Protective Effect of Naringin on Sodium Arsenite Induced Testicular Toxicity via Modulation of Biochemical Perturbations in Experimental Rats

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ABSTRACT
Background: Arsenicals induced testicular cancer is a serious debilitating epidemic affecting all social strata in developing as well as developed countries. Naringin possess antioxidant and free radical scavenging properties. Hence, the present investigation was design to elucidate the effect of naringin against sodium arsenite induced testicular toxicity in laboratory rats. Materials and Methods: Male Sprague-Dawley rats were pretreated with either vehicle (control), naringin (20, 40 and 80 mg kg\(^{-1}\), p.o.) and Co-enzyme Q\(_{10}\) (10 mg kg\(^{-1}\), p.o) for 7 days. Testicular toxicity was induced by sodium arsenite (10 mg kg\(^{-1}\), p.o., for two consecutive days). Drug treatment was continued up to 15th day. On 16th day reproductive organo-somatic indices, biochemical and histopathological determinations were carried out. Results: Rats pretreated with naringin (40 and 80 mg kg\(^{-1}\), p.o.) showed significant and dose dependant increased (p<0.01 and 0.001, respectively) in relative organ weight (epididymis, seminal vesicle and testis) and sperm count. It also showed significant increased (p<0.01 and 0.001, respectively) in level of serum protein, albumin, AST, ALT and activity of testicular endogenous enzymes (nitric oxide (NO) content superoxide dismutase (SOD) and reduced glutathione (GSH). The elevated levels of serum creatinine and glucose, testicular MDA and NO were significantly inhibited (p<0.01 and 0.001, respectively) by the naringin (40 and 80 mg kg\(^{-1}\), p.o.). It significantly (p<0.01) restored the alteration in serum lipid profile. Elevated levels of pro-inflammatory cytokines (Tumor necrosis factor-alpha (TNF-\(\alpha\)) and Interleukin-1beta (IL-1\(\beta\)) were significantly and dose dependently (p<0.01 and 0.001, respectively) inhibited by the naringin (40 and 80 mg kg\(^{-1}\), p.o.) treatment. It also restored the histological aberration induced by the sodium arsenite. Conclusion: Naringin exerted its protective effects against sodium arsenite induced testicular toxicity via modulation of endogenous antioxidant enzyme, inhibition of release of oxido-inflammatory mediators such as MDA and NO as well as pro-inflammatory cytokines.

Key words: IL-1\(\beta\), naringin, oxido-nitrosative stress, sodium arsenite, testicular toxicity, TNF-\(\alpha\)


INTRODUCTION
Arsenic is an inorganic compound which exists in environment in abundant amount and it has ability to cause multiple causative effects in animals as well as in human (Bates et al., 1992; Liu et al., 2001). There are various natural and man-made sources through which arsenic release in the environment like agricultural pesticides industries, coal fired power plants, glassware manufacturing, wood preservatives and smelting industries, cosmetic industries, etc. (Tchounwou et al., 2002). Water-soluble trivalent arsenic form (As III) and pentavalent arsenate form (As V) are the two most common forms of arsenic, however inorganic trivalent arsenic has more potential to cause toxicity than pentavalent arsenic (Bertolero et al., 1987). Water, food and drugs are the common ways through which exposure of arsenic easily occur and this arsenic is quickly absorbed through gastrointestinal tract results in the toxicity of multiple organs including liver, lung, testis, spleen, kidney, etc. (Pant et al., 2004; Sarkar et al., 2003). This toxic effect is mediated through an array of vicious processes including obstacles in DNA repair, changes in ubiquitous enzymatic activities, alteration in gene expression and involvement of oxidative stress (Das et al., 2009; Yousef et al., 2008).

Impaired male reproductive function is a result of arsenic intoxication and reflected by suppression of spermatogenesis and androgenesis, reduction of the weight of testes, imbalance of sex hormones and accessory sex organs (Pant et al., 2004; Sarkar et al., 2003). Exact mechanism behind the arsenic induced testicular
toxicity is not fully elucidated however, studies carried out in various animal models of testicular toxicity revealed the mechanism behind arsenic induced toxicity (Kandhare et al., 2011b). Various findings favour the role of oxidative stress and inflammation with the increment of generation of reactive oxygen species and production of pro-inflammatory cytokines (Chang et al., 2007; Ghosh et al., 2012a, b, c; Singh et al., 2010).

