



## Oxidative Stress: Role of *Eruca sativa* Extract on Male Reproduction in Rats

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**Abstract** | The possible protective effects of ethanolic extract of *Eruca sativa* leaves on reproduction was evaluated in male adult rats exposed to hydrogen peroxide. Forty adult male rats were randomly divided into four equal groups and treated daily for 60 days as follows: a control group (C) received tap water, group T<sub>1</sub> received tap water containing 0.5% H<sub>2</sub>O<sub>2</sub>, group T<sub>2</sub> received tap water containing 0.5% H<sub>2</sub>O<sub>2</sub> and administered 300 mg/kg. B.W. ethanolic extract of *Eruca sativa* leaves orally and group T<sub>3</sub> received the same dose of ethanolic extract only as in T<sub>2</sub>. At 0 (pretreated), 30 and 60 days of the experiment fasting blood samples was collected by cardiac puncture technique under anesthesia and serum was isolated to analyze hormonal profile i.e serum testosterone, follicular stimulating hormone (FSH) and leutinizing hormone (LH) and peroxynitrite concentrations, in addition to catalase activity, furthermore, semen parameters was studied. The results showed a significant decrease in catalase activity, with significant increase in peroxynitrite concentration in group T<sub>1</sub> compared to control and T<sub>3</sub> groups. In addition, a significant decrease in serum testosterone, FSH and LH concentrations was observed in group T<sub>1</sub> compared with other groups. Whereas, hormonal assay revealed a significant elevation in serum testosterone, LH and FSH concentrations at two experimental periods in group T<sub>3</sub> compared with control, T<sub>1</sub> and T<sub>2</sub> groups. Hydrogen peroxide resulted in a significant decrease in sperm concentration with increase in sperm abnormal morphology in group T<sub>1</sub> as compared to other experimental groups. These results supported that oxidative stress induced by hydrogen peroxide may have triggered some degree of testicular toxicity. The findings of the present study indicated that ethanolic extract of *Eruca sativa* leaves improved the testicular functions and spermatogenesis in stressed rats. Thus lends credence to it is folklore use of the plant in the management of testicular dysfunction.

**Keywords** | Hydrogen peroxide, *Eruca sativa*, Hormonal profile, Semen parameters, Rats

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## INTRODUCTION

Oxidative stress (OS) is a condition that reflects an imbalance between the systemic manifestation of reactive oxygen species (ROS) and the ability of biological system to detoxify or to repaired the resulting damage (Saalu, 2010; Birben et al., 2012). ROS has been linked to the pathogenesis of numerous disease (Alfadda and Sallam, 2012). Previous studies indicated that spermatozoa and seminal plasma contain a battery of ROS scavenger, including enzymes such as SOD, catalase (CAT) and the glu-

tathione peroxidase / redctase system, and also a variety of substances such as a tocopherol, ascorbic acid, glutathione, pyrovate, taurine hypotaurine and albumin (Alvarez and Storey, 1989; de Lamirande and Gagnon, 1992). The main source of endogenous ROS in semen are leukocytes (Aitken and Baker, 1995; Gharagozloo and Aitken, 2011) and abnormal spermatozoa (Sakkas et al., 2003). Saleh and Agarwal (2002) explained that low concentrations of ROS play an important role in normal physiological processes such as capacitation, hyperactivation and acrosome reaction (Ford, 2004). However, under pathological condition,

excessive generation of ROS from human defective spermatozoa appears to be related to male infertility (Henkel, 2011; Trussell, 2013; Agarwal et al., 2014), in addition, to cause sperm pathologies (ATP depletion) in the form of inadequate axonemal phosphorylation or lipidperoxidation (LPO) resulting in a loss of sperm motility and viability (Koppers et al., 2008; Bansal and Bilaspuri, 2011; Chen et al., 2013) and DNA oxidation (Fraga et al., 1991), which render the sperm cell unable to fertilize the oocyte. In addition, several life style factors such as excessive smoking, alcohol consumption, and environmental factors such as radiation and toxins can contribute to exogenous ROS (Esteves, 2002).

