

## Protective Effects of *Tribulus terrestris* and Vitamin C on Permethrin Induced Oxidative Stress in Goat Testis

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### Original Research Article

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**Abstract:** Present study was designed to evaluate the ameliorative effect of *Tribulus terrestris* (TT) and vitamin C against permethrin induced oxidative stress in goat testicular tissue in vitro. Testicular tissue was divided into control and three experimental groups EI, EII and EIII. All the groups were cultured in TCM-199 nutritive media at 95% humidity, 5% and 39°C in CO<sub>2</sub> incubator for 4hr. and 8hr. duration. In group EI, the testicular tissue was exposed to 100µg/ml and 200µg/ml of permethrin. EII group was exposed to 100µg/ml and 200µg/ml of permethrin along with 0.1mmol of vitamin C. While EIII group was tested for 100µg/ml and 200µg/ml of permethrin along with 100µg/ml hydro-alcoholic extract of TT. Present study revealed that the activity of antioxidant markers such as catalase (CAT), glutathione peroxidase (GPx) and reduced glutathione (GSH) were significantly ( $p<0.05$ ) decreased in permethrin treated groups as compared to control in both dose and time dependent manner. Maximum decline (62.69%, 39.4%, 51.5% decrease in level of CAT, GPx, GSH respectively) in the antioxidant status of the tissue was reported at 200µg/ml for the duration of 8hr. However, 100µg/ml of permethrin resulted in 29.3%, 14.3%, 29.5% decrease in CAT, GPx, and GSH level respectively for the same exposure duration i.e. 8hr. The activity of antioxidants was found to be improved in vitamin C (EII) and TT (EIII) supplemented groups ( $p<0.05$ ). From the present study, it is concluded that permethrin induce a decline in the status of antioxidant enzymes. Vitamin C and TT are potent antioxidants that mitigate the toxic effects of permethrin by elevating the level of antioxidants.

**Keywords:** Permethrin, oxidative stress, antioxidant enzymes, *Tribulus terrestris*, vitamin C.

### INTRODUCTION

Oxidative stress is root cause of several metabolic disorders and diseases which can potentially abates the efficiency of cellular antioxidant defense system. The antioxidant defense system includes non-enzymatic ingredients (e.g. vitamin E, C, and A, GSH, arginine, citrulline, taurine, selenium, etc.) and enzymes (e.g. catalase, glutathione peroxidase, superoxide dismutase etc.). This system works by neutralizing excessive ROS generation and keeping ROS level below a toxic threshold. Environmental pollutants including pesticides are established agents for enhanced ROS generation leading to several reproductive disorders [1].

Among pesticides, synthetic pyrethroids have wide spectrum uses against pests and embrace 25% of global insecticide [2]. It is reported that pyrethroids (such as fenvalerate, permethrin and cypermethrin) have competency to disrupt biochemistry [3], histopathology [4] and reproduction [5] in mammals. Pyrethroid toxicity leads to disruption of antioxidant enzyme profile [6]. Permethrin (3-phenoxybenzyl-(±)-*cis*, *trans*-3-(2,2-dichloroethenyl)- 2,2-

dimethylcyclopropane-1-carboxylate) is a non-cyano pyrethroid used in agriculture practices as pest control, insect repellent and termiticide. According to Zhang *et al.* [7] permethrin cause reproductive toxicity in mammals. In addition, Hu *et al.* [8] demonstrated that permethrin can induce oxidative stress in rat adrenal pheochromocytoma (PC12 cells).

Now-a-days, herbal drugs are preferred over synthetic drugs due to their high effectivity and low toxicity [9]. The antioxidant potential of several medicinal plants in reducing oxidative stress has been established [10]. *Tribulus terrestris* is an annual herb of family Zygophyllaceae having aphrodisiac, antiurolithic, immunomodulatory, antidiabetic, hypolipidemic, hepatoprotective, anti-inflammatory, antibacterial and anticariogenic properties. Studies have revealed that it has natural antioxidant formulations in the areas of medicine and nutrition [11]. TT imparts ameliorative effects either by scavenging free radicals or inflecting antioxidant defense system. Mitra *et al.* [12] found that both whole plant extract as well as fruit extract of TT have antioxidant property.

