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Original Article

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Abstract

Chronic renal failure (CRF) is associated with male gonadal dysfunction that may lead to impotence, reduced libido, reduced size of testes and impaired spermatogenesis. The present study assesses the protective impact of *Raphanus sativus* seed extract (RSE) on testicular dysfunction and infertility associated with CRF induced by adenine in male rats. Twenty adult male albino rats were divided into four groups (5 each) and received their treatment for 4 weeks: group I, control; group II, (RSE): (0.52 ml/kg) orally via stomach tube, group III, (AD): (0.75 % W/W in diet), group IV, (RSE+ AD): received RSE and AD the same dose and route as groups II and III daily. The results showed a significant decline in the body weight gain, testicles and epididymides weights, testosterone level, semen quality (sperm count, motility, and viability), reduced glutathione (GSH) level, superoxide dismutase (SOD) and catalase (CAT) activities, and increase in kidney weight, serum creatinine (SCr), BUN, uric acid, FSH & LH levels, sperm abnormality, malondialdehyde (MDA) & nitric oxide (NO) in AD treated rats, and histopathological alteration in kidney and testes tissue. But, RSE and AD co-administration partially successfully blocked these parameters and prevent histological structures abnormalities that nearly remain within normal levels in comparison with AD group.

1. Introduction

Chronic Renal Failure (CRF) – or chronic kidney disease (CKD) - is a public health issue that growing in developed and developing countries **(Perico and Remuzzi, 2016; Hsu and Powe, 2017)**, CRF is characterized by the presence of a markers of kidney damage, such as increasing of renal function markers (creatinine and urea) and a decreased glomerular filtration rate (GFR) for three months or more **(Bickel *et al.*, 2002 and Hsu *et al.*, 2004).** In CRF, retention of several metabolic end products is progressively occurs and the body excretes them difficultly due to kidney failure, but they can hardly remove from the body through dialysis techniques **(Vanholder *et al.*, 2003).** The progresses of CKD may lead to cardiovascular, respiratory and endocrine disorders **(Webster *et al.*, 2017).**

Adenine (AD) (6-amino-purine), which initially described by **Yokozawa *et al.* (1986)** is mainly lead to induction of CRF**,** had accepted as animal model to study CRF which resemble that occur in human CKD **(El-Habibi *et al.*, 2014 and Thakur *et al.*, 2018).**

Sexual and abnormal male gonadal dysfunction is mostly associated with CKD patients **(Finkelstein and Finkelstein, 2002).** These patients may suffer from impotence, reduced drive, diminished testicular size, decline sperm formation and gynaecomastia **(Edey, 2017).** Semen analysis in men with advanced CKD demonstrates low volume of ejaculate and decrease sperm motility **(Lessan-Pezeshki and Ghazizadeh, 2008; and Lundy and Vij, 2019).** Men with CKD have sex hormone profile which characterized by raised LH, FSH and markedly reduced testosterone **(Eckersten *et al.*, 2015 and Lehtihet and Hylander, 2015).** Also, rats with CRF induced by AD or 5/6 nephrectomy showed a decrease in testosterone level, increase in LH and FSH levels, sperm abnormalities, decrease in sexual behavior, and histopathological changes in the testis **(Adachi and Nakada, 1999; and Li *et al.*, 2020).**

Some therapeutic plants are broadly utilized as aphrodisiac to diminish abnormal gonadal action or fertility stimulating factors, through increasing nutritional value thereby improving sexual performance **(Sumalatha *et al.*, 2010).** Radish (*Raphanus sativus*) belongs to the family

Brassicaceae that has dietary and health protective properties. All parts of radish (seeds, roots and leaves) are used as a food and in treatment of a wide assortment of diseases **(Ghayur and Gilani, 2006).** In addition, different reports have recorded the antimicrobial **(Rakhmawati *et al.*, 2009),** anticancer **(Pocasap *et al.*, 2013)**, and antioxidant effect of radishes **(Kim *et al.*, 2017)**. Radish seeds contain alkaloid compounds like coumarins, saponins, flavonoids and anthocyanins **(El-Sayed, 2001).** Radish seed extract has protective impact against hypogonadism in male rats (**Tabassum and Khan, 2017)** and improve semen qualities **(Dafaalla *et al.*, 2017).** Also, **Yang *et al.* (2016)** concluded that, *Raphanus sativus* sprout extract enhanced sperm cell count in the testes with increased testosterone secretion in male rats intoxicated with bisphenol A. **El-Tohamy *et al.* (2010)** concluded that, radish seed meal increased the sperm motility, lower the abnormal sperm count, and exceed the normal spermatozoa in male rabbits.