It has been documented that diseases which are related to oxidative stress can be easily treated by applying nutritional antioxidants (Kamble et al., 2013; Kandhare et al., 2011a, 2013a). Isolated bioactive moieties from plant are known to exert their protective effects by scavenging free radicals and modulating antioxidant defense system (Kandhare et al., 2012a; Raygude et al., 2012a; Visnagri et al., 2014). Naringin (4',5,7-trihydroxy flavonone 7-rhamnoglucoside), a plant bioflavonoid is naturally present in grape fruits and other citrus species with the ability of positive effect on human health (Jagetia and Reddy, 2002; Jagetia et al., 2003; Kandhare et al., 2012b). Naringin has free radical scavenging property that attenuate induced molecular macromolecules such as reduction of cellular and chromosomal aberrations (Jagetia et al., 2003). Various pharmacological actions like that naringin antioxidant, antidiabetic, renoprotective, cardioprotective, anti-inflammatory, anticancer properties (Ali et al., 2004; Benavente-Garcia and Castillo, 2008; Choe et al., 2001; Gonzalez-Gallego et al., 2007; Martin et al., 1994; Punithavathi et al., 2008; Rajadurai and Prince, 2007; Singh et al., 2004) have been reported. Naringin caused inhibition of lipopolysaccharides which induced squeezing of tumour necrosis factor-α in macrophages of mice (Kasuguchi et al., 1999). Naringin possess preventive effect on lipoproteins, lipid as well as enzymes related with metabolism of lipid and lipid peroxides in isoprenaline-induced myocardial infarction in rats (Rajadurai and Prince, 2007). Kandhare et al. (2012a) reported that naringin possess neuroprotective effects in streptozotocin (STZ) induced neuropathic pain (Kandhare et al., 2012b). Moreover, naringin ameliorates acetic acid induced colitis through modulation of endogenous oxido-nitrosative balance and DNA damage (Kumar et al., 2013). Various plant bioflavonoids including curcumín, rutin and hesperidin have been shown to play ameliorating effect in sodium arsenite induced biochemical perturbations in tests of rats (Akondi et al., 2011; Taepongsorat et al., 2008; Yousef et al., 2008). Previous report of Akondi et al. (2011) reported that naringin exert its protective effect on testicular tissue via inhibition of oxidative stress in type I diabetes mellitus (Akondi et al., 2011). On the basis of above outcomes, the objective of present study was to study the effect of naringin in sodium arsenite induced testicular toxicity by assessing various biochemical, molecular and histological parameters.

MATERIALS AND METHODS

Animals: Adult male Sprague-Dawley rats (200-250 g) were purchased from the National Institute of Biosciences, Pune (India). They were housed at 24±1°C, with relative humidity of 45–55% and 12:12 h dark/light cycle. The animals had free access to standard pellet chow (Pramav Agro industries Ltd., Sangli, India) and water throughout the experimental protocol. All experiments were carried out between 09:00 and 17:00 h. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Poona College of Pharmacy, Pune (PCP/93/2012) and performed in accordance with the guidelines of Committee for Control and Supervision of Experimentation on Animals (CPCSEA), Government of India on animal experimentation.

Drugs and chemicals: Naringin was purchased from Sigma Chemical Co. (St Louis, MO, USA). Co-enzyme Q10 was supplied by Medicines Pvt. Ltd., (Mumbai, India) and sodium arsenite powder (Otto Chemicals, India). 1,1′,3,3′-Tetraethoxypropane, crystalline beef liver catalase, reduced glutathione (GSH), 5,5′-dithiobis (2-nitrobenzoic acid) were purchased from S.D. Fine Chemicals, Mumbai, India. Sulphanilamides, naphthaline diamine HCl and phosphoric acid were obtained from LobaChemie Pvt. Ltd., Mumbai, India. Rat Tumor necrosis factor-alpha (TNF-α) and Interleukin-1beta (IL-1β) ELISA kits were purchased from Thermo Scientific (Rockford, IL, Campus, USA).

Experimental design: Rats were randomly divided into following groups of 6 animals each as follows:

Group I: Normal [N]: Received double distilled water (10 mg kg⁻¹, p.o. for 15 days)

Group II: Arsenic Control [As]: Received sodium arsenite (10 mg kg⁻¹, p.o. for two consecutive days) and treated with distilled water (10 g kg⁻¹, p.o. for 15 days)

Group III: Arsenic+Naringin (20 mg kg⁻¹) [As+N (20)]: Received sodium arsenite (10 mg kg⁻¹, p.o. for two consecutive days) and treated with naringin (20 mg kg⁻¹, p.o. for 15 days)

Group IV: Arsenic+Naringin (40 mg kg⁻¹) [As+N (40)]: Received sodium arsenite (10 mg kg⁻¹, p.o. for two consecutive days) and treated with naringin (40 mg kg⁻¹, p.o. for 15 days)
Group V: Arsenic+Naringin (80 mg kg⁻¹) [As+N (80)]; Received sodium arsenite (10 mg kg⁻¹, p.o. for two consecutive days) and treated with naringin (80 mg kg⁻¹, p.o. for 15 days)

Group VI: Arsenic+Co-enzyme Q₀ (10 mg kg⁻¹) [As+CoQ₀ (10)]; Received sodium arsenite (10 mg kg⁻¹, p.o. for two consecutive days) and treated with Co-enzyme Q₀ (10 mg kg⁻¹, p.o. for 15 days)

Testicular damage was induced in rats of the second and third groups by sodium arsenite at a dose of 10 mg kg⁻¹, orally for two consecutive days (Das et al., 2009). Co-enzyme Q10 was prepared as aqueous solution with addition of Tween 80 (1%), while sodium arsenite was dissolved in normal saline. The three different dosage of naringin i.e., 20, 40 and 80 mg kg⁻¹ were selected on the basis of previous study (Kadkhade et al., 2012a). Everyday naringin solution was freshly prepared in distilled water. An appropriate stock solution was prepared to administer selected dosage of 20, 40 and 80 mg kg⁻¹ orally for 15 days. Naringin and Co-enzyme Q₀ were administered for 7 consecutive days before arsenite administration and continued up to 15 days. On 16th day rats were anesthetized by anesthetic ether and blood was withdrawn by retro orbital puncture. Each blood sample was collected into separate vials for determination of serum parameters. After blood collection the animals were sacrificed by cervical dislocation and testis were rapidly removed and stored at -80°C for biochemical parameters.