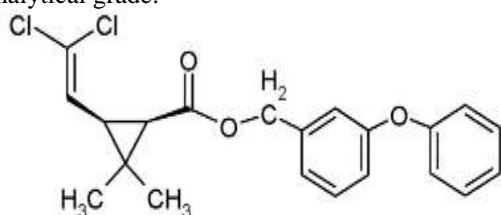
Ascorbic acid (vitamin C) is water-soluble vitamin and pivotal component of diet. It is considered to be one of the most prevalent antioxidative components of fruits/vegetables and could also impart chemopreventive effects [13]. It is known to play important role in pruning lethal effects of pesticides against the reproductive toxicity [14, 15]. Studies showed that vitamin C has antioxidant properties in quenching free radical [16, 17].

To best of our knowledge, the role of *Tribulus terrestris* and vitamin C against permethrin –induced biochemical alteration and antioxidant status in goat has not been studied yet. It is therefore, the present study was designed to evaluate attenuation potency of *Tribulus terrestris* and vitamin C against permethrin induced alterations in activity of antioxidant enzymes.

## MATERIALS AND METHODS

### Chemicals

Permethrin ( $C_{21}H_{20}Cl_2O_3$ ) having CAS no.52645-53-1, 96% purity was procured from Nanz Medical Science Pharma Pvt. Ltd., Himachal Pradesh, India. Chemicals such as L-Ascorbic acid ( $C_6H_8O_6$ ) with CAS no:50-81-7, EDTA, sodium azide, reduced glutathione, Ellman's reagents, hydrogen peroxide, Triton X-100, DTNB and all other chemical used were of analytical grade.



Chemical structure of permethrin

**Table-1: Different experimental group having different doses of permethrin and ameliorants**

Groups	Permethrin	Vitamin C	<i>Tribulus terrestris</i>
Control	-	-	-
Experimental group-I	100µg/ml(4hr.)	-	-
	100µg/ml(8hr.)	-	-
	200µg/ml(4hr.)	-	-
	200µg/ml(8hr.)	-	-
Experimental group-II	100µg/ml(4hr.)	0.1mM/ml(4hr.)	-
	100µg/ml(8hr.)	0.1mM/ml(8hr.)	-
	200µg/ml(4hr.)	0.1mM/ml(4hr.)	-
	200µg/ml(8hr.)	0.1mM/ml(8hr.)	-
Experimental group-III	100µg/ml(4hr.)	-	100µg/ml(4hr.)
	100µg/ml(8hr.)	-	100µg/ml(8hr.)
	200µg/ml(4hr.)	-	100µg/ml(4hr.)
	200µg/ml(8hr.)	-	100µg/ml(8hr.)

### Preparation of homogenate

Cultured testicular tissue was homogenized for 2 min in ice-cold PBS buffer (pH 7.4) at 4°C. Supernatant from different experimental groups, was used as enzyme source for the estimation of protein content and the activity of antioxidant enzymes i.e.

### Collection and Extraction of Plant Material

The fruits of *Tribulus terrestris* were collected from Kurukshetra University campus (29° 6' N, 76° 50'E) and authenticated by Dr. B. D. Vashista, Professor of Botany, Kurukshetra University, Kurukshetra. A copy of herbarium (Voucher specimen No. Herbarium/BOT.KU/ZOO-1-2016) was deposited to Department of Botany. The fruits of TT were shed dried and coarsely grinded. Hydro-alcoholic extract of TT was prepared by maceration of mixture in large mouthed bottles under constant agitation in rotary flask shaker for 72h. The filtrate was collected and concentrated in rotary evaporator (Rotavapour R-300, Buchi) under reduced pressure at 40±2°C. The extractive yield was calculated and crude extract was stored at 4°C for future use.