Along these lines, the current study aims to assess the protective impact of *Raphanus sativus* seed extract (RSE) on testicular dysfunction and infertility in CRF induced by adenine in male rats..

2. Materials and Methods

Cell line

Materials

Chemicals and Kits

Adenine ($C_5H_5N_5$) was purchased from agent of TM Media (Rajasthan, India). Methanol and Tween (80) were bought from Adwic Company, Cairo, Egypt. Kits for Kidney function tests [blood urea nitrogen (BUN), creatinine (Cr), and uric acid (UA)], antioxidant biomarkers [reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT)], and oxidative stress markers [malondialdehyde (MDA) and nitric oxide (NO)] were purchased from Biodiagnostic Company, Giza, Egypt. Kits for estimation of hormones (Testosterone, FSH & LH) were purchased from agent of Roche Diagnostic Company, Mannheim, Germany. All other chemicals were of analytical grade.

Preparation of *Raphanus sativus* Seed Extract (RSE)

Raphanus sativus seeds were purchased from a local market in Mansoura city, Egypt, and identified and authenticated in Department of Botany, Faculty of science, Mansura University.

Raphanus sativus seeds (1kg) were grained by a blender into a powder form, then the powder was soaked in pure methanol (3liter) three times for three weeks, then they were filtrated through a funnel containing cotton. The filtrate was dried in rotary apparatus to evaporate methanol, yielding a crude oily extract of *Raphanus sativus* seeds, and weighed (72.67 g). The extract kept in refrigerator at 4ºC until preparation of dose each week **(Tabassum and Khan, 2017).** The extract was dissolved in Tween 80 before administration.

Animals

Twenty male albino rats weighing 130-150g were purchased from the Egyptian Organization for Biological Products and Vaccines (VACSERA), Cairo, Egypt. They were kept at the Animal House of Faculty of Science, Mansoura University, Mansoura, Egypt, the rats were housed in stainless steel cages under controlled temperature (25 ± 2 °C), and 12h light-dark cycle. Animals were allowed to rodent chew diet and free access of water *ad libitum*. Procedures involving animals and their care were performed in an ethical clearance from Mansoura University Research Ethics Committee, and in conformity conducted with the Institution of laboratory animal resources guidelines, National Research Council (NRC) **(NRC, 1995).**

Experimental Protocol

After one week of acclimization, rats were divided into four groups (5 rats each), as follow:

I. **Control group:** Rats were fed on standard diet without any supplementation for 4 weeks.
II. **RSE group:** Rats were fed on standard diet and received *Raphanus sativus* oil extract orally at dose 0.52 ml/kg daily for 4 weeks **(Elshazly *et al.* 2016).**
III. **Adenine (AD) group:** Rats were fed on standard diet mixed with 0.75 % adenine (w/w) for 4 weeks **(El-Habibi, 2013 and Thakur *et al.* 2018).**
IV. **RSE and AD group:** Rats fed on AD-containing diet as group III as well as received the same dose of RSE as group II for 4 weeks.

At the start of the experiment, rats were weighed and weekly to obtain body weight gain and to adjust the dose of RSE.

Samples Collection and Tissue Preparation

At the end of experimental period, rats were fasted overnight; all rats weighed and anesthetized under diethyl ether, then sacrificed. Blood samples were collected in clean dry centrifuge tubes without anticoagulant, and then centrifugation was performed at 860 x g for 15 min to separate sera. The acquired sera were kept at - 20ºC for biochemical analysis. Rats were dissected; the two kidneys, testicles and epididymides were expelled from the rats and weighed. A known weight of each right testis was homogenized in 3ml cold normal saline solution (0.9%), then centrifuged at 860 x g for 15 min and the supernatants were kept at - 20ºC for biochemical analysis. Left testes and kidneys of all rats were preserved in 10 % neutral formalin for histopathological examination.

