar to those of previous works reported by other researchers with different herbs containing antioxidant property[18-19].

The group of rats treated with silymarin alone has demonstrated significant elevation of serum creatinine and urea levels when compared to those of Control group. The elevation of serum creatinine and serum urea level may be due to the use of high dose of silymarin or it might be due to other active ingredients of silymarin probably to produce pro-oxidant effect on kidney like certain antioxidant agents reported earlier[20].

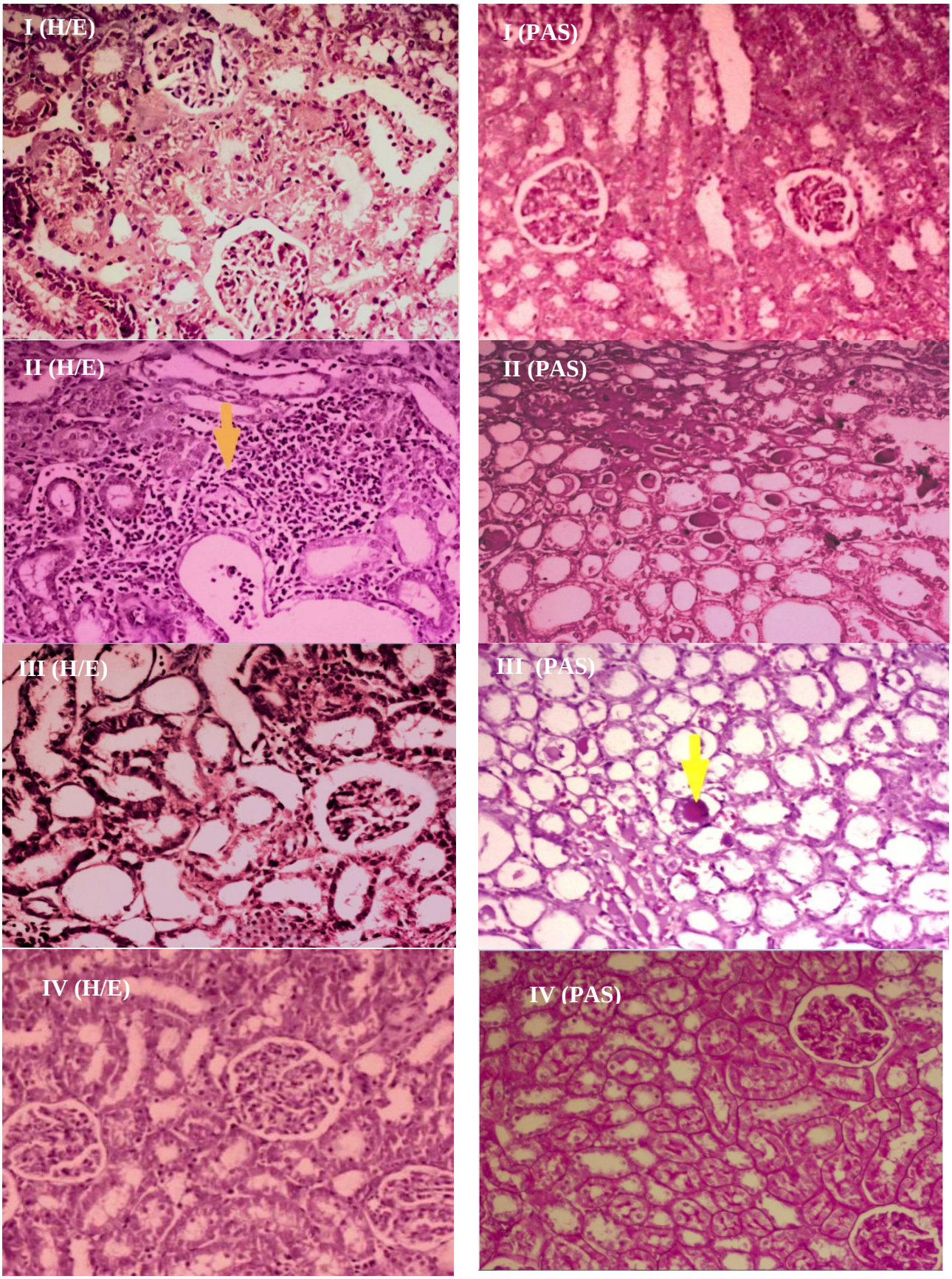

Figure II: Representative photograph of sections of renal cortex under light microscope of rat treated with distilled water (Control group I; microscopic plate I); treated with gentamicin (80mg/kg/day for 7 days) sacrificed on 15th day (group II; microscopic plate II); treated with gentamicin (80mg/kg/day for 7 days) along with silymarin (500mg/kg/day for 14 days) and sacrificed on 15th day (group III; microscopic plate III), treated with silymarin alone (500mg/kg/days,14 days) and sacrificed on 15th day (group IV; microscopic plate IV)(H/E & PAS, 200x).

Conclusion

The cumulating results of this study suggested that the oral administration of silymarin was able to produce considerable protection and alleviation from the nephrotoxic action of gentamicin in rats. These occurred possibly by inhibition of lipid peroxidation due to the antioxidant activities offered by the contained phytochemicals. Further studies with different doses for a longer duration and follow-up for any adverse effect are required to confirm these results.

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None

Conflict of Interest
The authors declare 'no conflict of interest.'

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Contribution to authors:
Hilmi SR, Dewan ZF, Kabir AKMN: Conception and design, or design of the research, the acquisition, analysis, or interpretation of data; Hilmi SR: involved in data input and data cleaning. Islam MM, Yusuf MA, Afreen KN, Akter M: drafted the manuscript. All authors reviewed and approved the final manuscript.

Data Availability
Any questions regarding the availability of the study's supporting d ata should be addressed to the corresponding author, who can provide it upon justifiable request.

Ethics Approval and Consent to Participate
The Institutional Review Board granted the study ethical approval. Since this was an animal study, it was not needed informed consent.

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