### Sample Collection

The testes from mature goat (*Capra hircus*) was obtained from slaughter houses around Kurukshetra (29° 6' N, 76° 50'E) and was brought to the lab. at 4°C in normal saline.

### Testicular tissue culture

After decapsulation, approximately 1mm<sup>3</sup> sized pieces were cut from goat testis. Testicular tissues were washed thrice with PBS having pH 7.0 and cultured in TCM-199 media having antibiotics (200 units of 100 IU/ml of penicillin and 100g/ml of streptomycin) at 39°C with 95% humidity and 5% CO<sub>2</sub> in the CO<sub>2</sub> incubator as per experimental layout.

### Experimental design

The testicular tissue was divided as follows.

catalase, glutathione peroxidase and reduced glutathione.

### Biochemical analysis

The protein content was estimated by the method of Lowry *et al.* [18] using bovine serum

albumin as standard. Catalase (CAT) activity was assayed as described by Aebi [19]. Glutathione peroxidase (GPx) activity was determined by the method of Rotruck *et al.* [20]. Reduced glutathione activity measured according to Tietze [21].

### STATISTICAL ANALYSIS

Data was analyzed using SPSS program version 16 (SPSS Inc., Chicago, IL). For comparison between treated groups and protective groups, One-Way analysis of variance (ANOVA) test followed by Dunccan's test for post-hoc analysis. Data are presented as mean $\pm$ SE and statistical significance analysis was acceptable to a level of  $p < 0.05$ .

### RESULTS

#### Extractive value

The extractive yield was 6.9% (w/w) for *Tribulus terrestris*.

#### Protein content

The protein content was found to be decreased in permethrin intoxicated groups both in dose and time dependent manner as compared to control groups (Figure:1A). 0.032 $\mu$ g/ml of protein content was reported in 200 $\mu$ g/ml dose of permethrin for 8hr. which was lower than 100 $\mu$ g/ml dose for same duration. Whereas ameliorative groups (EII and EIII) have increased protein content as compared to treated groups.

#### Antioxidant enzyme

The effect of permethrin, vitamin C and TT on the activity of antioxidant markers (CAT, GPx and GSH) were estimated (Table: 2). Decrease percentage of their activity was also calculated (Figure: 2A and 2B).

#### (A) Catalase

Table: 2 and Figure:1B depicts significant ( $p < 0.05$ ) decrease in CAT activity in permethrin treated group as compared to control in both dose and time

dependent manner. At higher dose i.e. 200 $\mu$ g/ml dose of permethrin, the decrease in CAT activity was 62.69% as compared to lower dose (100 $\mu$ g/ml of permethrin) with 29.3% decline from that of control value at 8hr. Group II showed restoring effect, as decline of only 7.32% (100 $\mu$ g/ml+vitamin C) and 17.1% (200 $\mu$ g/ml+vitamin C) to that of control value at 8hr. was estimated. TT supplemented groups, found to have high CAT activity i.e. 12.19% (100 $\mu$ g/ml+TT) and 25.4% (200 $\mu$ g/ml+TT) to that of control at 8hr.

#### (B) Glutathione peroxidase

A significant decrease ( $p < 0.05$ ) in GPx activity was observed in permethrin treated groups as compared to control and protective group in both time and dose dependent manner. At higher dose (200 $\mu$ g/ml of permethrin) the estimated decrease percentage was 39.4% higher than 100 $\mu$ g/ml of permethrin (14.3%) to that of control for 8hr. Whereas in group II decline of 20.79% (200 $\mu$ g/ml+vitamin C) and 10.1% (100 $\mu$ g/ml+vitamin C) to that of control was reported at 8hr. TT administered group, EIII showed high GPx activity as compared to treated groups where decline of 25.02% (200 $\mu$ g/ml+TT) and 19.9% (100 $\mu$ g/ml+TT) of control value was estimated at 8hr. (Table: 2 and Figure: 1C)