Methods

Determination of Kidney Function Parameters

Serum creatinine (SCr), BUN and UA were estimated by the methods of **Bartles *et al.* (1972),**

Fawcett and Soctt (1960), and **Barham and Trinder (1972)** respectively.

Determination of testosterone, FSH, and LH

Serum testosterone, FSH, and LH levels were estimated by using IMMULITE 2000 analyzer and kits purchased from Roche Diagnostic Company (Mannheim, Germany) based on the methods of **Wheeler (1995), Beastall *et al*. (1987),** respectively.

Determination of Semen Analysis

Weighed caudal epididymides were cut into pieces in 5 ml saline solution (0.9%) to obtain a sperm suspension to be used for semen analysis. Semen analysis was performed to estimate the epididymal sperm count, percentage of motility, viability percent, and abnormal morphology percent, by Computer Assisted Semen Analysis (Mira - 9000 CASA) in accordance with World Health Organization (WHO, 2010) recommendations.

Determination of Testicular Oxidative Stress and Antioxidants Biomarkers

Testicular malondialdehyde (MDA), NO **and** GSH concentrations, as well as the activities of SOD and CAT were determined as mentioned by **Ohkawa *et al*. (1979)**, **Montgomery and Dymock (1961), Beutler *et al. (*1963)**, **Nishikimi *et al.* (1972),** and **Aebi (1980),** respectively.

Histopathological Examination

The fixed testes and the kidneys were dehydrated in ascending series of ethyl alcohol, cleared in xylene and embedded in paraffin wax. The prepared blocks were cuts into 5 µm thick and stained with H& E **(Bancroft and Gamble, 2008).** Afterwards, the slides were examined under light microscope and images of illustrative fields of the histopathological changes were taken.

Statistical Analysis

Statistical analyses of the obtained results were performed by SPSS statistical software (IBM SPSS Statistics, version 16). One – way ANOVA (analysis of variance) test was used to analyze the difference between groups and followed by *Tukey* test for post comparison. The obtained data were reported as Mean ± SE and $p \leq 0.05$ are considered statistically significant **(Snedecor and Cochran, 1980).**

3. Results

Body Weight Gain:

Fig. 1 represents body weight gain that was declined significantly in AD-fed group when compared to control group. While, administration of RSE with AD-fed showed an increase in body weight gain compared to AD-fed rats.

Kidney, Testes, and Epididymis Weights:

As shown in Table 1, feeding of AD produced a significant increase in kidney weight associated with significant decrease in testicular and epididymal weights when compared to control group. On the other hand, RSE + AD-fed group showed a significant decrease in kidney weight, and increase in testes and epididymis weights compared to AD group.

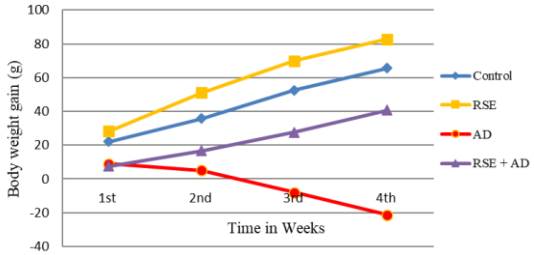

Fig. 1: Body weight gain in control and different treated rat groups

Table 1: kidney, testis, and epididymis weights in control group and different treated rat groups

Animal Groups	kidney weight (g)	testes weight (g)	epididymis weight (g)
I	0.84 ±0.03^a	1.47 ± 0.03^a	0.42 ± 0.02^a
II	0.82 ± 0.02^a	1.52 ± 0.02^a	0.42 ± 0.01^a
III	1.08 ± 0.01^b	0.48 ± 0.03^b	0.11 ± 0.01^b
IV	1.00 ± 0.02^c	0.78 ± 0.04^c	0.20 ± 0.01^c

Values are expressed as mean ± SE (n=5), different letters are significant at $P \leq 0.05$

Kidney Function Parameters:

Table 2 which showed a significant increase in SCr, BUN, and UA concentrations in AD- fed rats when compared to control group. However, RSE + AD group showed a significant decrease in the mentioned parameters if compared to AD fed rats.