Serum biochemistry: The serum was separated by centrifugation using Eppendorf Cryocentrifuge (model No, 5810, Germany), maintained at 4°C and run at speed of 7000 rpm for 15 min. Serum protein, albumin, creatinine, Aspartate Aminotransferase (AST), Alanine transaminase (ALT), glucose, cholesterol, HDL (High-Density Lipoprotein), LDL (Low-Density Lipoprotein) and triglyceride were measured by spectrophotometer (UV-Visible spectrophotometer, Jasco V-530, Japan) using reagent kits according to procedure provided by manufacturer (Accurex Biomedical Pvt. Ltd., Mumbai, India).

Biochemical estimation of testis homogenate

Tissue homogenate preparation: Animals were sacrificed at the end of study and testis were immediately isolated. Tissue homogenate was prepared with 0.1 M tris-HCl buffer (pH 7.4) and supernatant of homogenates was employed to estimate superoxide dismutase (SOD), reduced glutathione (GSH), lipid peroxidation (MDA content) and nitric oxide (NO content) as described previously (Kadkhade et al., 2012a, b, c; Visnagri et al., 2012).

Quantification of testicular TNF-α and IL-1β: The quantification of testicular TNF-α and IL-1β was performed in testis homogenate with the help and instructions provided by rat TNF-α and IL-1β ELISA kit manufacturer (Thermo Scientific, Rockford, IL, Campus, USA).

Histopathological analysis of testis: Specimen of other testis was stored in 10% formalin for 24 h. The specimen was dehydrated and placed in xylene for 1 h (3 times) and later in ethyl alcohol (70, 90 and 100%) for 2 h, respectively. The infiltration and impregnation was carried out by treating with paraffin twice, each time for one h. Tissue specimens were cut into sections of 3-5 μm thickness and stained with hematoxylin and eosin (H and E). The sections were mounted on slide by use of Distrene Phalate Xylene (DPX) as mounting medium. Sections were examined under a light microscope to inspect the histopathology features of specimen and infiltration of cells. The various changes in histological features were graded as Grade 0 (not present or mild); Grade 1 (mild); Grade 2 (moderate); and Grade 3 (severe).

Statistical analysis: Data were expressed as mean±standard error of mean (SEM). Data analysis was performed using Graph Pad Prism 5.0 software (Graph Pad, San Diego, CA, USA). Data of biochemical parameters were analyzed using one way analysis of variance (ANOVA) and Dunnett’s test was applied for post hoc analysis. A value of p<0.05 was considered to be statistically significant.

RESULTS

Effect of naringin and Co-enzyme Q10 on body weight and relative organ weights in male rats: There was significant (p<0.001) reduction in body weight, relative weight of epididymis, seminal vesicle and testis of arsenic control group as compared to normal group. On the other hand, there was significant (p<0.01 and 0.001) and dose dependant (40 and 80 mg kg⁻¹) increase in the body weight, relative weight of epididymis, seminal vesicle and testis in naringin treated group as compared to arsenic control group. In co-enzyme Q10 (10 mg kg⁻¹) treated group significant (p<0.001) increase in the body weight, relative weights of epididymis, seminal vesicle and testis as compared to arsenic control group was observed (Table 1).

Effect of naringin and Co-enzyme Q10 on sperm count in male rats: Sperm count in arsenic control rats was significantly decreased (p<0.001) after arsenite administration as compared to normal rats. Sperm count in naringin treated rats was significantly (p<0.01 and
Table 1: Effect of naringin treatment on body weight, relative organ weights and sperm count in male rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weight (g)</th>
<th>Epididymis weight</th>
<th>Seminal vesicle weight</th>
<th>Testis weight</th>
<th>Sperm count (million/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>278.8±5.68</td>
<td>0.022±0.0013</td>
<td>0.005±0.0002</td>
<td>0.0136±0.0015</td>
<td>48.5±5.48</td>
</tr>
<tr>
<td>A6</td>
<td>211.0±5.46</td>
<td>0.0102±0.0004###</td>
<td>0.0027±0.0010###</td>
<td>0.0066±0.0010###</td>
<td>44.5±5.36###</td>
</tr>
<tr>
<td>A6+N (20)</td>
<td>223.7±7.69</td>
<td>0.0112±0.0001</td>
<td>0.0058±0.0002</td>
<td>0.0078±0.0011</td>
<td>17.14±0.21</td>
</tr>
<tr>
<td>A6+N (40)</td>
<td>246.8±7.49**</td>
<td>0.0208±0.0027**</td>
<td>0.0076±0.0007**</td>
<td>0.0116±0.0011**</td>
<td>28.12±5.22**</td>
</tr>
<tr>
<td>A6+N (80)</td>
<td>266.5±7.37***</td>
<td>0.0259±0.0025***</td>
<td>0.0080±0.0001**</td>
<td>0.0145±0.0027**</td>
<td>42.21±1.58***</td>
</tr>
<tr>
<td>A6+CoQ10 (10)</td>
<td>277.3±8.78****</td>
<td>0.0334±0.0019****</td>
<td>0.0077±0.0007****</td>
<td>0.0104±0.0012****</td>
<td>43.51±3.12****</td>
</tr>
</tbody>
</table>