#### (C) Reduced glutathione

The content of GSH found to be decreased in treated group (EI) than control and ameliorative groups (EII and EIII) in both time and dose dependent manner. In EI group, the decline in GSH level was 29.5% (100 $\mu$ g/ml dose of permethrin) and 51.59% (200 $\mu$ g/ml of permethrin) to control for 8hr. Group II showed significant ( $p < 0.05$ ) increase in the GSH activity as the estimated decreased percentage was only 12.6% (100 $\mu$ g/ml+vitamin C) and 26.6% (200 $\mu$ g/ml+vitamin C) to that of control at 8hr. The decline percentage in group III was 18% (100 $\mu$ g/ml+TT) and 34.2% (200 $\mu$ g/ml+TT) to that of control at 8 hr. (Table: 2 and Figure: 1D).

**Table-2: Mean value of catalase, glutathione peroxidase and reduced glutathione in different Experimental groups**

Parameters (per mg protein)	Control	100 $\mu$ g/ml of permethrin	200 $\mu$ g/ml of permethrin	100 $\mu$ g/ml of permethrin along with (0.1mM) of vitamin C	200 $\mu$ g/ml of permethrin along with (0.1mM) of vitamin C	100 $\mu$ g/ml of permethrin along with (100 $\mu$ g/ml) of <i>Tribulus terrestris</i>	200 $\mu$ g/ml of permethrin along with (100 $\mu$ g/ml) of <i>Tribulus terrestris</i>
<b>4 Hours</b>							
CAT( $\mu$ mol)	5.27 <sup>a</sup> $\pm$ 0.09	4.53 <sup>c</sup> $\pm$ 0.08	3.1 <sup>c</sup> $\pm$ 0.11	5.02 <sup>ab</sup> $\pm$ 0.5	4.44 <sup>a</sup> $\pm$ 0.88	4.83 <sup>bc</sup> $\pm$ 0.11	4.03 <sup>b</sup> $\pm$ 0.12
GPx(nmol)	35.0 <sup>a</sup> $\pm$ 0.57	30.6 <sup>b</sup> $\pm$ 0.88	25.3 <sup>c</sup> $\pm$ 0.33	32.6 <sup>ab</sup> $\pm$ 0.88	30.0 <sup>b</sup> $\pm$ 0.57	31.3 <sup>b</sup> $\pm$ 1.2	29.6 <sup>b</sup> $\pm$ 0.88
GSH(nmol)	25.0 <sup>a</sup> $\pm$ 0.57	19.0 <sup>b</sup> $\pm$ 0.57	17.0 <sup>c</sup> $\pm$ 0.57	22.6 <sup>ab</sup> $\pm$ 0.04	19.3 <sup>b</sup> $\pm$ 0.88	21.3 <sup>ab</sup> $\pm$ 0.88	17.6 <sup>bc</sup> $\pm$ 0.33
<b>8 Hours</b>							
CAT( $\mu$ mol)	4.1 <sup>a</sup> $\pm$ 0.06	2.9 <sup>c</sup> $\pm$ 0.05	1.53 <sup>d</sup> $\pm$ 0.08	3.8 <sup>b</sup> $\pm$ 0.14	3.4 <sup>b</sup> $\pm$ 0.05	3.6 <sup>b</sup> $\pm$ 0.05	3.06 <sup>c</sup> $\pm$ 0.08
GPx(nmol)	31.3 <sup>a</sup> $\pm$ 0.33	26.3 <sup>b</sup> $\pm$ 0.33	18.6 <sup>c</sup> $\pm$ 1.8	27.6 <sup>ab</sup> $\pm$ 1.8	24.3 <sup>b</sup> $\pm$ 0.33	25 <sup>b</sup> $\pm$ 1.5	23.0 <sup>b</sup> $\pm$ 0.57
GSH(nmol)	21.0 <sup>a</sup> $\pm$ 0.57	16.0 <sup>c</sup> $\pm$ 0.57	10.3 <sup>c</sup> $\pm$ 0.88	18.6 <sup>b</sup> $\pm$ 0.88	15.0 <sup>b</sup> $\pm$ 1.0	17.3 <sup>bc</sup> $\pm$ 0.05	14.0 <sup>b</sup> $\pm$ 1.1