Table 2: kidney function parameters (SCr, BUN and UA) in control group and different treated rat groups

Animal Groups	SCr (mg/dl)	BUN (mg/dl)	UA (mg/dl)
I	0.90 ±0.01^a	40.58 ± 0.73^a	2.00 ± 0.01^a
II	0.90 ± 0.01^a	41.40 ± 0.66^a	2.02 ± 0.01^a
III	1.79 ± 0.01^b	160.31 ± 0.73^b	3.02 ± 0.01^b
IV	1.28 ± 0.01^c	136.83 ± 0.55^c	2.88 ± 0.01^c

Values are expressed as mean ± SE (n=5), different letters are significant at $P \leq 0.05$

Histopathological Observation of Kidney:

In AD-fed rats, renal cortex showed shrunken renal corpuscle with loss of its capsular space and vascular component, atrophy of glomerulus, dilated renal tubules with empty areas inside it, obliteration of some tubule's lumen, and presence of massive inflammatory cells at parts of degenerated tubules relative to control group (Plate 1. C). On the other hand, co-administration of RSE + AD showed almost normal structure of renal corpuscle and Bowman's space, some tubules still with wide lumen and others have normal shape compared to AD-fed group (Plate 1. D).

Male Sex Hormones Levels:

The data in Table 3 demonstrated that feeding of AD was significantly diminished testosterone, while FSH and LH levels were increased when compared to control group. Furthermore, in RSE + AD group, testosterone level was significantly increased accompanied by significantly decrease in the levels of FSH and LH contrasted with AD group.

Table 3: testosterone FSH and LH levels in control group and different treated rat groups

Animal Groups	Testosterone (ng/mL)	FSH (mIU/mL)	LH (mIU/mL)
I	4.10 ±0.07^{a}	0.12 ± 0.004^{a}	0.13 ± 0.01^{a}
II	4.14 ± 0.09^{a}	0.13 ± 0.002^{a}	0.14 ± 0.01^{a}
III	1.45 ± 0.13^{b}	0.21 ± 0.01^{b}	0.22 ± 0.01^{b}
IV	2.19 ± 0.16^{c}	0.16 ± 0.005^{c}	0.17 ± 0.004^{c}

Values are expressed as mean ± SE (n=5), different letters are significant at $P \leq 0.05$

Semen Analysis:

The presented data in table 4, recorded that in rats fed AD there was a significant decline in semen analysis of epididymis sperms (count, motility, viability, and excessive abnormality) when compared to control group. In RSE + AD group, there was a significant increase in sperm count, sperm motility, sperm viability, and a significant decrease in sperm abnormality when compared to AD-treated group.

Table 4: Semen analysis in control group and different treated rat groups

Animal Groups	Sperm Count (10^{6}/g)	Sperm Motility %	Sperm Viability %	Sperm Abnormality %
I	2.22 ±0.12^{a}	58.28 ± 0.49^{a}	77.20 ± 0.58^{a}	10.22 ± 0.02^{a}
II	2.34 ± 0.12^{a}	59.95 ± 0.35^{a}	78.60 ± 0.40^{a}	9.99 ± 0.03^{a}
III	0.13 ± 0.01^{b}	18.68 ± 0.52^{b}	34.00 ± 0.71^{b}	49.70 ± 0.64^{b}
IV	0.53 ± 0.03^{c}	29.38 ± 0.55^{c}	43.20 ± 0.97^{c}	25.25 ± 0.53^{c}

Values are expressed as mean ± SE (n=5), different letters are significant at $P \leq 0.05$

Testicular Oxidative Stress and Antioxidants Biomarkers:

The introduced data in Table 5, AD administered group showed a significant increase in testicular oxidative stress biomarkers (MDA, NO), associated with a significant decrease in testicular antioxidant biomarkers (GSH levels, SOD and CAT activities) in comparison with control rats. However, co-administration of RSE and AD showed a significant decline in MDA, NO levels and a significant increase in GSH level, SOD and CAT activities when compared to AD group.

