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ORIGINAL ARTICLE

Comparison of Effect of Silymarin and Ethanol Extract of *Trigonella foenum-graecum* on Gentamicin Induced Nephrotoxicity in Rats

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Abstract

Background: A variety of drugs and chemicals possess nephrotoxic potentials; therefore it is of keen interest to the researchers to obtain ways and means for alleviation of nephrotoxicity. **Objective:** The present study was designed to compare the ameliorative effect of silymarin with ethanol extract of *Trigonella foenum-graecum* in an experimental model of gentamicin- induced nephrotoxic rats. **Methodology:** This animal study was conducted by giving distilled water intraperitoneally to control group for seven days (1ml/rat/day) and was sacrificed on 8th day. To induce nephrotoxicity, gentamicin (GM) was administered (80 mg/kg/day for 7 days) intraperitoneally and sacrificed on 15th day. The ethanol extract of *T. foenum-graecum* was administered (500 mg/kg/day for 14 days) orally concomitantly with GM (7 days) and sacrificed on 15th day. To another group of rats, silymarin was administered (500 mg/kg/day for 14 days) orally concomitantly with gentamicin (7 days) and sacrificed on the 15th day. Biochemical indices like serum creatinine and serum urea levels were estimated to determine nephrotoxicity and amelioration of nephrotoxicity in all rat groups. To determine the status of oxidative stress and lipid peroxidation, the renal cortical glutathione (GSH) and renal cortical malondialdehyde (MDA) levels were estimated. **Results:** Statistically significant amelioration was observed in all the biochemical parameters in *T. foenum-graecum* and silymarin treated groups. **Conclusion:** The ameliorating effect of *T. foenum-graecum* is much more effective in comparison to that of silymarin in nephrotoxicity. [*Journal of Current and Advance Medical Research 2019;6(1):23-27*]

Keywords: *Trigonella foenum-graecum*; silymarin; gentamicin; nephrotoxicity

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Introduction

The epidemiology of renal failure differs in different region of the world. Studies indicate that it may be more in South East Asia than in the Western world. The young people are more affected in South East Asia whereas elderly male people are more affected in Western countries[1-2].

Renal impairment indicates disordered functions of the kidney which if untreated may lead to renal failure. Renal failure may also occur through nephrotoxicity which can be mediated by nephrotoxic drugs or chemicals to which the kidneys may be exposed. Report is available that, death within 90 days among hospital admitted patients was 13.1% other than acute renal failure, 34.5% with acute renal failure as principal diagnosis and 48.6% with acute renal failure as a secondary diagnosis[2]. The aetiology of acute renal failure is due to ischemia, nephrotoxic injury, acute tubular interstitial nephritis and acute glomerular nephritis[3]. Moreover 50.0% of hospital acquired acute renal failure were due to drug causes such as aminoglycosides, non-steroidal anti-inflammatory drugs (NSAIDs) or angiotensin converting enzyme inhibitor (ACE-I) administration[4].

Gentamicin, a renowned member of the aminoglycoside group of drugs, has proven its efficacy against many aerobic Gram negative organisms[5]. The nephrotoxic effect of gentamicin is established which limits its clinical use but still they are preferred for their potent bactericidal activity, post antibiotic effect and low cost[6]. About 20% patients treated with this drug may show signs of nephrotoxicity[7]. Gentamicin induced acute renal tubular necrosis is associated with the production of reactive oxygen species which may culminate in renal failure[8,9]. As a result of oxidative stress, the endogenous antioxidants such as reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) decrease in concentrations while products of oxidation such as malondialdehyde (MDA) would increase in concentration[10-13].