Values are expressed as mean±SE. Where superscript ‘a’ versus superscript ‘b’; superscript ‘b’ versus superscript ‘c’ and superscript ‘d’. Significant difference in Catalase, are indicated ( $p < 0.05$ ) in

different doses of permethrin and ameliorants (vitamin C and *Tribulus terrestris*). CAT=Catalase, GPx= Glutathione peroxidase, GSH= Reduced glutathione.

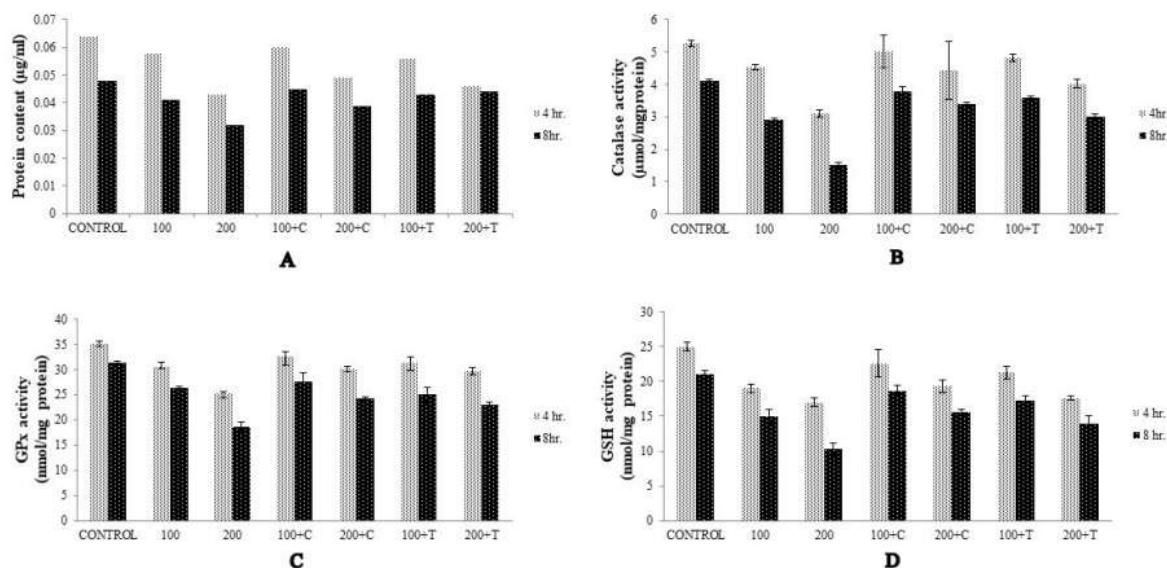


Fig-1: Ameliorative effect of vitamin C (0.1mmol) and *Tribulus terrestris* (100µg/ml) against permethrin induced alteration in protein content (A), enzymatic activity of CAT (B) GPx (C) and GSH (D) in control, treated and protective groups. Data are expressed as mean values±S.D ( $p < 0.05$ ). CAT: Catalase; GPx: Glutathion peroxidase; GSH: Reduced glutathione.