Table 5: Testicular oxidative stress and antioxidants biomarkers in control group and different treated rat groups

Animal Groups	MDA (nmol/g)	NO (µmol/g)	GSH (mmol/g)	SOD (U/g)	CAT (U/g)
I	239.53 ±0.71^{a}	84.00 ± 5.89^{a}	2.61 ± 0.02^{a}	329.82 ± 0.47^{a}	99.78 ± 0.42^{a}
II	241.50 ± 0.82^{a}	85.00 ± 4.25^{a}	2.66 ± 0.02^{a}	331.81 ± 0.57^{a}	100.23 ± 0.23^{a}
III	723.69 ± 0.54^{b}	303.00 ± 5.30^{b}	1.00 ± 0.01^{b}	127.31 ± 0.61^{b}	50.80 ± 0.73^{b}
IV	390.08 ± 0.69^{c}	155.00 ± 4.56^{c}	1.22 ± 0.01^{c}	200.83 ± 0.55^{c}	70.09 ± 0.56^{c}

Values are expressed as mean ± SE (n=5), different letters are significant at $P \leq 0.05$

Histopathological Observation of Testes:

Testis section of AD-fed group showed degenerated seminiferous tubules with disorganized spermatogenic cells, separations among the spermatogenic cells and decrease of spermatozoa and widening of the intertubular space compared to control group (Plate 2. C). However, RSE + AD treated group showed seminiferous tubules with intact histological structure, and spermatogenic cells are normally aligned and spermatozoa heavily present compared to AD-fed group (Plate 2. D).

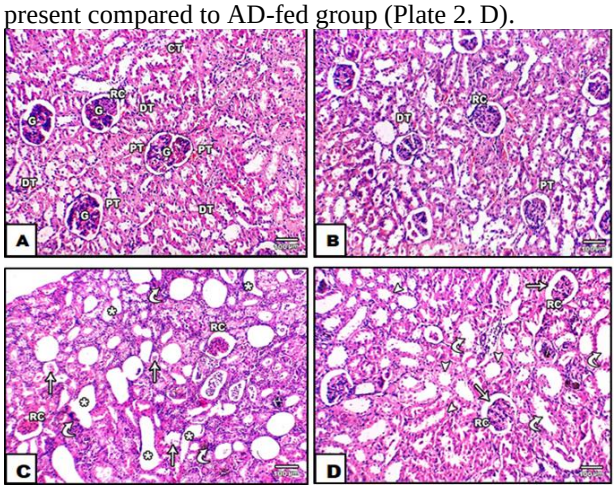

Plate1: (A): A photomicrograph of kidney section in the renal cortex of control group showing normal structure of the renal corpuscle (RC) consists of glomerulus (G) surrounded by Bowman's capsule, normal proximal convoluted tubules (PT), distal convoluted tubules (DT), and collecting tubules (CT). (H&E x 100). **(B):** A photomicrograph of kidney section through the renal cortex of RSE treated group showing the renal corpuscle (RC) and the tubules are preserving their histological structure. (H&E x 100). **(C)** A transverse section of the kidney tissue in AD-fed group: revealed shrunken renal corpuscle (RC), dilated renal tubules with empty areas inside it(asterisks), lumen of some proximal tubules (PTs) contained remnants of degenerated epithelial cells (arrows), interstitial mononuclear cells infiltration is found between tubules (curved arrows). (H&E x 100). **(D):** Renal cortex of RSE +

AD treated group showing almost normal structure of renal corpuscle (RC) and intact Bowman's space (arrows). Some tubules still degenerated with wide lumen (curved arrows) and others have normal shape (arrow heads). (H&E x 100).