Medicinal plants play an important role in complementary and alternative medicines duo to better acceptability and fewer side effects. In folkloric management *Eruca sativa* (Rocket) is considered a medicinal plant with many reported properties, including its strong aphrodisiac effects (Font et al., 2003). Several studies found that the presence of flavonoids, saponine and alkaloids in rocket extract (Pasini et al., 2012) caused a significant increase in sperm activity (Barillari et al., 2005), as well as increase spermatogenesis (Homady et al., 2000). The plant possesses hypoglycemic properties (El-Missiry and El-Gindy 2000; Hetta et al., 2014), anticancer activity (Melchini et al., 2009), diuretic (Shalaby and Hammouda, 2014) and anti-inflammatory effects (Yehuda et al., 2009). Hussien et al. (2010) reported that *E. sativa* showed improvement the liver functions with a potent antioxidant activities (Kim and Ishii, 2006; Alam et al., 2007). To our knowledge no information is available about the effect of *Eruca sativa* on reproduction oxidative stressed male rats. So the current work was aimed to investigate the effect of an herbal plant *Eruca sativa* leaves to improve some aspects of male reproduction and hormonal profile in oxidative stressed rats induced by hydrogen peroxide.

## MATERIALS AND METHODS

### *Eruca sativa* PREPARATION

Ethanollic extract of *Eruca sativa* leaves was prepared according to the method described by Jin et al. (2009), the yield equal of 10 g of extract was stored in a dark sterile screw bottle at (4°C) until used.

### ANIMALS, DIETS AND EXPERIMENTAL DESIGN

Forty male Albino adult Wistar rats, weighing 200-275 gram body weight were used. The animals were maintained at animal house of the College of Veterinary Medicine / Baghdad University from November 2013 to January 2014 in polypropylene under conditions of controller temperature and well ventilation room. Rats received normal pellet diet (according to the laboratory rodent diet 5001) and tap

water *ad libitum* through the experimental period.

Rats were randomly divided into four main equal groups (10/each) and given orally the following treatment for 60 days: control group, received tap water, group (T<sub>1</sub>) were received tap water contain 0.5% H<sub>2</sub>O<sub>2</sub>, group (T<sub>2</sub>) received tap water contain 0.5% H<sub>2</sub>O<sub>2</sub> and oral administration of 300 mg/kg. B.W ethanollic extract of *Eruca sativa* leaves via epigastric tube, while group (T<sub>3</sub>) administered *Eruca sativa* extract only at the same dose of T<sub>2</sub>. Fasting blood samples were collected at 0, 30, 60 days of the experiment by cardiac puncture technique (cardiocentesis) by using vacuum tube with gel colt activator. Serum was collected and kept at -18°C until analysis for measurement of serum testosterone (T), follicular stimulating hormone (FSH), luteal hormones (LH) using Kits-Monobind Inc, USA and peroxynitrite radical concentrations (Vanuffelen et al., 1998), as well as, catalase activity was determined according to Goth (1991). At the end of experiment left testis was excised and covered with physiological normal saline, cleaned from attached fat and connective tissue, tail of the epididymis was taken for preparation of epididymal sperm suspension as described by Sakamoto and Hashimoto (1986) to perform routine sperm evaluation.

### STATISTICAL ANALYSIS

The data were arranged and represented as mean ± standard error of mean (S.E.). The significances between the mean values were calculated using One and Two-Way Analysis of Variance (ANOVA). P value less than 0.05 were considered to be significantly by using LSD test (Snedecor and Cochran 1973).

