Increase of glutathione, testosterone and antioxidant effects of *Jurenia dolomiaeae* on CCl₄ induced testicular toxicity in rat

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**Abstract**

**Background:** Root of *Jurenia dolomiaeae* is used traditionally in various disorders involving oxidative injuries i.e. rheumatism, gout and as stimulant. Earlier we have investigated in vitro antioxidant and DNA protective ability. In this investigation we have evaluated protective potential of *J. dolomiaeae* root against the oxidative injuries induced with carbon tetrachloride (CCl₄) in testes of rat.

**Methods:** Dried roots of *J. dolomiaeae* were powdered and extracted with 95% methanol and residue was fractionated in escalating polarity of solvents. On the basis of potent antioxidant ability; the ethyl acetate fraction (JDEE) was selected to evaluate the in vivo antioxidant activity against CCl₄ induced oxidative stress in rat. Sprague Dawley male rats (42) were equally divided into 7 groups: control, vehicle control, JDEE (400 mg/kg; p.o.) alone, CCl₄ (1 ml/kg; 1:10 v/v in olive oil) alone, JDEE (200 mg/kg, 400 mg/kg) with CCl₄, and silymarin (200 mg/kg) with CCl₄ on alternate days for 60 days. Testes samples were investigated for antioxidant enzymes, biochemical markers and histopathology while the serum samples were analyzed for the testosterone level.

**Results:** Administration of CCl₄ to rats depleted the activity level of antioxidant enzymes viz.; CAT, POD, SOD, GST, GPx, and GR, and the concentration of protein and GSH while enhanced the level of lipid peroxides (TBARS), H₂O₂ and nitrite in testes samples of rat. Concentration of testosterone in serum of rat decreased with CCl₄ treatment. Co-treatment of silymarin and the JDEE (200 mg/kg, 400 mg/kg) lessened the toxic effects of CCl₄ and reversed the level of these parameters towards the control group. An admirable increase (*P* < 0.05) in the level of GSH in testes, testosterone in serum and thickness of germinal layers in testes with JDEE (400 mg/kg) alone was recorded. Histopathological observation of testes samples endorsed the alterations induced with different treatments.

**Conclusions:** JDEE co-treatment to rats ameliorated the toxic effects of CCl₄ in testes samples. Enhanced level of GSH, thickness of germinal layers in testes and testosterone in serum with JDEE (400 mg/kg) treatment alone to rats demanded the evaluation of JDEE for sexual behavior.

**Keywords:** *Jurenia dolomiaeae*, Antioxidant enzymes, Lipid peroxides, Gsh, Testosterone

**Background**

Male infertility is a major clinical problem affecting 30% of the world population [1]. Among several factors affecting the fertility are the erectile dysfunction, premature ejaculation, quality and quantity of sperms. These effects might be caused due to the production of free radicals in testes. Clinical disorders such as hypertension and atherosclerosis which are usually induced with reactive oxygen species (ROS) also provoke the erectile dysfunction. Male sexual characteristics are regulated by androgens; hypogonadism induced with decrease of natural antioxidant capacity (glutathione) and/or increase of ROS may cause loss of libido in adults which badly affected the sexual behavior [2].

Various reports revealed that carbon tetrachloride (CCl₄) a well-established hepatotoxin also causes injuries in testes [2, 3]. CCl₄ is metabolized in the tissues to highly reactive trichloromethyl radical which starts free...
radical induced lipid peroxidation of cytoplasmic mem-
brane phospholipids and brings pathological changes in
the cell membrane by accumulation of lipid derived ox-
idants. Exposure of rats to CCl₄ causes oxidative injuries
in testes of rat leading to decrease the activity level of
antioxidant enzymes and substances such as glutathione
(GSH) while enhanced the lipid peroxidation. Histopa-
thological investigation of testes revealed the dam-
aging action of CCl₄ in rat. Treatment of CCl₄ causes hypo-
gonadism and decrease the level of testosterone in
serum of rat [3, 4]. To cope with such reactive species
and the clinical disorders, it is essential to obtain dietary
antioxidants which counter measure the excessive gener-
ation of free radicals [3, 4].