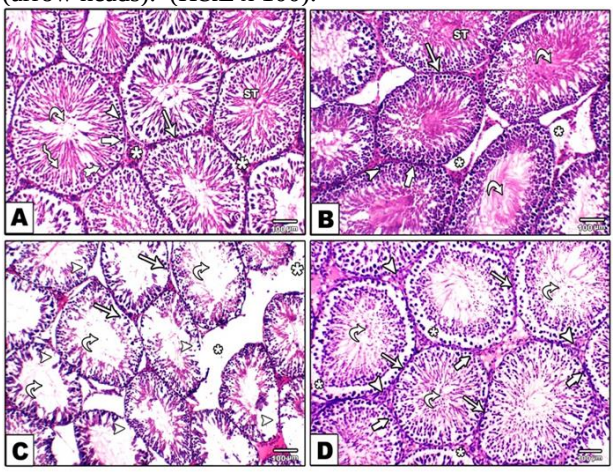

Plate2: (A): A photomicrograph of testis section of control group showing seminiferous tubules (ST) and the interstitial tissue (asterisks) in between the Leydig cells. Each tubule is surrounded by a basement membrane (arrow), lined with Sertoli cells (arrow head) and series of spermatogenic cells which include spermatogonia (thick arrow), primary spermatocytes (tailed arrow), spermatids (zigzag arrow) and many spermatozoa (curved arrow) filling the tubular lumen. (H&E x100). **(B):** A photomicrograph of testis section of RSE treated group showing seminiferous tubules (ST) with intact histological structure. Interstitial tissue (asterisks), basement membrane (arrow), Sertoli cells (arrow head), spermatogonia (thick arrow), spermatozoa (curved arrows). (H&E x 100). STs with disorganized spermatogenic cells, widening of the intertubular space (asterisks), irregular and deformed with few disorganized spermatogenic cells (arrows), separations (arrow heads) are also seen among the spermatogenic cells. STs showed decline of spermatozoa (curved arrows). (H&E x 100). **(D):** A photomicrograph of testis section of RSE + AD treated group showing ST with intact histological structure. The interstitial tissue and Leydig cells have preserved morphology (arrows heads) and almost proper structure of basement membrane surrounding the tubules (arrow). A spermatogenic cells are normally aligned (thick arrows) and spermatozoa still heavily present (curved arrows). (H&E x 100).

4. Discussion

The main target for development of novel drugs is In this study the obtained results showed that feeding of rats with AD at 0.75% (w/w) for 4 weeks lead to the development of CRF which characterized by worsened kidney functions as demonstrated by significant elevations of renal function markers (SCr, BUN, and UA) concentrations. Also, AD-induced CRF caused marked abnormalities in testicular weight, male sex hormones levels, semen parameters, and testicular antioxidant markers that affected male fertility.

It seem that, AD caused a decline in body weight gain and exceed kidney weight, these results are in agreement with **Ogirima *et al.* (2006)** who reported that, AD-induced CRF in male rats had significantly lower weight gain than normal group which started from the second week, and **El-Habibi (2013)** and **Dalal (2019)** who reported that, AD-induced CRF in male rats decreased body weight and increased the kidney weight. These results may partially due to reduced food intake accompanied by excess albuminuria leading to malnutrition in AD-fed group **(Tong *et al.*, 2010).** The kidneys enlargement may be due to the marked elevation in the levels of monocytes, inflammatory infiltrates and crystalline tubulointerstitial deposits in renal tissues **(Brulé *et al.*, 1988)**. This suggestion was confirmed by the histopathological findings.

AD administration was resulted in exceed SCr, BUN, and UA. These results are in consistent with **Nakano *et al.*, (2016).** These obtained increases in kidney functions parameters means the kidneys damage **(Wang *et al.*, 2011)**. The outcomes which affirmed by the histopathological observation of renal section in the current study which run parallel with **Yang *et al.* (2018)** who concluded that, AD-induced CRF in male rats showed changes in the renal tubules; swelling and cystic renal tubule dilatation, with interstitial chronic inflammation.