**Table 1:** Effect of *Eruca sativa* and hydrogen peroxide on serum peroxynitrite radical (µmol/L) in rats

Group Day	Control group (C)	Group T1 (0.05% H <sub>2</sub> O <sub>2</sub> )	Group T2 (H <sub>2</sub> O <sub>2</sub> + ES)	Group T3 (ES)
Zero	290.94 ± 32.18	286.2 ± 30.97a	289.51± 22.59	298.6 ± 28.09
30	293.41 ± 26.31AB	243.39± 14.80Bab	262.00 ± 28.20AB	315.92 ± 24.36 A
60	291.41 ± 16.25 AB	200.33 ± 9.76 Cc	255.30 ± 13.36 BC	323.69 ± 14.55 A

Values are expressed as mean ± SE; n: 10/group; Capital letters denote differences between groups: P<0.05 vs control; Small letters denote significant differences within time P<0.05 zero time

## RESULTS

The results of peroxynitrite concentration are reported in Table 1. Daily exposure of rats to hydrogen peroxide (T<sub>1</sub>) caused significant (P<0.05) increase in serum peroxynitrite concentration at 30 and 60 days of experiment as compared to other groups (T<sub>2</sub> and T<sub>3</sub>). A significant (P<0.05)

decrease in the serum peroxynitrite concentration was observed at two experimental periods in T<sub>2</sub> and T<sub>3</sub> groups as compared to T<sub>1</sub> group and alcoholic extract normalize the value of peroxynitrite in group T<sub>3</sub> through the experimental période.

Results in Table 2 indicated that treatment of normal rats with H<sub>2</sub>O<sub>2</sub> in drinking water (T<sub>1</sub>) showed a significant (P<0.05) decrease in serum catalase activity after 60 days of the experiment compared with control and other treated groups.

**Table 2:** Effect of *Eruca sativa* and hydrogen peroxide on serum catalase (KU/I) in rats

Groups Days	Control group	Group T <sub>1</sub> (0.05% H <sub>2</sub> O <sub>2</sub> )	Group T <sub>2</sub> (H <sub>2</sub> O <sub>2</sub> + ES)	Group T <sub>3</sub> (ES)
Zero	1.33± 0.12a	1.26± 0.15 a	1.31± 0.10 a	1.30± 0.12 a
30	1.15± 0.06Ba	0.97± 0.04Bab	1.14± 0.08Ba	2.18± 0.11A b
60	1.22± 0.10Ba	0.84± 0.03Cb	1.08± 0.04CBa	3.95± 0.25A b

Note: For details see Table 1

**Table 3:** Effect of *Eruca sativa* and hydrogen peroxide on serum testosterone (ng/ml) in rats

Groups Days	Control group	Group T <sub>1</sub> (0.05 H <sub>2</sub> O <sub>2</sub> )	Group T <sub>2</sub> (H <sub>2</sub> O <sub>2</sub> + ES)	Group T <sub>3</sub> (ES)
zero	37.98± 1.62	38.31± 1.95c	38.47± 2.01b	38.35± 1.56
30	38.22± 1.12BC	47.10± 1.99Ab	41.26± 2.00B ab	37.21± 1.65BC
60	38.97± 1.68C	52.89± 2.08A a	43.71± 1.43B a	36.70± 1.31C

Note: For details see Table 1

Regarding the effect of hydrogen peroxide on serum testosterone concentration in rats (group T<sub>1</sub>), H<sub>2</sub>O<sub>2</sub> caused a marked decrease (P<0.05) in testosterone concentration at the end of the experiment compared to control and T<sub>3</sub> groups (Table 3). Lack of significant differences (P>0.05) in testosterone concentration among control and T<sub>2</sub> groups was detected after 60 days of experiment when compared between each other. The results showed that the serum testosterone concentration in *Eruca sativa* extract treated group (T<sub>3</sub>) were significantly (P<0.05) higher than that of control and other treated groups throughout the two treated periods (30 and 60 day).

Data in Table 4 revealed a significant (P<0.05) increase in serum FSH concentration in group T<sub>1</sub> after 30 days of treatment with H<sub>2</sub>O<sub>2</sub> in drinking water compared with the control and T<sub>2</sub> treated groups. However, rats treated with 0.5% H<sub>2</sub>O<sub>2</sub> (T<sub>1</sub>) showed a significant (P<0.05) decrease

in serum FSH concentration at the end of experiment compared to control and T<sub>2</sub> groups. After 60 days of treatment the result also showed a significant increase (P<0.05) in FSH concentration in T<sub>2</sub> and T<sub>3</sub> groups compared to group T<sub>1</sub>.

