INTRODUCTION

Kidneys have an important role in the excretion of medicines and poisons that is why they are prone to numerous forms of damaging insults.¹ Non-steroidal anti-inflammatory drugs (NSAIDS) including acetylsalicylic acid are well known to produce nephrotoxicity.² Recent use of acetylsalicylic acid in the US is reported to be 10,000-20,000 tons per year, as an antiplatelet agent.³ It acts on the body by inhibiting cyclooxygenase (COX) enzymes activity.⁴ Afferent and efferent arterioles contain sites for the action of COX1 and COX2 enzymes.⁵ COX2 inhibition in kidneys lead to hypertension.⁶ It irreversibly inhibits the prostaglandins and thromboxanes through cyclooxygenase enzyme system making it different from other NSAIDS.⁶ Its excess usage can produce hepatic and renal toxicity.⁷ It has damaging effects on kidneys by producing vasoconstriction of glomerular blood vessels and salt retention.⁸ Within about two hours of oral or rectal administration, highest plasma levels of acetylsalicylic acid are obtained dispersing throughout the body, having maximum concentrations in the kidneys, lungs and heart.⁸ Single intravenous injection of acetylsalicylic acid had been shown to produce renal tubular necrosis in rats.⁹ In an experimental study, acetylsalicylic acid and chloroquine were co-administered in rats with resultantly aggravated levels of urea and creatinine as compared to chloroquine alone.⁶ In another study, rate of reduced clearance of creatinine and uric acid was indicated by the use of low dose of acetylsalicylic acid. In approximately 20% of elderly persons, more than 50% reduced rate of creatinine clearance indicated its hazardous effects on renal tubular functions. That is why, its prolonged use should be avoided.¹⁰

Nigella sativa (NS) is an herbaceous plant of the ranunculaceae family. It is being used as a herbal medicine for many years. It is also known by the name Kalonji and black cumin.¹¹ Its fruit capsule contains white seeds which turn black on exposure to air.¹² It contains volatile and fixed oils, reducing sugars and proteins.¹³ In traditional medicine, it is being used for the treatment of cough, asthma, abdominal pain and diarrhea.¹⁴ Its active components, especially thymoquinone and alpha-hederin, due to their anti-inflammatory and antioxidant properties, possess tremendous pharmacological activities.¹⁴ Alemi et al. described that the anti-inflammatory and anti-oxidant role of NS was due to its thymoquinone contents and showed its value as an anti-inflammatory agent.¹⁵ In 1997, three types of flavonoids were separated from the seeds of NS.¹⁶ Flavonoids, such as quercetin and kaempferol, contain various anti-oxidant and anti-inflammatory properties.¹⁷ Use of NS

ABSTRACT

Objective: To see the effects of Nigella sativa on acetylsalicylic acid-induced nephrotoxicity in albino rats.
Study Design: An experimental study.
Place and Duration of Study: The Anatomy Department of University of Health Sciences, Lahore, from January 2014 to December 2015.
Methodology: Thirty-two female albino rats were divided into four groups. Group A (control) was given single dose of 10 mg/100 gm body weight of 1% methylcellulose, orally. Group B and C were treated with oral 1000 mg/kg acetylsalicylic acid as a single dose. Group D was given 250 mg/kg ethanolic extract of Nigella sativa (NSE) by oral gavage followed by single dose of 1000 mg/kg acetylsalicylic acid on 1st day of experiment; after that only NSE was continued till 7 days. Animals of groups A, C and D were sacrificed on day 8 and that of group B on day 2 of experiment. Cardiac puncture was performed to draw blood from each animal for renal function tests. Animals were evaluated for gross (Paired kidney weight, body weight, relative tissue body weight index) as well as for biochemical (Serum urea and creatinine) parameters.
Results: Gross as well as biochemical parameters were markedly impaired in group B, but statistically significant improvement was noticed in Nigella sativa treated group. There was no self recovery in group C.
Conclusion: Ethanolic extract of Nigella sativa has a protective role against acetylsalicylic acid-induced nephrotoxicity in albino rats.


Department of Anatomy, University of Health Sciences, Lahore.

Correspondence: Dr. Sania Asif, Assistant Professor, Department of Anatomy, University of Health Sciences, Lahore. E-mail: sania_asif@yahoo.com

Received: May 05, 2017; Accepted: October 02, 2017.
seeds in different types of inflammatory disorders is due to their free radical scavenging properties.\textsuperscript{18}

NS is known to have anti-oxidant, anti-inflammatory, anti-ulcerogenic and anti-microbial effects.\textsuperscript{19} It increases glutathione levels thus producing anti-oxidant activity.\textsuperscript{20}

Use of NS in any form is harmless for liver and kidneys.\textsuperscript{21}

Segal \textit{et al.} proved the adverse effects of acetylsalicylic acid on renal function tests.\textsuperscript{10} Similar findings were seen in another study conducted by Owen and Heywood.\textsuperscript{2}

Protective effects of NS against gentamicin, amphotericin and cisplatin-induced nephrotoxicity had already been studied. Studies have shown its role in the improvement of serum urea and creatinine levels.\textsuperscript{22}

The present study was, therefore, conducted to determine the protective effects of NS on acetylsalicylic acid-induced nephrotoxicity in adult albino rats.