Jurinea dolomiaea Boiss (syn. Jurinea macrocephala
Royle) has a place with Family Asteraceae (Compositae).
It is a prostrate perennial herb with a thick terminal
group of large flower heads and a rosette of long spread-
ing lobed leaves with purple mid veins, root long, tuber-
ous. It is found in scattered places in Byas and Darma
valley in Northern areas of Pakistan. Root is utilized in
the form of poultice as antiseptic in skin eruptions while
its decoction is given in colic. Additionally, it is recog-
nized cordial and is given in puerperal fevers. Roots are
acknowledged to be immuno-stimulant and given in
fever after labor [5, 6]. Roots are also used by the local
population for loose bowels and stomachache [6]. Local
communities use the crushed roots for skin eruptions
[7, 8] while the aromatic oil obtained from roots is used
in gout and rheumatism [9]. Ahmad and Habib [10] re-
ported that local communities use the extract of roots as
 tonic for weakness of the bones. Roots are cooked with
maize flour and used for the treatment of bone fractures.
Antimicrobial activity of the leaf extracts of J. dolomiaea
have been reported by Dwivedi and Wagay [11]. Antioxi-
dant and antibacterial activities of the J. dolomiaea plant
have also been evaluated [12]. Earlier we have reported in
vitro antioxidant and DNA protection ability of the
methanol extract and its derived fractions. Among the
extract/fractions ethyl acetate fraction exhibited the
admirable antioxidant and DNA protective activities [13].
In vivo evaluation of the plant was needed to ensure its
protective effects against CCl₄ induced toxicity in testes of
rat. In this regard we have assessed the activity level of
antioxidant enzymes and lipid peroxidation in testes sam-
pies whereas the concentration of testosterone in serum
of rat. Histopathological investigations on testes samples
were also carried out to endorse the effect of various

Methods
Plant collection
Plants of J. dolomiaea were collected in 2011 from Nazar
zera area of Kohistan, Pakistan. The plants were
recognized by their local names and then confirmed by
Dr. Mir Ajab Khan, Department of Plant Sciences,
Quaid-i-Azam University, Islamabad. Voucher specimen
with Accession No. 27823 was deposited at the Herbarium,
Quaid-i-Azam University, Islamabad.

Extract preparation
After collection, roots were shade dried till the complete
removal of moisture and samples were made to mesh
sized powder by using plant grinder and powder (5 kg)
was soaked in crude methanol (10 L) for extraction for
72 h. The extraction was repeated two times with above
procedure. For the purpose of filtration, Whatman No. 1
filter was used and methanol was evaporated on a rotary
evaporator at 40 °C under reduced pressure to get the
viscous material and fractionated on escalating polarity
basis. Ethyl acetate fraction (JDEE) exhibited the most
promising antioxidant abilities for various assays [13]
thus was selected for in vivo evaluation against CCl₄
induced testicular in a rat model.

Acute toxicity studies
For acute toxicity study 18 Sprague–Dawley male rats of
good health were randomly divided into six groups
(3 rats in each). Animals were off feed but had open
access to water 15 h prior of test samples. The control
group orally received 15% DMSO in olive oil. However,
rats of other groups 2–6 orally received 250, 500, 1000,
2000, and 4000 mg/kg of JDEE. General behavior of
animals was noted after 120 min of treatment. Food and
water were given ad libitum. Animals were screened for
mortality and morbidity for 14 days [14].

CCl₄ induced toxicity studies in rat
For in vivo evaluation of JDEE, CCl₄ was used to induce
toxicity in testes of rats, used as an animal model.
Guidelines of National Institutes of Health, Islamabad
were strictly followed in order to conduct experiments
effectively. The designed protocol (Bch#248) was then
approved by the Ethical Committee of Quaid-i-Azam
University, Islamabad, Pakistan. Animals were kept at
room temperature (25 ± 3 °C) with a 12 h dark/light
cycle in ordinary cages. Animals were properly fed on
standard laboratory feed and water.