**Table 4:** Effect of *Eruca sativa* and hydrogen peroxide on serum FSH (mIU /ml) in rats

Group Days	Control group	Group T <sub>1</sub> (0.05% H <sub>2</sub> O <sub>2</sub> )	Group T <sub>2</sub> (H <sub>2</sub> O <sub>2</sub> + ES)	Group T <sub>3</sub> (ES)
Zero	5.11± 0.27 a	5.05± 0.13 a	5.31± 0.33 a	4.91± 0.26 a
30	4.88± 0.37B a	5.82± 0.29A a	4.74± 0.33B a	5.44± 0.35AB a
60	5.09± 0.26A a	3.05± 0.24B b	5.10± 0.29A a	5.70± 0.40A a

Note: For details see Table 1

**Table 5:** Effect of *Eruca sativa* and Hydrogen peroxide on serum LH (mIU /ml) in rats

Groups Days	Control group	Group T <sub>1</sub> (0.05% H <sub>2</sub> O <sub>2</sub> )	Group T <sub>2</sub> (H <sub>2</sub> O <sub>2</sub> + ES)	Group T <sub>3</sub> (ES)
Zero	2.37± 0.12	2.41± 0.14bc	2.29± 0.16	2.31± 0.14b
30	2.59± 0.25B	3.45± 0.16A a	2.61± 0.18B	3.42± 0.25A a
60	2.55± 0.25B	1.97± 0.18C c	2.25± 0.17B	3.31± 0.16A a

Note: For details see Table 1

Serum concentration of LH pointed a marked significant (P<0.05) decrease in rats subjected to H<sub>2</sub>O<sub>2</sub> (group T<sub>1</sub>) after 60 days of the experiment as compared to other experimental groups (Table 5). Lack of significant (P>0.05) differences in LH concentration was observed at two treated period in group T<sub>2</sub> as compared to the control group. Meanwhile, group T<sub>3</sub> ( treated with *Eruca sativa* extract) induced- a significant increase (P<0.05) in serum LH concentration as compared with the control and T<sub>2</sub> treated groups after 30 and 60 days of experiment.

Table 6 reported that treatment of rats with hydrogen peroxide at concentration 0.05% in drinking water –induced a significant decrease (P<0.05) in sperm concentration in comparison with the other experimental treated groups (C, T<sub>2</sub> and T<sub>3</sub>). Furthermore, there was a significant (P<0.05) increase in this parameter in group T<sub>2</sub> as compared to control. Whereas, a significant (P<0.05) increase was recorded in sperm concentration of rats treated with extract alone (T<sub>3</sub>) as compared with T<sub>1</sub> and T<sub>2</sub> groups. In contrast, the results showed a significant (P<0.05) increase in abnormal morphology of sperm after 60 day of experiment in group T<sub>1</sub> as compared to control, T<sub>2</sub> and T<sub>3</sub> groups. Daily oral administration of *E. sativa* leaves extract, alone and in com-

bination along with hydrogen peroxide to rats, caused a significant ( $P < 0.05$ ) decrease of sperm abnormal morphology at the end of the experiment comparing to groups  $T_3$ . However, rats in group  $T_3$  showed a significant ( $P < 0.05$ ) decrease of sperm abnormal morphology at the end of the experiment comparing to control,  $T_1$  and  $T_2$  groups.