Data are expressed as Mean±SEM (n = 6) and analyzed by one-way ANOVA followed by Dunnett’s test multiple range test. *p<0.05, **p<0.01 and ***p<0.001 as compared with the vehicle control group. ###p<0.001 as compared to arsenic control group, N: Normal group, A6: Arsenic control group, N (20): Naringin (20 mg kg⁻¹, p.o.) treated group, N (40): Naringin (40 mg kg⁻¹, p.o.) treated group, N (80): Naringin (80 mg kg⁻¹, p.o.) treated group and CoQ10 (10): Co-enzyme Q10 (10 mg kg⁻¹, p.o.) treated group

Table 2: Effect of naringin treatment on serum protein, albumin, creatinine, AST, ALT and serum glucose levels in male rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Protein (mg/L)</th>
<th>Albumin (g/L)</th>
<th>Creatinine (mg/dL⁻¹)</th>
<th>AST (IU/L⁻¹)</th>
<th>ALT (IU/L⁻¹)</th>
<th>Serum glucose (mg/dL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>10.22±1.05</td>
<td>1.65±0.45###</td>
<td>0.30±0.01</td>
<td>132.65±3.11</td>
<td>132.50±2.07</td>
<td>117.14±3.88</td>
</tr>
<tr>
<td>A6</td>
<td>5.02±0.78###</td>
<td>1.05±0.45###</td>
<td>0.50±0.05###</td>
<td>74.33±3.44###</td>
<td>98.62±2.30###</td>
<td>244.33±3.54###</td>
</tr>
<tr>
<td>A6+N (20)</td>
<td>5.89±1.52</td>
<td>1.84±0.12</td>
<td>0.85±0.07</td>
<td>79.15±0.78</td>
<td>194.62±4.32</td>
<td>198.62±2.15</td>
</tr>
<tr>
<td>A6+N (40)</td>
<td>7.15±0.78**</td>
<td>2.10±0.15**</td>
<td>0.50±0.02**</td>
<td>95.63±5.07**</td>
<td>124.30±1.70**</td>
<td>163.82±3.08**</td>
</tr>
<tr>
<td>A6+N (80)</td>
<td>9.52±1.08**</td>
<td>3.12±0.78**</td>
<td>0.38±0.04**</td>
<td>120.4±3.07**</td>
<td>144.20±2.09**</td>
<td>132.50±2.80**</td>
</tr>
<tr>
<td>A6+CoQ10 (10)</td>
<td>9.87±0.78**</td>
<td>4.08±1.04**</td>
<td>0.32±0.09**</td>
<td>128.15±4.33**</td>
<td>148.62±2.63**</td>
<td>140.41±3.04**</td>
</tr>
</tbody>
</table>

Data are expressed as Mean±SEM (n = 6) and analyzed by one-way ANOVA followed by Dunnett’s test multiple range test. *p<0.05, **p<0.01 and ***p<0.001 as compared with the vehicle control group. ###p<0.001 as compared to arsenic control group, N: Normal group, A6: Arsenic control group, N (20): Naringin (20 mg kg⁻¹, p.o.) treated group, N (40): Naringin (40 mg kg⁻¹, p.o.) treated group, N (80): Naringin (80 mg kg⁻¹, p.o.) treated group and CoQ10 (10): Co-enzyme Q10 (10 mg kg⁻¹, p.o.) treated group

Effect of naringin and Co-enzyme Q10 on lipid profile in male rats: There was significant (p<0.001) increase in serum cholesterol, triglyceride and LDL-C level in arsenic control group as compared to normal group. The increased level of cholesterol and LDL-C due to arsenic administration was significantly (p<0.05 and 0.01) and dose dependent (40 and 80 mg kg⁻¹) by naringin treatment as compared to arsenic control group. There was significantly (p<0.01 and 0.001) and dose dependent (40 and 80 mg kg⁻¹) reduction in the serum triglyceride level by naringin treatment as compared to arsenic control animals. Treatment with Co-enzyme Q10 (10 mg kg⁻¹) significantly decreased the serum cholesterol (p<0.001), triglyceride (p<0.001) and LDL-C (p<0.01) as compared to arsenic control group (Table 3).

The level of serum HDL-C in arsenic control rats was significantly (p<0.001) decreased after arsenic administration as compared to normal rats. Serum LDL-C level in naringin (80 mg kg⁻¹) treated rats was significantly (p<0.01) increased as compared to arsenic control rats. When compared with arsenic control rats, naringin (20 and 40 mg kg⁻¹) treated rats failed to produce any significant change in serum HDL-C. The reduced serum HDL-C level was significantly (p<0.05) increased by Co-enzyme Q10 (10 mg kg⁻¹) treated rats as compared to arsenic control rats (Table 3).