South East Asia is a centre for natural herbs and plants rich in anti-oxidant ingredients. The local doctors of these countries including Bangladesh have been using these herbs and plants as medicines through centuries from time immemorial. One such plant is *Trigonella foenum-graecum* (Fenugreek), locally known as 'methi' by the rural people of Bangladesh and also to countries around. *Trigonella foenum-graecum* (FG) is an annual plant of the family Fabaceae. *T. foenum-graecum* seed extracts have antioxidant property and protects cell from oxidative damage as well as exhibits its health benefits and potential medicinal properties in various indications and has little or no side effect[14, 15]. Among all extracts of *T. foenum-graecum*, the ethanolic extract is reported to show the highest antioxidant activity[16].

Another such plant is *Silybum marinum*, commonly known as milk thistle which is a member of Asteraceae family. Silymarin is extracted from the seeds of milk thistle and this plant is native to the Mediterranean region in Europe, in North Africa and in the Middle East[17]. Milk thistle is used mostly in liver and gallbladder disorders and is well recognized as a hepatoprotective herbal medicine[18]. Silymarin have antioxidant effect and this effect is mediated by scavenging of free radicals, decreasing formation of reactive oxygen species and inhibition of fatty acid peroxidation and have significant protective or ameliorative effect against drug-induced kidney disease based on findings from animal studies[19-23].

As oxidative stress is an important factor in producing gentamicin induced nephrotoxicity which leads to acute tubular necrosis; it may be assumed that any drug or disease which produces acute tubular necrosis could be prevented by silymarin and ethanol extract of *T. foenum-graecum*.

Methodology

This was an experimental study conducted in the Department of Pharmacology at Bangabandhu Sheikh Mujib Medical University. **Chemicals and reagents:** Gentamicin (80 mg/ml) was obtained from Opsonin Pharma Ltd. (Bangladesh). Kits for estimation of serum creatinie 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 tetraethoxypropane (MDA standard) was obtained from Sigma Aldrich Chemie Gmbh (Germany) for estimation of MDA. 50 gm of silymarin was obtained from Square Herbal & Nutraceuticals Ltd. Bangladesh. *Trigonella foenum-graecum* was purchased from local grocery shop.

Ethanol extract of *Trigonella foenum-graecum:* 2 kg of FG seeds were cleaned shed dried and grinded by grinding machine. 1.19 kg powder was suspended in 7.35 l of 70% ethanol for 72 hrs then they were filtered and the filtrate was concentrated

in rotary vacuum evaporator. 122 gm of condensed ethanol extract of FG was obtained. A dose of 500 mg/kg/day was selected for oral administration to the rats[24]. **Silymarin:** Based on detailed studies a non-toxic concentration of 500 mg/kg/day was selected for oral administration to the rats[19, 25].

Animals: Adult Long Evans Norwegian rats weighed between 150-250g and aged between 8-12 weeks 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 80mg/kg/day for seven days and were sacrificed on the fifteenth day. **Group III (GM+FG)** rats received concomitant treatment with intraperitoneal (i.p) injection of Gentamicin at a dose of 80 mg/kg/day for seven days and ethanol extract of *Trigonella foenum-graecum* 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 (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 V (FG):** Rats received ethanol extract of *Trigonella foenum-graecum* (500 mg/kg/day) orally for fourteen days and sacrificed on fifteenth day. **Group VI (SM):** Rats received silymarin (500 mg/kg/day) orally for fourteen days and sacrificed on the fifteenth day.

Biochemical measurements: Serum creatinine concentration by alkaline picrate method[26], Serum urea concentration by enzymatic method[27], renal cortical GSH concentration of renal cortex by Ellman's method[28], renal cortical MDA concentration[29].

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 mlondialdehyde concentration (Table I) when compared to the control group (group I). This suggests that these rats were made model for nephrotoxicity. The ethanol extract of *Trigonella foenum-graecum* treated nephrotoxic rat groups showed significant ($P<0.001$) reduction of serum creatinine, urea and significant ($P< 0.001$) elevation of renal cortical glutathione and reduction ($P< 0.001$) of MDA level when compared to the corresponding gentamicin treated nephrotoxic rat group II (Table I). Silymarin treated nephrotoxic rat groups also 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 (Table I).