The rats were then left for adaptation to laboratory con-
dition for 7 days before the commencement of experi-
ment. The sixty day experiment was designed according
to Patrick et al. [15] with minor modifications. For this
purpose 42 male Sprague Dawley (Rattus novergicus) rats
(180–200 g) were randomly divided in to 7 groups with 6
rats in each. JDEE and silymarin after dissolving in DMSO
(500 mg/ml) was mixed with olive oil (1:1; v/v). Animals
of Group I were remained untreated while of Group II
were treated with the vehicle (1 ml/kg). Group III were
treated (i.p.) with 1 ml/kg of CCl₄ (1:10; v/v) after dissolving in olive oil and DMSO (1:1; v/v). The rats of Group IV were treated with CCl₄ and co-administered with silymarin (200 mg/kg; p.o.); however, the rats of Group V and VI were treated with CCl₄ and co-administered (p.o.) with JDEE (200 mg/kg; 400 mg/kg, respectively). Rats of Group VII were administered (p.o.) alone with 400 mg/kg of JDEE. All these treatments were given in the morning for 60 days. Rats were remained unfed for 24 h after the last treatment. Animals were euthanized after chloroform light anesthesia and dissected from ventral side. Blood was collected for testosterone measurement in the serum. Testes were excised from each animal and placed in liquid nitrogen.

Analysis of testes homogenates

Testes samples were homogenized and mixed with 10 volume of 100 mM potassium phosphate buffer containing 1 mM EDTA (pH 7.4). Centrifugation of the homogenates was done for 30 min at 12000 g at 4 °C. The supernatant was collected which was used for further analyses. The total amount of soluble proteins in tissue homogenates of testes was determined by using crystalline BSA as standard [16].

Catalase (CAT) activity

CAT activity determination was based on the process which depends on the decomposition of H₂O₂. The reaction mixture was prepared by the addition of 100 μl of 5.9 mM H₂O₂, 625 μl of 50 mM potassium phosphate buffer (pH 5.0) and 25 μl of tissue homogenate. Disappearance of H₂O₂ by catalase was measured in the reaction mixture at 240 nm spectrophotometrically. Absorbance change of 0.01 as units/min defines one unit CAT activity [17].

Peroxidase (POD) activity

Chance and Maehly [17] method was used to determine POD activity in the the samples. For this the reaction mixture contained 25 μl of tissue homogenate, 25 μl of 20 mM guaiacol, 75 μl of 40 mM H₂O₂ and 625 μl of 50 mM potassium phosphate buffer (pH 5.0). At 470 nm change in absorbance was recorded. Change in absorbance of 0.01 as units/min defines one unit POD activity.

Superoxide dismutase (SOD) activity

SOD activity was determined by Kakkar et al. [18] method. Tissue homogenates were first centrifuged at 1500 g for 10 min and then for 15 min at 10000 g. From the supernatant a volume of 150 μl was added to the reaction mixture containing 50 μl of 186 μM phenazine methosulphate and 600 μl of 0.052 mM sodium pyrophosphate buffer (pH 7.0). In order to start the reaction 100 μl of 780 μM NADH was added and then 500 μl of glacial acetic acid was added after 1 min to stop the reaction. Absorbance of the reaction mixture was recorded at 560 nm. Finally the results obtained were expressed in units/mg protein.

Glutathione-S-transferase (GST) activity

According to Habig et al. [19] activity of glutathione-S-transferase was based on the formation of conjugate between GSH and 1-chloro-2,4-dinitrobenzene (CDNB). The reaction mixture was prepared by the addition of 150 μl of tissue homogenate, 12.5 μl of 1 mM CDNB, 100 μl of 1 mM GSH and 720 μl of sodium phosphate buffer. At 340 nm absorbance of the reaction mixture was recorded and with molar extinction coefficient of 9.6 × 10³/M/cm, GST activity was determined, expressed as nM CDNB conjugate formed/min/mg protein.

Glutathione peroxidase (GPx) activity

According to Mohandas et al. [20], NADPH was used as substrate in order to determine GPx activity. For this purpose the reaction mixture was constituted by the addition of 50 μl of tissue homogenate in 50 μl of 1 mM sodium azide, 50 μl of 1 mM EDTA, 25 μl of glutathione reductase (1 unit/ml), 25 μl of 1 mM GSH, 5 μl of 0.25 mM H₂O₂ and 740 μl of 0.1 M sodium phosphate buffer (pH 7.4). To initiate the reaction 50 μl of 0.2 mM NADPH was added and decline in absorbance was recorded at 340 nm at 25 °C for 20 min. Blank tubes contained only distilled water. By using molar extinction coefficient (6.22 × 10³/M/cm) activity of GPx was assessed and expressed as nM of NADPH oxidized/min/mg protein.