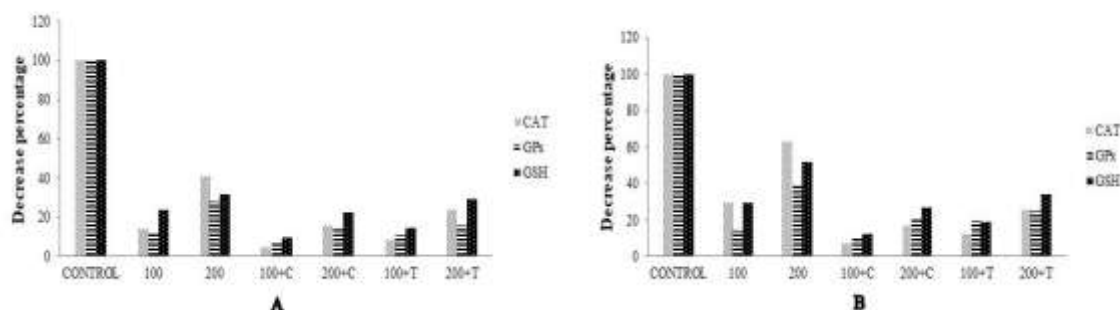


Fig-2: (A) Showing the decrease percentage (with respect to control) of catalase, glutathione peroxidase, reduced glutathione for the duration of 4hr. (B) Showing the decrease percentage (with respect to control) of catalase, glutathione peroxidase, reduced glutathione for the duration of 8hr

### Decreasing Percentage

The decreasing percentage of antioxidant enzyme for 4hr. and 8hr. durations has been depicted in Figure 2 (A) and (B) respectively.

### DISCUSSION

Present study showed that permethrin has adverse effects on antioxidant enzymes (CAT, GPx, GSH) of goat testis in both dose and time dependent manner. Significant decrease in the activity of antioxidant enzyme was reported in treated groups (E1) because of scavenging action of these enzymes against permethrin induced ROS. These observations are in consonance with the findings of De La Haba *et al.* [22]

who have concluded that permethrin treatment impaired antioxidant enzyme system and induced oxidative stress. Catalase is the marker enzyme which decomposes  $H_2O_2$  produced by peroxisome oxidases [23]. Present work documented a significant decrease in CAT activity (62.69%) at maximum dose of 200µg/ml of permethrin for 8hr. Catalase along with glutathione (a tripeptide thiol) is an important antioxidant system which is a crucial cellular defence against reactive free radicals [24]. Present studies have observed a significant decrease in GSH (51% decline to that of control) and GPx (39% decline to that of control) level at 200µg/ml of permethrin for 8hr in treated groups as compared to control group. Decline in glutathione



activity is due to utilization of GSH for detoxification of permethrin induced free radicals. The decrease in intracellular concentration of CAT, GSH and GPx is an indicator of permethrin induced oxidative stress. Present study is in consistence with the studies of Nasuti *et al.* [25], who have revealed that permethrin intoxicated striatum of rat showed decreased GSH level. El-Demerdash [26] also reported that permethrin treatment reduced the level of GSH and GPx in rats.

Result of present study revealed that fruit extract of TT has phenolic compounds which act effectively against permethrin induced ROS thus capable in reducing oxidative stress. TT supplemented group (EIII) estimated to have high antioxidant enzymes activity than that of treated groups. Previous studies showed that TT have antioxidant properties and flavonoids of fruit extract of TT, reacted with the free radicals either converting them into more stable products or terminating free radical chain reaction [27]. Zheleva-Dimitrova *et al.* [11] documented that TT has phenolic compounds like furostanol and spirostanol saponins which help in scavenging free radicals.

Vitamin C (L-ascorbic acid) is a non-enzymatic, water-soluble and well-known antioxidant which function against free radicals and pollutants [28]. Present study showed that vitamin C has the ability to mitigate permethrin induced toxicity in goat testis by increasing the activity of antioxidant markers in both dose and time dependent manner. It is also documented that vitamin C supplemented groups showed elevated activity of CAT, GPx, GSH as compared to treated groups but relatively lower than control group. The findings are in agreement with Nasuti *et al.* [25] who stated that GSH and CAT activity were slightly lower than control in permethrin intoxicated rat's erythrocytes along with vitamin C. Sulak *et al.* [29] demonstrated that vitamin C is potent scavenger of free radicals in extracellular fluids.