**Table 6:** Effect of *Eruca sativa* and Hydrogen peroxide on sperm parameters in rats

Groups Parameters	Control group	Group $T_1$ (0.05% $H_2O_2$ )	Group $T_2$ ( $H_2O_2$ + ES)	Group $T_3$ (ES)
Sperm concentration ( $\times 10^7/ml$ )	29.62 $\pm$ 1.17 AB	22.25 $\pm$ 1.84 C	28.12 $\pm$ 1.23 B	33.50 $\pm$ 1.38A
Sperm abnormal morphology(%)	23.75 $\pm$ 1.80 B	30.12 $\pm$ 1.31 A	24.25 $\pm$ 1.82 B	13.62 $\pm$ 1.19 C
Sperm viability (%)	79.12 $\pm$ 1.38 B	52.37 $\pm$ 3.41 C	76.50 $\pm$ 1.26 B	89.75 $\pm$ 1.22 A

**Note:** For details see Table 1

In Table 6, statistical analysis showed a significant ( $P < 0.05$ ) decrease in the percentage of sperms viability ( $P < 0.05$ ) rats received hydrogen peroxide in drinking water (group  $T_1$ ) for 60 consecutive days as compared with the others groups. Also, the results showed a significant increase ( $P < 0.05$ ) in sperm viability in groups  $T_2$  and  $T_3$  as compared with the control. A significant changes was observed in this parameter between  $T_3$  and control group (Table 6).

## DISCUSSION

A significant elevation in serum peroxynitrite and significant decrease in catalase activities in group  $T_1$  were observed. A marked increase in ROS and RNS generation is implicated in the pathogenesis of many diseased conditions (Halliwell, 2006) with impairment of nitric oxide (NO) production (Tiefenbacher, 2001). These results are similar to other studies that showed an increase in lipid peroxidation and peroxynitrite as a results of an alteration in the antioxidants status due to exposure to xenobiotic like  $H_2O_2$  (Khudiar, 2000; Al-Shafeay, 2013). The generation of peroxynitrite over a long periods will react at a relatively slow rate with biological molecules and is able to transverse cell membrane and ion channels leading to disruption of cell signaling pathway and cell damage (Macfadyen, 1999; Pacher et al., 2007). Moreover, Tiefenbacher (2001) reported that oxidative stress is a common mechanism underlying impairment of nitric oxide (NO) production, where NO can react with  $O_2^-$  or  $H_2O_2$  to form peroxynitrite (Barber and Shaw, 2010). Besides, NO inhibits catalase activity and increases intracellular ROS levels in macrophages (Borutaite and Brown, 2003) concomitant with elevation in peroxynitrite radical concentration. This possible mechanism is supported by Mora-Esteve and

Shin (2013) whom showed that catalase aids in the decomposition of  $H_2O_2$  into water and oxygen, accordingly, the loss of CAT activity impairs cellular protection against the endogenously generated  $H_2O_2$  (Cimen, 2008). However, Hussein (2008) found that catalase protect the tissues from highly reactive hydroxyl radical which is generated from  $H_2O_2$ . This explain its depletion after  $H_2O_2$  exposure. At the same time, treatment of rats with extract of *eruca sativa* exhibited a significant decrease in serum peroxynitrite and significant increase in catalase activities in groups  $T_2$  and  $T_3$  as compared to  $T_1$ . These results are in agreement with other researches using different medicinal plants like ginger (Ahmed et al., 2000), black current (Al-Zubaidi, 2007; Al-Shammary and Al-Okaily, 2009) and pomegranate seed oil (Abudalah, 2013). Thus, this might be reflect the antioxidant effects of *Eruca sativa* (El-Missyry and El-Gindy, 2000; Ganie et al., 2011) due to the presence of phenolic compounds which exerted their modulatory actions (Saleh et al., 1995) and improves the status of antioxidant enzymes (Martinez-Sanchez et al., 2007; Etebong and Nwafor, 2009).

Generally, the results indicated a consistent decrease in serum hormonal profile (testosterone, FSH and LH) in rats received hydrogen peroxide (group  $T_1$ ). It has been well known that testicular tissue characterized by synthesis, secretion of androgens (Ganong, 2011) with normally generation of small amounts of ROS in the steroidogenic pathway and spermatozoa which are necessary for fertilizing capabilities (Aitken, 1997). Several studies demonstrated that lipid peroxidation can cause an increase in the activity of all the lysosomal enzymes in the testis which can be lead to decrease the antioxidant enzymes and finally inhibit the steroidogenesis (Sikka, 2001; Ismail and Al-Nahri, 2009; Tijani et al., 2010). Castilla-Cortazar et al. (2000) found that hydrogen peroxide caused advanced cirrhosis which may cause hypogonadism and low serum testosterone. Therefore, this may give an indication that any agent caused dysfunctions of Sertoli cells may have profound effects on spermatogenesis.