Glutathione reductase (GR) activity

NADPH was used as substrate in order to determine GSR activity. To 50 μl of tissue homogenate, 25 μl of 1 mM oxidized glutathione, 50 μl of 0.1 mM NADPH, 50 μl of 0.5 mM EDTA and 825 μl of 0.1 M sodium phosphate buffer (pH 7.6) were added. Decomposition of NADPH was measured spectrophotometrically at 340 nm (25 °C). With the help of the molar extinction coefficient (6.22 × 10³/M/cm), enzymatic activity (GSR) was expressed as nM NADPH oxidized/min/mg protein [21].

Reduced glutathione (GSH)

The method of Jollow et al. [22] was followed to measure GSH activity in the testes samples. Briefly, 500 μl of testes supernatant was mixed with 500 μl of sulfosalicylic (4%) to carry out precipitation. The reaction mixture was incubated for 1 h at 4 °C and then centrifuged at 1200 g for 20 min. Supernatant was collected and 33 μl of it was added to the reaction mixture containing 66 μl of 100 mM of 5,5′-dithio-bis (2-nitrobenzoic acid
(DTNB) and 900 μl of 0.1 M potassium phosphate buffer (pH 7.4). The yellow colored complex was formed due to the reaction of reduced glutathione with DTNB. At 412 nm absorbance was immediately read and the GSH activity was presented by μM GSH/g tissue.

**Lipid peroxidation assay (TBARS)**
The method of Ohkawa et al. [23] was used to measure the TBARS (thiobarbituric acid reactive substances) in testes samples. The reaction mixture consisted of 100 μl of tissue homogenate, 10 μl of 100 mM FeCl₃, 100 μl of 100 mM ascorbic acid and 290 μl of sodium phosphate buffer (pH 7.4). The reaction mixture was incubated for 1 h at 37 °C in a shaking water bath at 37 °C. In order to stop the reaction 500 μl of 10% trichloro-acetic acid (TCA) was added and after an addition of 500 μl of 0.67% thiobarbituric acid (TBA) the mixture was placed in boiling water bath for 15 min. Then it was shifted on crushed ice for 5 min and centrifuged at 2500 g for 10 min. In order to determine the amount of TBARS formed, the absorbance of the supernatant was recorded at 535 nm. With the help of the molar extinction coefficient (1.560 × 10⁵/M/cm), lipid peroxidation activity (TBARS) was expressed as TBARS formed/min/mg tissue.

**Nitrite assay**
With the help of the Griess reagent nitrite assay was carried out according to the method of Green et al. [24]. The homogenate was treated with an equal volume of (100 μl) of 0.3 M NaOH and 5% ZnSO₄. The mixture was then centrifuged at 6400 g for 20 min to get the protein free supernatant. Griess reagent (1 ml) was added into the cuvette to blank the spectrophotometer. Then 20 μl of supernatant was added to the cuvette containing reagent and change in color was recorded at 540 nm. Nitrite concentration was calculated in the tissue samples by using standard curve of sodium nitrite.

**Hydrogen peroxide (H₂O₂) assay**
The protocol of Pick and Keisari [25] was followed to assess the level of hydrogen peroxide (H₂O₂) in the testes samples. The oxidation of phenol red was carried out by H₂O₂-mediated horseradish peroxidase enzyme. The reaction mixture was prepared by the addition of 1 ml of 0.28 nM phenol red, 2.0 ml of sample homogenate, 5.5 nM dextrose, 0.05 M phosphate buffer (pH 7) and horseradish peroxidase (8.5 units) and incubated for 60 min at 37 °C. To stop the reaction 0.01 ml of 1 N NaOH was added and centrifuged at 800 g for 5–10 min. The absorbance of the sample was noted at 610 nm by using the reagent as a blank. The concentration of H₂O₂ was given as nM H₂O₂/min/mg tissue based on the standard curve of H₂O₂ oxidized phenol red.

**Analysis of testosterone in serum**
The concentration of testosterone in serum was estimated through Astra Biotech kit purchased from Immunotecnique Company. Sensitivity of the kit is 0.2 nmol/L – 50 nmol/L. The experiment was performed according to the instruction.