**METHODOLOGY**

This experiment study was conducted at the Anatomy Department of University of Health Sciences, Lahore, from January 2014 to December 2015.

Acetylsalicylic acid was obtained from Sigma company, USA and suspended in 1% methylcellulose. The seeds of NS were purchased from a local herb store. Seeds were washed, dried, crushed and soaked in absolute alcohol for four days, at room temperature, with intermittent stirring. The seeds were then filtered with the help of filter paper. Alcohol was evaporated by using rotary evaporator. The ethanolic extract was stored in refrigerator at 4°C.\textsuperscript{23}

Thirty-two female albino rats were procured from Animal House of the University of Health Sciences, Lahore. Rats were kept under controlled environment (temperature 23 ±2°C, humidity 50 ±5%) and light and dark cycle of 12 hours each. They were divided into four groups A, B, C and D.

Group A was given single dose of 10 mg/100g body weight of 1% methylcellulose by oral gavage. Group B and C were given 1000 mg/kg single dose of acetylsalicylic acid suspended in 10 mg/100g body weight of 1% methylcellulose, orally. Group D was treated with 250 mg/kg ethanolic extract of \textit{Nigella sativa} (NSE) followed by 1000 mg/kg single dose of acetylsalicylic acid orally on the first day of experiment then only NSE was continued till seven days.

Animals were weighed at the end of experiment. After completely anesthetizing the animal, cardiac puncture was performed and 5 ml blood was withdrawn from the animals of groups A, C and D on 8th day of the experiment while from group B on the second day of the experiment. Then rats were dissected out. Kidneys were removed and paired kidney weight was determined by the help of digital balance (EK-300i). Kidneys were then washed with normal saline and cut into 3 to 5 millimeter cube-size pieces, which were then placed for fixation in 10% buffered formalin solution for 48 hours.

Serum urea and creatinine levels were measured by using kits of Human company. Reagents, standard and sample, were used. Absorbance was measured against blank using Merk semiautomatic (Microlab, 300) spectrophotometer.

Data was analyzed by using SPSS version 20.0. Shapiro Wilk test was used to test the normality of data. Mean ±SD was given for quantitative variables like urea, creatinine, body weight, kidney weight and relative tissue body weight index. One way ANOVA was applied to compare the biochemical parameters among groups. Post-hoc Tukey’s test was applied for pair-wise comparison. P-value of ≤0.05 was considered as statistically significant.

**RESULTS**

The rats were observed daily for the assessment of health and were found to be healthy during the entire period of the experiment. Paired kidney weight was significantly increased in group B. Gain in body weight was normal in group D. Relative tissue body weight index was significantly increased in group B. There was no statistically significant self-recovery regarding these gross parameters in group C (Table I).

Serum analysis of rats was performed by using spectrophotometer and absorbance was calculated. Serum urea levels were significantly increased in acetylsalicylic acid treated groups B and C as compared to control group (p <0.001); significant improvement was observed in NS treated group D (p=0.049) when comparison was made with group B (Table I).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A Mean ±SD</th>
<th>Group B Mean ±SD</th>
<th>Group C Mean ±SD</th>
<th>Group D Mean ±SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paired kidney weight (gms)</td>
<td>1.37 ±0.09</td>
<td>1.64 ±0.18</td>
<td>1.40 ±0.12</td>
<td>1.47 ±0.10</td>
<td>0.001</td>
</tr>
<tr>
<td>Body weight of animal (gms)</td>
<td>178.87 ± 2.41</td>
<td>179.50 ±14.09</td>
<td>167.37 ±16.93</td>
<td>192.25 ±15.13</td>
<td>0.010</td>
</tr>
<tr>
<td>Relative tissue body weight index</td>
<td>0.76 ±0.05</td>
<td>0.91 ±0.08</td>
<td>0.83 ±0.07</td>
<td>0.76 ±0.03</td>
<td>0.000</td>
</tr>
<tr>
<td>Serum urea mg/dl</td>
<td>32.75 ±4.33</td>
<td>45.25 ±4.92</td>
<td>44.62 ±4.86</td>
<td>38.37 ±5.82</td>
<td>0.000</td>
</tr>
<tr>
<td>Serum creatinine mg/dl</td>
<td>0.56 ±0.05</td>
<td>0.80 ±0.07</td>
<td>0.63 ±0.09</td>
<td>0.51 ±0.13</td>
<td>0.000</td>
</tr>
</tbody>
</table>

* p ≤ 0.05 is considered statistically significant.
Increase in serum creatinine levels in acetylsalicylic acid treated groups B and C were statistically significant (p <0.001) when comparison was made with NS treated group D (Table I). So, significant improvement in the levels of serum creatinine was observed by NSE in group D (p < 0.001).