Administration of AD to rats is oxidized to 2, 8-dihydroxyadenine (DHA), which precipitates as tubular crystals leading to CRF **(Ali *et al.*, 2014).** Both AD and its oxidized form 2, 8-DHA form crystals due to low solubility and precipitation in the renal tubules. The consumption of AD thus causes the occlusion of renal tubules that prevents nitrogenous substances from excretion leading to a biochemical and physiological status resembling CKD in humans **(Yu *et al.*, 2017).**

Meanwhile, co-administration of RSE with AD caused an increase in body weight gain and diminished the raised kidney weight compared to AD group, which agree with **Shehzadi *et al.* (2020),** who showed that, co-administration of *Raphanus sativus* seed extract recovered the loss in body weight and decreased the kidney weight of nephrotoxicity rats induced by Carbon tetrachloride (CCl_4). The ameliorated effect of RSE on body weight may be attributed to the renal function improvement which appeared in the outcomes results while kidney weight improvement may be due to seeds content of antioxidant compounds.

The enhancement of histopathological changes in renal tissue in this study was attributed to the protective effect of *Raphanus sativus*, supported by the findings of **Bojana *et al.* (2016)** who reported that, *Raphanus sativus* root extract showed nephroprotective activity on galactosamine (GaIN)-induced kidney damage due to its extract content of

antioxidants as phenolic. Likewise, rats given RSE + AD showed significant decline in SCr, BUN, and UA concentrations, indicating improvement in renal function. The lower uric acid concentration in RSE group is agreement with **Zaman, (2004)** and may be attribute to its sulforaphene content. The improvement in renal functions may be due to the phytoconstituents detected in *Raphanus sativus* extracts which may be responsible for its nephroprotective activity **(Bojana *et al.*, 2016).**

The present study demonstrated that, AD feeding decreased the weights of testicles and epididymides, these results agree with **Yu *et al.* (2014),** who showed that, CRF caused by AD in rats decreased both testicular and epididymal weights. The decrease of testicular weights in rats with CRF accompanied by lower testicular function due to decline of both spermatogenesis and steroidogenesis, since the testicular weight correlates with spermatogenesis and testosterone biosynthesis **(Takihara *et al.*, 1987).**

The co-administration of RSE with AD caused significant increase in testicular and epididymal weight compared to AD group, these results agree with **Dafaalla *et al.* (2017)** who indicated that, *Raphanus sativus* seeds extract was significantly - dose dependent- increase rats testes weight. As testosterone stimulates secretion and growth of the reproductive organs **(Singh *et al.*, 1995)**, the increased count and function of both testis somatic and germinal cells in this group could be due to excess testosterone synthesis by increased the testis and epididymis weights **(Parandin *et al.*, 2012).**

Sperm count, sperm viability, sperm motility were showed significant decline in the rats fed on AD, while sperm abnormality was increased significantly, these results run parallel with **Yu *et al.* (2014)** who observed decrease of sperm count and motility, increased sperm malformation in AD-treated rats. The resulted increased sperm abnormalities and sperm morphological alterations may be due to decline in serum erythropoietin (EPO) levels and down regulated EPO protein expression in kidney and testes in AD treated rats as reported by **Li *et al.*, (2020).** The decrease of caudal epididymal sperm count in CRF rats may be also attributed to deficiency of both Leydig and Sertoli cells secretion as testosterone stimulates spermatogenesis and activates maturation of spermatid **Yamamoto *et al.* (1996)**. Low testosterone levels along with high FSH levels leads to impaired spermatogenesis **(Anantharaman and Schmidt, 2007)**. While administration of RSE with AD caused a significant improvement in sperm count, motility, viability and decline sperm abnormalities compared to AD group, these results agreed with **Dafaalla *et al.* (2017),** and may be due to enhance of spermatogenesis and increased of testosterone level due to antioxidant components of radish seeds extract which contain alkaloid like coumarins, saponins, flavonoids and anthocyanins **(El-Sayed, 2001).**

The current study demonstrated that, AD feeding significantly decline testosterone concentration and a significantly increase the concentration of FSH and LH, a result which are in consistent with **Chen *et al.* (2014)**. The hypothalamic-pituitary-testicular axis has strongly affected by CRF that lead to hormonal disturbances and testicular function deterioration **(Eckersten *et al.*, 2015).** Uremia affect the release of gonadotropin releasing hormone through its effect on amino acids neurotransmitters out flow, hence affecting GnRH formation or secretion as showed by **Schaefer *et al.*, (2001).**