**Histopathological study of testes**
The testes samples were fixed in fixative sera for 3–4 h, then with the help of ascending grades of alcohol (80%, 90%, and 100%) tissues were dehydrated and finally shifted in cedar wood oil. Tissues after becoming clear were embedded in paraplast. Thin slices (3–4 μm) were prepared with the help of the microtome and then after removing wax, these were stained with hematoxylin-eosin stain, examined under microscope at 40×.

**Statistical analysis**
All parametric values were expressed as means ± standard deviation (SD) of six observations. To determine the difference among various treatments one way analysis of variance was estimated by using the Statistix 8.1. Multiple comparisons among various treatments were determined by using Boneferroni post hoc comparison test. A P value <0.05 was considered significant.

**Results**

**Effect of JDEE on antioxidant enzymes of testes**
In this study the activity level of CAT, POD, SOD in testes samples of CCl₄ treated rats decreased as compared to the control group (Fig. 1). Administration of silymarin in combination with CCl₄ ameliorated the toxic effect of CCl₄ and increased the activity level of CAT, POD, SOD in testes samples and nonsignificant difference with the control was recorded. In case of JDEE co-treatment with CCl₄, the activity level of CAT, POD, SOD, dose dependently, elevated in the testes samples as compared to CCl₄ alone treated group. The higher dose of JDEE (400 mg/kg) along with CCl₄ ameliorated the toxic effects of CCl₄ significantly enhanced the level of CAT, POD, SOD and non significant (P > 0.05) difference was observed in comparison to that of the control group. However, the activity level of CAT, POD, SOD in testes of rat with JDEE (400 mg/kg) treatment alone was not changed as compared to the control group.

Activity level of GST, GPx and GR after various treatments is shown in Fig. 1. The activity level of GST, GPx and GR in testes samples treated with CCl₄ decreased (P < 0.05) as comparison to the control group. Co-treatment of silymarin reference drug in combination with CCl₄ significantly (P < 0.05) increased the level of these enzymes as compared to the CCl₄ group. However, the level of these enzymes with co-treatment of silymarin was significantly (P < 0.05) lower as compared to...
the control group. In groups of CCl₄ along with JDEE treatment activity level of GST, GPx and GR in testes samples was increased, dose dependently, as compared to the CCl₄ group. The higher dose of JDEE (400 mg/kg) produced similar protective effects ($P > 0.05$) for these enzymes to that of the silymarin co-treated group. Rats when orally treated with JDEE (400 mg/kg) alone did not alter the activity level of these enzymes as compared to the control group.

**Effect of JDEE on biochemical profile of testes**

The profile of testes protein, TBARS, H₂O₂ and nitrite content of different groups is shown in Fig. 2. It is apparent from Fig. 2 that concentration of protein in testes of rat was significantly ($P < 0.05$) decreased in CCl₄ treated group as compared to the control group. Co-administration of JDEE along with CCl₄, dose dependently, increased the level of protein in testes samples. However, complete protection was not attained even at the highest dose of JDEE (400 mg/kg) and significantly lower level of protein was recorded in comparison to that of the control group. Rats treated with CCl₄ in combination with silymarin exhibited the protein level similar ($P > 0.05$) to that of the control group. Treatment of JDEE (400 mg/kg) alone to rats did not change the level of protein as compared to the control group.

The level of TBARS, H₂O₂ and nitrite was significantly ($P < 0.05$) elevated in CCl₄ treated group as compared to the control group (Fig. 2). Concentration of TBARS, H₂O₂ and nitrite was diminished, dose dependently, and at higher dose of JDEE (400 mg/kg) co-administered with CCl₄ similar level of these parameters to that of the control group.

**Fig. 1** Effect of different treatments of *J. dolomiaeae* ethyl acetate fraction (JDEE) on (a) catalase (b) peroxidase (c) superoxide dismutase (d) glutathione-S-transferase (e) glutathione peroxidase (f) glutathione reductase in testes of rat. (1) Control, (2) Vehicle control, (3) CCl₄ treated control, (4) CCl₄ + Silymarin (200 mg/kg), (5) CCl₄ + JDEE (200 mg/kg), (6) CCl₄ + JDEE (400 mg/kg), (7) JDEE (400 mg/kg). Bars with different letters indicate significant difference ($P < 0.05$).
control group was observed. However, co-administration of JDEE at higher dose of 400 mg/kg showed more protection in terms of nitrite content than the reference silymarin treated group.