Effect of naringin and Co-enzyme Q10 on testicular SOD and GSH in male rats: Testicular SOD and GSH levels of arsenic control rats were significantly (p<0.001) decreased after administration of sodium arsenite as
Table 3: Effect of naringin treatment on lipid profile in male rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cholesterol (mg dl⁻¹)</th>
<th>Triglyceride (mg dl⁻¹)</th>
<th>HDL-C (mg dl⁻¹)</th>
<th>LDL-C (mg dl⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>22.54±2.84</td>
<td>54.57±1.95</td>
<td>62.52±3.85</td>
<td>2.30±0.84</td>
</tr>
<tr>
<td>As</td>
<td>64.02±2.70**</td>
<td>153.20±4.02</td>
<td>20.05±1.18**</td>
<td>5.45±1.43**</td>
</tr>
<tr>
<td>As+N (20)</td>
<td>61.23±2.02</td>
<td>18.05±0.75</td>
<td>24.30±2.75</td>
<td>4.02±1.08</td>
</tr>
<tr>
<td>As+N (40)</td>
<td>50.21±2.42**</td>
<td>48.52±4.28**</td>
<td>35.62±3.07</td>
<td>3.11±0.89**</td>
</tr>
<tr>
<td>As+N (80)</td>
<td>32.02±3.42**</td>
<td>62.02±3.02**</td>
<td>42.52±1.95**</td>
<td>3.00±1.02**</td>
</tr>
<tr>
<td>As+CoQ10 (10)</td>
<td>24.60±0.27***</td>
<td>59.30±2.15***</td>
<td>40.30±3.27**</td>
<td>2.08±0.65**</td>
</tr>
</tbody>
</table>

Data are expressed as Mean±SEM (n = 6) and analyzed by one-way ANOVA followed by Dunnett’s test multiple range test. *p<0.05, **p<0.01 and ***p<0.001 as compared with the vehicle control group, ###p<0.001 as compared to arsenic control group, N: Normal group, As: Arsenic control group, N (20): Naringin (20 mg kg⁻¹, p.o) treated group, N (40): Naringin (40 mg kg⁻¹, p.o) treated group, N (80): Naringin (80 mg kg⁻¹, p.o) treated group and CoQ10 (10): Co-enzyme Q10 (10 mg kg⁻¹, p.o) treated group.

Table 4: Effect of naringin treatment on testicular SOD, GSH, MDA and NO levels in male rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SOD (U mg⁻¹ of protein)</th>
<th>GSH (µg mg⁻¹ of protein)</th>
<th>MDA (nmol mg⁻¹ of protein)</th>
<th>NO (µmol mg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>29.27±2.85</td>
<td>3.21±0.88</td>
<td>12.55±4.02</td>
<td>98.66±5.84</td>
</tr>
<tr>
<td>As</td>
<td>8.95±2.22**</td>
<td>0.98±0.33**</td>
<td>48.62±2.15**</td>
<td>243.33±10.24**</td>
</tr>
<tr>
<td>As+N (20)</td>
<td>9.65±3.12</td>
<td>1.02±0.98</td>
<td>42.56±1.84</td>
<td>200.15±8.62</td>
</tr>
<tr>
<td>As+N (40)</td>
<td>14.57±3.84**</td>
<td>2.45±0.19**</td>
<td>30.12±1.81**</td>
<td>163.22±7.38**</td>
</tr>
<tr>
<td>As+N (80)</td>
<td>18.21±2.65***</td>
<td>4.12±1.02***</td>
<td>18.62±2.22**</td>
<td>138.62±10.43**</td>
</tr>
<tr>
<td>As+CoQ10 (10)</td>
<td>19.05±0.22***</td>
<td>4.55±0.98***</td>
<td>15.00±3.32***</td>
<td>121.66±8.55**</td>
</tr>
</tbody>
</table>

Data are expressed as Mean±SEM (n = 6) and analyzed by one-way ANOVA followed by Dunnett’s test multiple range test. *p<0.05, **p<0.01 and ***p<0.001 as compared with the vehicle control group, ###p<0.001 as compared to arsenic control group, N: Normal group, As: Arsenic control group, N (20): Naringin (20 mg kg⁻¹, p.o) treated group, N (40): Naringin (40 mg kg⁻¹, p.o) treated group, N (80): Naringin (80 mg kg⁻¹, p.o) treated group and CoQ10 (10): Co-enzyme Q10 (10 mg kg⁻¹, p.o) treated group.

compared to normal rats. The testicular SOD level in naringin treated rats was significantly (p<0.05 and 0.001) and dose dependently (40 and 80 mg kg⁻¹) increased as compared to arsenic control rats. Whereas naringin treatment showed significant (p<0.01 and 0.001) and dose dependent inhibition (40 and 80 mg kg⁻¹) in the arsenic induced reduction in testicular GSH level when compared with arsenic control rats. When compared with arsenic control rats, naringin showed significant (p<0.001) increase in testicular SOD and GSH level (Table 4).

Effect of naringin and Co-enzyme Q10 on testicular MDA and NO level in male rats: Lipid peroxidation (MDA) and NO levels were significantly increased (p<0.001) in arsenic control rats after administration of sodium arsenite as compared to normal rats. The testicular MDA and NO levels in naringin treated rats were decreased significantly (p<0.01 and 0.001) and dose dependently (40 and 80 mg kg⁻¹) as compared to arsenic control rats. When compared with arsenic control rats, Co-enzyme Q10 (10 mg kg⁻¹) treated rats also showed significant (p<0.001) inhibition in this elevated level of testicular MDA and NO (Table 4).