Table 1: Levels of biochemical indices of experimental and control rat groups

Variables	I(C)	II(GM)	III(GM+FG)	IV(GM+SM)	V(FG)	VI (SM)
S. creatinine (mg/dl)	0.38 ± 0.08	2.93± 0.19^{a}	1.30 ±0.09^{ade}	1.45±0.12^{ad}	0.45±0.08^{c}	0.51±0.11^{bh}
S. urea (mg/dl)	25.49± 0.71	109.09±2.85^{a}	52.76±1.80^{ade}	57.14±3.28 ^{ad}	26.30± 0.71^{c}	27.81±1.19^{ag}
Cortical GSH (mg/g)	2.30± 0.08	1.04±0.06^{a}	1.68±0.09^{adf}	1.65±0.08^{ad}	4.66±0.13^{a}	4.67±0.09^{ah}
Renal MDA (µmol/l)	0.31± 0.05	1.28±0.09^{a}	0.63±0.06^{adf}	0.62±0.09^{ad}	0.34±0.06^{c}	0.35±0.05^{ch}

$^{a}P< 0.001$,$^{b}P< 0.05$, $^{c}p >0.05$ when compared to control (I) group; $^{d}P< 0.001$when compared to group II; $^{e}P< 0.05$, $^{f}P >0.05$ when compared to group IV; $^{g}p< 0.05$, $^{h}p >0.05$ when compared to group V

The ameliorating effect of *Trigonella foenum-graecum* when compared to silymarin; *Trigonella foenum-graecum* treated group (III) of rats showed a significant ($P< 0.05$) reduction of serum creatinine and urea levels in comparison to that of silymarin treated group (IV) of rats, while there were no significant ($P> 0.05$) differences in renal cortical glutathione and MDA levels in between these two groups.

Rats treated with *Trigonella foenum-graecum* alone (group V) showed no significant change in serum creatinine, serum urea and renal cortical MDA levels, whereas significant ($P< 0.001$) elevation of renal cortical glutathione level (Table I) when compared to control group (I).

Rats treated with silymarin alone (group VI) 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 was no significant changes in renal cortical MDA levels (Table I) when compared to control group (I).

Discussion

Nephrotoxicity in rats were induced by using a widely used aminoglycoside antibacterial, gentamicin at a specified and well- tried dosage[30]. Nephrotoxicity was evident by significant increase in serum creatinine, serum urea, renal cortical MDA and depletion of renal cortical glutathione. These biochemical observations were similar to previous research work done by other researchers[7, 31].

Silymarin and the ethanol extract of *Trigonella foenum-graecum* possess strong anti-oxidant properties[9,20-21]. So, both of them were used in the present study to antagonize the oxidative damage of the renal cortex by free radicals as a result of gentamicin administration. Silymarin is a well-known ready available medicine for treatment of disease involving oxidants; so, its renoprotective effect was observed and compared to *Trigonella foenum-graecum* to see that how much the effect differs from each other.

The results of biochemical observations indicate that the nephrotoxic group of rats which were treated with silymarin *and Trigonella foenum-graecum* showed significant alleviation of toxic effects when compared to those of corresponding gentamicin treated nephrotoxic groups. These observations appear similar to those of previous works reported by other researchers[18, 23-24, 32].

When *Trigonella foenum-graecum* treated nephrotoxic group compared to silymarin treated nephrotoxic group; there was significant reduction of serum creatinine and urea levels in *Trigonella foenum-graecum* treated group in comparison to silymarin treated group. Non-significant difference in case of renal MDA and renal cortical glutathione level in between silymarin and fenugreek treated nephrotoxic rat groups; so, the antioxidant properties were almost similar. These observations suggest that probably the nephrotoxicity–alleviating effects of fenugreek was superior to that of silymarin.

Only silymarin treated groups of rats 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[33].

Conclusion

The present study indicates that probably the ameliorating effect of toxic damage to the renal tubules of *Trigonella foenum-graecum* (Fenugreek) extract was more effective than that of silymarin.

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