## CONCLUSION

From the above results, it is concluded that permethrin treatment elicits a significant imbalance in antioxidant enzymes level of goat testis in dose and time dependent manner. The higher dose for higher exposure duration induce more deleterious effects than lower dose for less exposure duration resulting in reproductive toxicity. The decrease in antioxidant level in treated groups is due to the combating action of marker enzymes in response to elevated level of ROS. Whereas elevated level of marker enzymes in protective groups showed that *Tribulus terrestris* and vitamin C have antioxidant properties against permethrin induced oxidative stress, thus they can be used as potent antioxidants.

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**Conflict of interest:** Authors have none to declare.

## REFERENCES

1. Abdollahi, M., Ranjbar, A., Shadnia, S., Nikfar, S., & Rezaiee, A. (2004). Pesticides and oxidative stress: a review. *Medical Science Monitor*, 10: 141-147.
2. Barr, D.B., Olsson, A.O., Wong, L.Y., Udunka, S., Baker, S.E., Whitehead Jr, R.D., Magsumbol, M.S., Williams, B.L., & Needham, L.L. (2010). Urinary concentrations of metabolites of pyrethroid insecticides in the general US population: National Health and Nutrition Examination Survey 1999–2002. *Environmental Health Perspectives*, 118: 742.
3. Sadowska-Woda, I., Wójcik, N., Karowicz-bilińska, A., & Bieszczad-bedrejczuk, E. (2010). Effect of selected antioxidants in  $\beta$ -cyfluthrin-induced oxidative stress in human erythrocytes in vitro. *Toxicology in Vitro*, 24: 879-884.
4. Bhardwaj, S., & Sharma, R.K. (2017). Ameliorative Effects of Phytoandrogen and antioxidant on histoarchitecture of permethrin induced Changes in Goat Testis. *International Journal of Pharmacy and Pharmaceutical Sciences*, 9: 183-187.
5. Yousef, M.I., Abdallah, G.A., & Kamel, K.I. (2003). Effect of ascorbic acid and vitamin E supplementation on semen quality and biochemical parameters of male rabbits. *Animal Reproduction Science*, 76: 99-111.
6. Madhubabu, G., & Yenugu, S. (2014). Allethrin induced toxicity in the male reproductive tract of rats contributes to disruption in the transcription of genes involved in germ cell production. *Environmental Toxicology*, 29: 1330-45.
7. Zhang, S.Y., Ito, Y., Yamanoshita, O., Yanagiba, Y., Kobayashi, M., Taya, K., Li, C., Okamura, A., Miyata, M., Ueyama, J. & Lee, C.H. (2007). Permethrin may disrupt testosterone biosynthesis via mitochondrial membrane damage of Leydig cells in adult male mouse. *Endocrinology*, 148: 3941-3949.
8. Hu, F., Li, L., Wang, C., Zhang, Q., Zhang, X., & Zhao, M. (2010). Enantioselective induction of oxidative stress by permethrin in rat adrenal pheochromocytoma (PC12) cells. *Environmental toxicology and chemistry*, 29: 683-690.
9. Mady, N.I., Allam, A.F., & Salem, A.I. (2001). Evaluation of the addition of *Nigella sativa* oil triclabendazole therapy in the treatment of human fascioliasis. *Journal of Egyptian Society of Pharmacology and Experimental Therapeutics*, 20: 807-27.
10. Krishnaiah, D., Sarbatly, R., & Nithyanandam, R. (2011). A review of the antioxidant potential of