The administration of RSE with AD was significantly increase testosterone concentration and significantly decreased of serum FSH and LH concentration. These results are consistent with previous study confirming that *Raphanus sativus* seed extract co- administration with CCl_4 to male rats ameliorated the hypogonadism by increasing serum testosterone level **(Tabassum and Khan, 2017),** through its curative effects by its antioxidant potential, scavenging free radical ability and may have important role in overcoming central-dysfunction by direct stimulating central nervous system and hypothalamus pituitary-gonadal axis due to its phytochemicals. So, increased level of testosterone stimulates pituitary gland to decrease release LH and FSH.

The present study showed that AD administration significantly decrease antioxidant enzymes (SOD and CAT) activities as well as GSH level in the testes homogenate with a significant increase in oxidative stress biomarkers (MDA and NO) levels compared to control group which reveal oxidative stress (OS) occurrence in testes. This attributed to increased production of ROS in CRF as uremic toxins increase ROS generation **(Descamps-Latscha *et al.*, 2001).** Obtained increase in MDA concentration in AD treated rats testes may be due to elevation of TGFβ1 which is a fibrosis cytokine marker regulate Sertoli, Leydig and germ cell growth and differentiation, where the interplay between oxidative stress and transforming growth factor-beta1 (TGFβ1) appears **(Sánchez-Capelo, 2005)**. Also, TGFβ1 can stimulate the production of ROS as mentioned by **Liu and Gaston Pravia (2010).**

Both sperm and testicular cells are able to oxidative damage by free radicals as the polyunsaturated fatty acids contents in their cellular membrane are very abundant **(Alvarez and Storey, 1995)**. Free radicals attacks may make blood vessel impediment and serious damage to the reproductive system cells, and consequently defects in spermatogenesis **(Halliwell, 2006).**

The co-administration of RSE with AD increased the testicular GSH level, SOD and CAT activities, and decline in MDA and NO concentration relative to AD- treated group. These results are in

harmony with **Tabassum and Khan, (2017)** who concluded that, *Raphanus sativus* seeds extract enhanced the oxidative stress of CCl_4 by increment of SOD, CAT, and GSH and decrease lipid peroxides. The ability of *Raphanus sativus* seeds extract to directly scavenge oxygen free radicals such as singlet oxygen, hydroxyl radical, hypochlorous acid, hydrogen peroxide and peroxyl radicals may lead to these amelioration **(Umamaheswari *et al.*, 2012)** because radish seeds contain alkaloid like coumarins, saponins, flavonoids and anthocyanins **(El-Sayed, 2001).** The dietary antioxidant anthocyanins prevent living cells from disease and protect from oxidative damage **(Matsufuji *et al.*, 2003).** Flavonoids are the main polyphenol compounds that inhibit lipid peroxidation or elevate level of GSH as antioxidants **(Achyut and Sirisha, 2015)** and they are good scavenger of ROS and hydroxyl radicals **(Kand'ár *et al.*, 2006)**.

The outcomes of testicular histopathological changes in this study were affirmed by **Diab *et al.* (2019)** and **Dalal (2019)** who revealed that, rats treated with AD showed degenerative changes in seminiferous tubules and reduction of spermatogenic cells, which clarified also by **Yu *et al.* (2014)**, who reported that AD treatment successfully induced the generation of spermatogenesis obstruction model, AD may reduce spermatogenesis and testosterone synthesis through a generation of free radicals by the xanthine oxidase reaction. The ameliorative effect of RSE on testicular histopathological changes in the present study was supported by the findings from **Tabassum and Khan, (2017)** who reported that co-administrated of *Raphanus sativus* seed extract ameliorated the toxicity in testes induced with CCl_4-induced hypogonadism, this beneficial effect may be attributed to the biological membranes adjustment through its antioxidant potential and ability to scavenge free radical.

CONCLUSION

The current study demonstrated the protective efficacy of radish seed oily extract against infertility induced by chronic renal failure in male rats, mostly through modulating the oxidative stress and enhancing hormonal levels and spermatogenesis.

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