The level of GSH in testes samples of various groups is shown in Fig. 2. The results indicated that administration of CCl₄ to rats cause toxicity and depleted the level of GSH in testes as compared to the control group. Co-treatment of reference drug silymarin with CCl₄ was able to maintain the level of GSH in testes sample similar to that of the control group. On the other hand co-administration of JDEE ameliorated the toxicity of CCl₄ and increased the level of GSH in testes samples in a dose dependent manner. However, the lower and the higher dose did not totally ameliorated the toxicity of CCl₄ thus the level of GSH obtained in testes samples was significantly (P < 0.05) lower as compared to the control group. Further, administration of JDEE (400 mg/kg) alone to rats significantly (P < 0.05) increased the level of GSH as compared to the control group.

Effect of JDEE on testosterone

Level of testosterone in serum of different groups is displayed in Fig. 2. Treatment of CCl₄ exhibited significant (P < 0.05) reduction in testosterone level of serum as compared to the control group of rat. Co-administration of silymarin as a reference drug in this experiment showed an elevation in the level of testosterone in comparison to that of the CCl₄ treated group but its level was significantly (P < 0.05) less to that of the control group. Treatment of JDEE in combination with CCl₄, dose dependently, increased the level of testosterone and at the higher dose (400 mg/kg) nonsignificant (P > 0.05) difference was observed in comparison to that of the control group.
control group. However, when rats were treated with JDEE (400 mg/kg) alone, significantly \( (P < 0.05) \) higher level of testosterone was recorded in comparison to that of the control group.

**Protective role of JDEE on histoarchitecture of testes**

Effect of JDEE on testes histoarchitecture in different groups is illustrated in Fig. 3. The control group and the negative control group did not exhibit any alteration and displayed normal architecture of seminiferous tubules normal developmental stages and concentration sperms in the seminiferous tubules. CCl\(_4\) treatment resulted in rupturing, displacement and alteration in shapes of seminiferous tubules. Vacuolization of germinal epithelium and prominent decrease in germ cells was also recorded. Silymarin co-treatment showed marked protection in terms of morphology of the seminiferous tubules and the density of germ cells. In JDEE co-treated groups ameliorative effects against CCl\(_4\) induced toxicity were observed on the architecture of the seminiferous tubules in a dose dependent fashion. JDEE at low dose illustrated some vacuoles in seminiferous epithelium which were absent at the higher dose (400 mg/kg). JDEE when treated alone demonstrated high density of germinal cells and sperms in the seminiferous tubules.

**Discussion**

Development of spermatozoa from spermatogonial stem cells is regulated by various hormones and this whole process in controlled by the hypothalamic-pituitary-testicular axis. Any minor disturbance either imposed internally or from the external environment such as chemicals, lead to fertility problems in males. Accumulation of ROS in testis induces hypogonadism [3]. Exposure of rats to CCl\(_4\) prompted significant reduction in CAT, POD, SOD and GPx profile, exhausted the GSH level and elevated TBARS in testes [2–4]. Higher production of H\(_2\)O\(_2\) and nitrite in testes samples on account of CCl\(_4\) treatment suppresses the antioxidant defense while enhances the cellular injuries [2–4]. JDEE constituted significant amount of total flavonoids 807.0 ± 7.2 mg rutin equivalent/g of JDEE. It can be argued that protection provided against oxidative stress by JDEE can be attributed by the presence of flavonoids and other antioxidant constituents. As JDEE interfere with the oxidation process by scavenging, reducing and chelating free radical [13] that is reflected in decrease of TBARS and increase in total antioxidant capacity. It is suggested that the presence of flavonoids in JDEE might play a major role in treating or retarding oxidative stress related fertility disorders; and can improve fertility rate in men.

Enhanced level of GSH in the rats treated with JDEE (400 mg/kg) alone was recorded in this study. As GSH regulate the level of antioxidant enzymes, it may be intriguing that JDEE can be helpful to control the oxidative status and consequently the libido and erectile function in males. Increase in GSH level with juice and methanol extract of pomegranate peel has been determined in rat [26]. In another study use of pomegranate juice increased the GSH in testis of rat [27].