- medicinal plant species. *Food and Bioproducts Processing*, 89: 217-33.
11. Zheleva-Dimitrova, D.I., Obreshkova, D.A., & Nedialkov, P. (2012). Antioxidant activity of *Tribulus terrestris*-a natural product in infertility therapy. *International Journal of Pharmacy and Pharmaceutical Sciences*, 4: 508-11.
  12. Mitra, N., Mohammad-Mehdi, D., & Reza, Z.M. (2012). *Tribulus terrestris* L. (Zygophyllaceae) Flavonoid Compounds. *International Journal of Modern Botany*, 2: 35-9.
  13. Lee, K. W., Lee, H. J., Surh, Y. J., & Lee, C. Y. (2003). Vitamin C and cancer chemoprevention: reappraisal. *The American journal of clinical nutrition*, 78: 1074-1078.
  14. Alhazza, L.M., & Bashandy, S.A. (1998). Influence of vitamin C on the toxicity of Pifpaf (containing permethrin) to gonads of male rats. *Saudi Journal of Biological Sciences*, 5: 31-7.
  15. Uzunhisarcikli, M., Kalender, Y., Dirican, K., Kalender, S., Ogutcu, A., & Buyukkomurcu, F. (2007). Acute, subacute and subchronic administration of methyl parathion-induced testicular damage in male rats and protective role of vitamins C and E. *Pesticide biochemistry and physiology*, 87: 115-122.
  16. Kojo, S. (2004). Vitamin C: basic metabolism and its function as an index of oxidative stress. *Current Medicinal Chemistry*, 11: 1041-64.
  17. El-Demerdash, F.M., Yousef, M.I., & Zoheir, M.A. (2005). Stannous chloride induces alterations in enzyme activities, lipid peroxidation and histopathology in male rabbit: antioxidant role of vitamin C. *Food and Chemical Toxicology*, 43: 1743-52.
  18. Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of biological chemistry*, 193(1), 265-275.
  19. Aebi, H. (1984). Catalase *in vitro*. *Methods in Enzymology*, 31: 121-6.
  20. Rotruck, J. T., Pope, A. L., Ganther, H. E., Swanson, A. B., Hafeman, D. G., & Hoekstra, W. (1973). Selenium: biochemical role as a component of glutathione peroxidase. *Science*, 179: 588-590.
  21. Tietze, F. (1969). Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. *Analytical Biochemistry*, 27: 502-22.
  22. de la Haba, C., Palacio, J. R., Martínez, P., & Morros, A. (2013). Effect of oxidative stress on plasma membrane fluidity of THP-1 induced macrophages. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, 1828: 357-364.
  23. De Duve, C.A., & Baudhuin, P. (1966). Peroxisomes (microbodies and related particles). *Physiological Reviews*, 46: 323-57.
  24. Haque, R., Bin-Hafeez, B., Parvez, S., Pandey, S., Sayeed, I., Ali, M., & Raisuddin, S. (2003). Aqueous extract of walnut (*Juglans regia* L.) protects mice against cyclophosphamide induced biochemical toxicity. *Human & experimental toxicology*, 22: 473-480.
  25. Nasuti, C., Falcioni, M. L., Nwankwo, I. E., Cantalamessa, F., & Gabbianelli, R. (2008). Effect of permethrin plus antioxidants on locomotor activity and striatum in adolescent rats. *Toxicology*, 251: 45-50.
  26. El-Demerdash, F.M. (2011). Oxidative stress and hepatotoxicity induced by synthetic pyrethroids-organophosphate insecticides mixture in rat. *Journal of Environmental Science and Health*, 29: 145-58.
  27. Patel, S., Patel, T., Parmar, K., Patel, B., & Patel, P. (2011). Evaluation of antioxidant activity, phenol and flavonoid contents of *Momordica charantia* Linn fruit. *Advance Research in Pharmaceutical and Biological*, 1: 120-9.
  28. Iqbal, K., Khan, A., & Khattak, M.M. (2004). Biological significance of ascorbic acid (vitamin C) in human health—a review. *Pakistan Journal of Nutrition*, 3: 5-13.
  29. Sulak, O., Altuntas, I., Karahan, N., Yildirim, B., Akturk, O., Yilmaz, H. R., & Delibas, N. (2005). Nephrotoxicity in rats induced by organophosphate insecticide methidathion and ameliorating effects of vitamins E and C. *Pesticide Biochemistry and Physiology*, 83: 21-28.