Effect of naringin on Co-enzyme Q10 testicular TNF-α and IL-1β level in male rats: There were significant (p<0.001) increase in the testicular TNF-α and IL-1β levels in arsenic control rats as compared with normal rats. These elevated testicular TNF-α and IL-1β levels were significantly (p<0.01 and p<0.001) and dose-dependently (40 and 80 mg kg⁻¹) inhibited by naringin treatment when compared with arsenic control rats. Treatment with Co-enzyme Q10 (10 mg kg⁻¹) showed significant (p<0.001) reduction in testicular TNF-α and IL-1β levels as compared to arsenic control group (Fig. 1).

Effect of naringin and Co-enzyme Q10 on histopathological analysis of testis in male rats: Histopathological investigation of testis from normal rat showed well structured features including the appearance of normal spermatogenesis and spermiation cycles, presence of uniformly arranged longitudinal to transversely placed seminiferous tubules with minimal interstitial spaces (Fig. 2a). It showed normal Leydig cells with presence of few congested blood vessels (Grade 1). However, sections of testis from arsenic control animals showed ruptured follicles and few follicles showing reduction in the number of spermatozoa and degenerated inter follicular septa (Fig. 2b). It also exhibits the prominent features like necrosis in seminiferous tubules (Grade 4), interstitial edema (Grade 3), maturation arrest, failure in spermatogenesis and vacuolization of spermatid nuclei (Grade 4). Histopathological investigation of testis tissue from Co-enzyme Q10 (10 mg kg⁻¹) treated rat showed normal spermatogenesis characterized by the presence of mature secondary spermatids at the luminal edges of epithelia but presence of moderate interstitial edema (Grade 1) with degenerative changes (Grade 1) in histological findings of testis (Fig. 2c). Naringin (80 mg kg⁻¹) treatment improved testicular aberration induced after arsenic toxicity. It showed the restoration of normal morphological structures of seminiferous tubules but
Fig. 1: Effect of naringin treatment on testicular tumour necrosis factor-α and interleukin-1β (IL-1β) in rats. Data are expressed as Mean±SEM and analyzed by one-way ANOVA followed by Dunnett’s test multiple range test. *p<0.05, **p<0.01 and ***p<0.001 as compared with the vehicle control group, ###p<0.001 as compared to Arsenic control group, N: Normal group; As: Arsenic control group, N (20): Naringin (20 mg kg⁻¹, p.o.) treated group, N (40): Naringin (40 mg kg⁻¹, p.o.) treated group, N (80): Naringin (80 mg kg⁻¹, p.o.) treated group and CoQ₀ (10): Co-enzyme Q₀ (10 mg kg⁻¹, p.o.) treated group.

Fig. 2(a-d): Effect of naringin treatment on histopathological analysis of testis in male rats. Photomicrographs of sections of testis tissue from each group stained with H and E. Testis microscopic image of normal rat, (a) Arsenic control rat, (b) Co-enzyme Q₀ (10 mg kg⁻¹, p.o.) treated rat, (c) Naringin (80 mg kg⁻¹, p.o.) treated rat and (d) Normal spermatogenesis (blue arrow), Necrosis (Red arrow) and Interstitium edema (black star). Images (×100 magnification) are typical and representative of each study group.

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presence of degenerated (Grade 1) and reduced number of Leydig cells. The numbers of spermatozoa were increased, tubular basement membrane thickness was reduced and the intertubular space was decreased (Fig. 2d). Restoration of active spermatogenesis in most of seminiferous tubules were seen in naringin (80 mg kg\(^{-1}\)) treated animals. The interstitial tissue edema (Grade 2) and necrosis (Grade 1) were decreased and histology showed minimal injury of testicular tissue.

DISCUSSION

Arsenic has wide range of application in manufacturing industries in an array of domains across insecticides, preservatives and dyes. Products from the industries are used by all strata of society and an imperative possibility of arsenic mediated pathobiology lurks in the background. The indispensable use of their products in the daily life promulgate the needs for agents possessing adequate property of quenching the toxic aberrations precipitative due to its chemical properties. It is present ubiquitously in the environment but in minuscule proportion (Ramanathan et al., 2003). IARC (International agency for research and cancer) has christened arsenic as group-I carcinogen reflecting the impact levied by arsenic toxicity on global health (IARC, 2004). However, well directed research initiatives have not produced or identified molecules or targets that could be used as cynosure of international efforts to alleviated arsenic induced toxicity.

Exposure to high levels of arsenic are liable to develop lung, testis, skin, bladder cancer and occlusive vascular disease thus withered populations are at elevated risk for these conditions (Bates et al., 1992; Smith et al., 1992). Arsenic is metabolized by recurring reduction and oxidative methylation. Various arsenic metabolic intermediates such as monomethylarsonous acid and dimethylarsinous acid were also found in urine, which are highly toxic than inorganic arsenicals (Kobayashi et al., 2005). Arsenic exposure leads to commencement of oxidative stress, increased lipid peroxidation, depletion of antioxidant defences and increased production of pro-inflammatory cytokines (Fouda et al., 2011). There are various facets of arsenic induced toxicity in encompassing oxidative, nitrosative and molecular perturbation due to mono, di and tri methylated forms which have enhanced toxic potential as compared to the parent molecules.