CCl\(_4\) impelled testes damages have been connected with high nitrite generation in this study. Peroxynitrite

![Fig. 3](image_url)
anions have been created by the response of nitric oxide and superoxide anion. These peroxynitrite anions oxidize biomolecules, which finally prompts lipid peroxidation [3, 4]. Free radical scavenging capability of JDEE may be the reason in defensive impact against CCl₄ harmfulness. JDEE hold the active constituents (flavonoids, terpenoids, saponins) which directly or indirectly scavenge the oxidative harm to various cells and organs while normalizing their oxidative status [3, 4]. Deterioration of seminiferous tubules and germ cells, interstitial in part was vanished and replaced by fibroblast and inflammatory cells were recorded in testes of CCl₄ treated group of this study. Presence of flavonoids in JDEE could be included in ameliorating the impacts of CCl₄ prompted injuries and correspondingly close to typical histology of testis. Similar histopathological alterations were recorded while assessing the defensive impact of various extracts of plants on testes against CCl₄ induced toxicity [3, 4]. Administration of JDEE alone to rats caused the increase in thickness of germinal layer of seminiferous tubules. Increase in the diameter of seminiferous tubules and increase in germinal thickness has been demonstrated in rat with pomegranate juice and alcoholic extract of Nigella sativa seeds [27, 28].

In the present study, testosterone concentration was quantified and was observed altogether low in correlation to that of the standard control groups. Treatment of animals with CCl₄ in combination with JDEE ameliorated the harmful impacts of CCl₄ and the level of testosterone was increased, in a dose dependent way. JDEE hold the constituents (flavonoids, terpenoids and saponins) which directly or indirectly ameliorate the oxidative harm to distinctive cells and organs. Protective effect of plants against the CCl₄ induced testicular toxicity have been reported [3, 4]. However, the administration of JDEE (400 mg/kg) alone to rats significantly enhanced the level of testosterone in serum as compared to the control group. Presence of saponins in the JDEE might be responsible for the release of pituitary LH while the flavonoids might have been implicated in the synthesis of androgens. Higher testosterone level with pomegranate juice and alcoholic extract of Nigella sativa seeds has been determined in rat [27, 28]. It is suggested that the enhanced level of testosterone with JDEE in male rats might be beneficial to increase libido and sexual function of human being.

Conclusions
The results of the present investigation suggested the antioxidant effects of the JDEE against the oxidative stress induced with CCl₄ in testes of male rates. Diminished level of CAT, POD, SOD, GPx, GST, GR and GSH while enhanced level of TBARS, nitrite and H₂O₂ in testes samples of male rats was reversed back to the control, dose dependently, by the co-administration of JDEE. The altered histopathological changes induced with CCl₄ were also diminished with co-treatment of JDEE. However, an admirable effect of JDEE alone to male rats was the enhanced level of GSH in testes and testosterone in serum samples. The thickness of the germinal epithelium was also increased. These results suggested the evaluation of JDEE for sexual behavior.

Abbreviations
CAT: Catalase; CCl₄: Carbon tetrachloride; GPx: Glutathione peroxidase; GR: Glutathione reductase; GSH: Glutathione; GST: Glutathione-S-transferase; H₂O₂: Hydrogen peroxide; JDEE: Jurenia dolomiaeae ethyl acetate fraction of crude methanol extract; POD: Peroxidase; SOD: Superoxide dismutase; TBARS: Thiobarbituric acid reactive substances

Acknowledgements
MRK is intensely acknowledged for his kind supervision, expert guidance and substantial facilitations of all necessary materials and equipment.

Funding
The project was funded by the Department of Biochemistry Quaid-i-Azam University Islamabad Pakistan.

Availability of data and materials
All the data is contained in the manuscript.

Authors’ contributions
NAS made significant contribution to experimentation, acquisition and drafting of the manuscript. MRK has made substantial contribution to designing, analyzing and drafting of the manuscript. Both authors read and approved the final manuscript.

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MRK did his Diploma in Unani Medicine and Surgery (DUMS) and is a registered practitioner of the National Council for Tibb of Pakistan. He is working as Associate Professor at the Department of Biochemistry, Quaid-i-Azam University, Islamabad, Pakistan.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Not applicable.

Ethics approval and consent to participate
This study makes use of rats and the experimental protocol for the use of animal was approved (Bch#0248) by the ethical board of Quaid-i-Azam University Islamabad Pakistan.

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Received: 22 November 2016 Accepted: 4 April 2017
Published online: 08 April 2017

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