A highest elevation in testosterone and LH hormones at the end of experiment in group  $T_2$  comparing to  $T_1$  may be related to the effect of polyphenolic flavonoid compounds in leaves extract on pituitary-testicular axis and led to alleviate the negative effects of  $H_2O_2$  (Ismail and Al-Nahri, 2009; Khan et al., 2012; Arafa et al., 2014). Few observations reported that the presence of sterols, flavonoids, quercetin and saponins in *E. sativa* which scavenging or remove FRs and secondary acts to an improvement the fertility and testicular functions (Agarwal and Allamaneni, 2011; Awoniyi et al., 2012; Ansari et al., 2014) and, thereby, augmentation of sexual desire (Ates and Erdogru, 2003).

Recently Al-Tohamy et al. (2010) showed that the presence of glucosinolates (the major glucosinolate is Erucin) and other stimulant materials in *Eruca sativa* seeds have

several biological activities and potentially capable for protecting the cells against oxidative stress led to ameliorate semen characters and fertility in male rabbits (Talalay and Fahey, 2001; Martinez-Sanchez et al., 2007), accordingly, the positive actions of *E. sativa* on the hormonal profile support the folkloric beneficial effect of the plant in the management of reproductive dysfunction.

A significant decrease in sperm concentration in group T<sub>1</sub> may be due to oxidative insult, that affect sperm quality and male fertility and could be caused spermatogenic arrest and changes in steroid biosynthesis lead to azoospermia (du Plessis et al., 2010; Helfenstein et al., 2010). Worthwhile, some evidence suggested that the loss of sperm motility may be attributed to the fact that H<sub>2</sub>O<sub>2</sub> inhibit sperm ATP production by different ways (Armstrong et al., 1999). While rats received hydrogen peroxide and extract (group T<sub>2</sub>) point out a significant increase in sperm concentration, this may be attributed to the antioxidant effects of *E. sativa* which is responsible for its protective effect against hydrogen peroxide toxicity, so spermatozoa are protected by various antioxidant agents belongs to the phytochemical constituent of extract and then normalized the sperm concentration (Ahlbom et al., 2001; Gharagozloo and Aitken, 2011). These finding cooperate with the researches of Salem and Moustafa (2001) and Hussein (2013) that concluded capability of *E. sativa* improve healthy sperm characteristics and fertility.

Increase of abnormal sperm morphology and decrease in viability in group T<sub>1</sub> may serve as a useful indicator of potential damage to sperm by intubation of H<sub>2</sub>O<sub>2</sub> (Said et al., 2005), or may be due to an impaired of Leydig's cell functions that can lead to enhances alteration of testosterone synthesis (Ben Abdallah et al., 2012). In the same manner, result in group T<sub>2</sub> explained the effect of *E. sativa* extract on scavenging system, which is essential to counteract the effects of ROS generated by hydrogen peroxide (Agarwal and Prabakaran, 2005). An improvement in male fertility and hormonal profile in T<sub>3</sub> treated group has been documented (Pakarainen et al., 2005; Abediet al., 2012).

## CONCLUSION

From the results of the present study, it could be concluded that this study provide an evidence for a prophylactic and stimulatory effects of *E. sativa* on pituitary-gonadal axis. Thus the potential of *E. sativa* leaves extract improved male reproduction in oxidative stressed rats is confirmed.

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## CONFLICT OF INTEREST

There is no conflict of interest.

## AUTHORS' CONTRIBUTION

Baraa Najim designed the experiment . Ahmed Jasim analyzed and interpreted the data and performed the experiment. Baraa Najim gave technical support , wrote the paper and conceptual advice.

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