The present investigation was undertaken to evaluate the effect of naringin to reverse arsenic induced toxicity in laboratory rats. The literature is punctuated with evidences proving antioxidants Reactive Oxygen Species (ROS) scavenging properties of naringin as the principle components of arsenic toxicity is oxidant-nitrosative stress leading to DNA damage hence the selection of naringin in this animal model is justifiable (Kandhare et al., 2012b, c; Kumar et al., 2013). Oxidative stress has a plethora of components which comprise various enzymes involved in the electron transport chain (Kandhare et al., 2013b, c).

The administration of sodium arsenite leads to decrease in the absolute weight of the testes, epididymis and seminal vesicle which indicate the toxic effects of arsenite on these reproductive organs, this observation is in agreement with a earlier finding (Morakinyo et al., 2010). Sperm count and sperm morphology are key features of male fertility, as these are the principal markers in testicular spermatogenesis and epididymal maturation (Sarkar et al., 2003). Moreover, undesirable effect of arsenite on sperm count may be accountable due to low testosterone concentration and it has been reported that high level of testosterone in testis is crucially required for usual spermatogenesis, maintenance of sperm morphology and normal composition of seminiferous tubules (Sharpe et al., 1988, 1992). In present investigation, the dwindling of sperm count by sodium arsenite treatment is corroborated with the findings of others (Jana et al., 2006; Sarkar et al., 2003). Treatment with naringin significantly inhibited alteration in absolute weight of testis, epididymis and seminal vesicle and improved the sperm count. The increase in sperm count is also evident by the restoration of active spermatogenesis in seminiferous tubules observed in histopathological investigation of testis of naringin treated animals. The results of present investigation are in line with the finding of previous researcher (Akondi et al., 2011).

The demur in plasma total protein due to induction with sodium arsenite was essentially due to diminished albumin fraction. The inhibitory effect of sodium arsenite on total protein is in accordance with the finding of previous researchers (Kalrider et al., 2001; Nandi et al., 2005) reported that arsenic inhibited the aptitude of glucocorticoid and its receptor to turn on genes, which plays an essential role in protein metabolism. The reduced level of plasma protein in rodents due to environmental pollutants could be endorsed to changes in protein as well as free amino acid metabolism and their synthesis in the liver. Treatment of naringin showed prominent effect by increasing the plasma total protein and albumin levels. In addition, the decrease in concentration of serum protein may be due to excessive loss of proteins, which leads to decrease in plasma proteins. Sodium arsenite caused reduction in the activities of serum AST, ALT levels (Yousef et al., 2008). However, treatment of naringin significantly restored the AST and ALT levels in serum.

The imbalance in glucose production and utilization leads to hyperglycaemia. The present study reveals the increase in glucose levels of sodium arsenite administered rats. It has been reported that methylated metabolites of arsenic are responsible for induction of
diabetes mellitus (Tseng, 2004). Moreover, Oxidative Stress (OS) has been considered as a major link to insulin resistance and β-cell dysfunction which thwart the release of insulin into circulation along with damage of insulin secreting β cells after prolonged exposure to arsenic (Hayden, 2002). Treatment of naringin significantly reduced the blood glucose level which is in accordance with the previous study (Kandhare et al., 2012b). The present study indicated that administration of sodium arsenite caused noteworthy increase in serum creatinine, which is due to decrease in the clearance from urine and leads to minimize an ability of the kidneys to filter waste products from the blood and excrete them in the urine. Naringin treatment show significant decrease in serum creatinine.

The present data showed that plasma cholesterol, triglyceride and LDL were significantly increased by sodium arsenite administration, while HDL levels were decreased, which are in accordance with (Mann et al., 2005) who reported that liver X receptor (LXR) lead to hypercholesterolemia. It is also reported that lipid and carbohydrate metabolism are primarily regulated by LXR genes (Steefensen and Gustafsson, 2004). Oral treatment of naringin promisingly reduced the serum cholesterol and triglyceride levels and increased HDL levels. The finding of present investigation is in accordance with the results of previous researcher where oral administration of naringin restored the altered level of serum lipid in paracetamol induced hepatotoxicity (Javed et al., 2013).

ROS generation leads to damage of macromolecules and DNA (Raygude et al., 2012b). In a typical organ system, the overall metabolism of formation of ROS involves damage to the physiological quenching of the superoxide radical anion (O²⁻). Superoxide anion is converted to H₂O via glutathione peroxidase or catalase (Patil et al., 2012c; Visnagri et al., 2013). Another pathway for diminution of superoxide free radical anion is via lipid conjugation (Kandhare et al., 2012d). This pathway involves β-oxidation by lipid peroxides leading to formation of malonaldehyde and 4-hydroxynoneol. This physiological balance is disregulated under exogenous stress like arsenic leading to elevation of MDA and reduction of GSH and SOD.