One-way ANOVA showing comparison in mean values of serum urea and serum creatinine levels among groups A, B, C and D.

DISCUSSION

NS is a rich source of fatty acids, minerals, and vitamins; its active ingredient is thymoquinone which is known to have anti-inflammatory, anti-oxidant, anti-microbial and gastro-protective effects. Studies have shown that acetylsalicylic acid has damaging effects on kidneys. The current study was, therefore, designed to see the gastro-protective effects. Studies have shown that acetylsalicylic acid has damaging effects on kidneys. NS is a rich source of fatty acids, minerals, and vitamins; its active ingredient is thymoquinone which is known to have anti-inflammatory, anti-oxidant, anti-microbial and gastro-protective effects.

Role of NS as a medicinal herb is well documented. In the current study, its protective role on acetylsalicylic acid-induced nephrotoxicity was studied by evaluating various gross parameters, i.e. paired kidney weight, body weight, relative tissue body weight index, and biochemical parameters, such as serum urea and serum creatinine levels.

Due to the toxic effects of acetylsalicylic acid, there was inflammatory reaction in the body, leading to leakage of fluid from the damaged tubules into the interstitium resulting in increased paired kidney weight in experimental group B. Increased paired kidney weight was statistically significant in group B when compared with group C (Recovery group) and A (Control group). In protective group D, the increase in paired kidney weight was not statistically significant, thus NS had shown its protective effect in this parameter (Table I). In case of relative tissue body weight index, statistically significant increase was observed in group B from group A and D; but no significant difference was found in group C when compared with other groups (Table I). Body weight of animals in group D was significantly increased when compared with group C (Table I), meaning thereby that in group D gain in body weight was more than the control due to NS. In the experimental group B, there was no statistically significant increase in body weight due to acetylsalicylic acid as compared to group D.

In the current investigation, acetylsalicylic acid-induced nephrotoxicity was shown to have statistically significant increased levels of serum urea and creatinine. Segal in 2006 showed that the impaired clearance of urea and creatinine was due to the effects of acetylsalicylic acid on tubular transport mechanisms. The raised levels were presumably due to the inflammatory and atrophic changes in kidneys, resulting from ischemia due to vasoconstriction of renal arterioles. In another study, acetylsalicylic acid-induced raised urea and creatinine levels were observed with proteinuria and tubular necrosis. Covalent binding of salicylates with mitochondria was reported to be an important causative factor for the salicylate-induced nephrotoxicity in 12 months old rats. Role of NS as a medicinal herb is well documented. With NSE, significant improvement was observed with respect to biochemical markers in group D as compared to acetylsalicylic acid treated groups B and C. In another study, improvement in these biochemical markers was observed when NS was given for gentamicin-induced nephritoxicity for it being antioxidant which protected kidneys against drug-induced damage. Improvement in serum urea and creatinine levels was observed when NSE was given against amphotericin B induced nephrotoxicity. The protective effects of NSE were possibly due to its free radical scavenging activities. NS has protective role on glomerular filtration rate of kidney by regulating the serum creatinine levels. It was reported that anti-inflammatory role of NS, especially of its active ingredient thymoquinone, may be due to its inhibitory effects on cyclo-oxygenase and lipoxygenase enzymes. As acetylsalicylic acid produced inflammatory changes, so the protective effects of NSE in this study were due to its anti-inflammatory effects on kidneys.

The main limitation of this study was that prostaglandins assay could not be performed due to limited funds. In future, prostacyclins and leukotriens kits can be used to determine more accurate results.

CONCLUSION

There was marked impairment in gross parameters and biochemical parameters of groups B and C. In group D, where NSE was given before and after the administration of acetylsalicylic acid, statistically significant improvement was observed in gross as well as biochemical markers. So, it was concluded that NS has protective effects against acetylsalicylic acid-induced nephrotoxicity in albino rats.

REFERENCES

5. Caspi D, Lubart E, Graff E, Habot B, Yaron M, Segal R. The effect of mini-dose acetyl salicylic acid on renal function and
Nigella sativa for acetylsalicylic acid-induced nephrotoxicity