MDA is a reproducible index of oxidative stress in biological system produced due to lipid peroxidation (Gosavi et al., 2012a; Patil et al., 2012b). This yard stick has been reported to mirror loss of testicular function and sperm damage owing to oxidative damage mediated by arsenic (Morakinyo et al., 2010; Sikka, 2004). Glutathion is a pivotal components of cell growth differentiation and protection comprising cellular thiol redox buffer which mediates and maintains thiol/disulphide redox balance (Sies, 1999). Severe oxidative stress corresponds to decrease in glutathione levels. Glutathione has major role in suppression and scavenging of ROS (Willcox et al., 2004). Arsenic has been a proven agent disturbing the pro-oxidant-antioxidant balance owing to its intrinsic affinity for sulfhydryl groups of GSH (Yamanaka et al., 1991). GSH exerts protective activity against toxic infiltrators viz., arsenic metabolism by donating electrons to convert arsenic³⁺ to arsenic⁵⁺ thus GSH depletion due to arsenic is a hallmark of arsenic induced cellular damage (Ramanathan et al., 2002). Naringin elucidate a discernable up-regulation of GSH and down-regulation of MDA conferring its ability to reverse arsenic induced oxidative damage.

SOD accentuate the dismutation of superoxide to H₂O₂ which is regulated by catalase (Usouh et al., 2005). SOD acts as primary line of defence to free radical induced aberrations. The reduction in levels of SOD corresponds to reduction of cellular defensive mechanism (Kandhare et al., 2012a). In present investigation, naringin elevated the levels of SOD in a dose dependent manner demonstrating its ability to restore cellular oxidoreductive balance.

It has been demonstrated that administration of sodium arsenite leads to elevate nitric oxide levels which produce cytotoxicity and oxidative damage (Bera et al., 2010; Flora et al., 2009). Superfluous of nitric oxide interact with superoxide anion to produce peroxynitrite radical which causes additional cell damage by nitrating and oxidizing cellular macromolecules. NO has dual role in cellular stress management mechanism (Gosavi et al., 2012b; Patil et al., 2012a). There are three isoforms of nitric oxide synthase (NOS) viz., eNOS (endothelial NOS), nNOS (neuronal NOS) and iNOS (inducible NOS). iNOS play a major pathological role in mediating inflammation host defence mechanism and maintenance of nitrosative balance in the cellular environments (Patil et al., 2012d). NO is produced by iNOS which in turn blind with O²⁻ leading to formation of ONOO⁻ (Beckman and Crow, 1993). This radical is termed as peroxinitrites which damages intra-mitochondrial and cellular oxidative balance (Radi, 2004). In the present investigation, naringin reduced the elevated nitric oxide levels thus evidencing its potential to restore disregulated nitrosative balance.

It has been reported that ROS are produced by cytokines. The production of hydroxyl radicals and lipid peroxidation is increased in tumorigenic fibroblast cells and mesangial cells by TNF-α (Bohler et al., 2000). Inorganic form of arsenic is considered to trigger inflammatory response. Exposure to arsenite in rats have been found to increase serum levels of TNF-α (Mukherjee et al., 2006). Over-expression of TNF-α was reported in humans chronically exposed to inorganic arsenite. TNF-α production is remarkably increased by cytopoietic effects of arsenite in murine micophages (Sakurai et al., 1998). Due to arsenic induced pro-inflammatory cytokines there may be increased level
of oxidative stress which leads to produce toxic effect of arsenite in present study. However, treatment of naringin prominently reduced the TNF-α level which is in line with the study of previous findings (Kandhare et al., 2012b). IL-1β is a member of pleiotropic family, utterly formed by macrophages (Bergh and Soder, 1990; Cannon et al., 1990) and influences Leydig cells in a paracrine manner. Additionally, testosterone secretion was noteworthy increased by the administration of IL-1β into rat testis (Gerendai et al., 2005). Moreover, inhibition of Leydig cell steroidogenesis during inflammation is reported by IL-1β (Calkins et al., 1988). Aforementioned mechanism of IL-1β may be responsible in present investigation to produce testicular toxicity in sodium arsenite treated rats which was significantly restored by the naringin treatment.

Co-enzyme Q10 (ubiquinone or 2, 3 dimethoxy-5 methyl-6-decaprenyl benzoquinone) is a liquid-soluble potent antioxidant and anti-inflammatory substance. It has been previously reported that treatment with Co-enzyme Q10 significantly decreased the arsenic-induced expression of Fas ligand and caspase-3 via its free radical scavenging activity, anti-inflammatory action with reduced tumor necrosis factor-α production and inhibition of nuclear factor-kB expression thus prevent testicular injury and dysfunction induced by arsenic exposure (Fouad et al., 2011). Treatment with naringin also showed similar effect in amelioration of arsenic-induced testicular toxicity via down-regulation of oxidative-inflammatory mediators and pro-inflammatory cytokines.

CONCLUSION

In conclusion, results of present study suggested that sodium arsenite altered the activities of oxidative-nitrosative stress, pro-inflammatory cytokines in testis along with biochemical parameters. On the basis of this study, the use of naringin may be useful in the protection from sodium arsenite induced toxicity. Hence, naringin exerted its protective effects against sodium arsenite induced testicular toxicity via modulation of endogenous antioxidant enzyme, inhibition of release of oxidative-inflammatory mediators such as MDA and NO as well as pro-inflammatory cytokines.

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