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Protective role of ginger (*Zingiber officinale*) against toxicity of paraquat on testis of mice with special reference to biochemical and histopathological study

Suhaib Mohd Malik, Vinoy Kumar Shrivastava, Ab Qayoom Naik & Tufaila Manzoor
Laboratory of Endocrinology, Department of Bioscience, Barkatullah University, Bhopal, Madhya Pradesh, India

Abstract
Male reproductive health has deteriorated significantly in the last few decades. Nutritional, socioeconomic way of life and environmental factors (among others) have been attributed to compromising male reproductive health. In recent years, a large volume of evidence has accumulated suggesting that the trend of declining male fertility (in terms of sperm count, quality and other changes in male reproductive health) might be due to exposure of environmental toxicants. Current study was framed to explore the toxicological effect of sub-lethal concentrations of paraquat (Herbicide) on biochemical and histopathological parameters of male mice *Mus musculus* and protective role of Ginger *(Zingiber officinale)* against paraquat toxicity. During the present study it was found that the protective effect of ginger extract against testicular damage induced by paraquat may be attributed to its antioxidant properties.

Keywords: paraquat, ginger, male mice, ALP, ACP, protein, creatinine, testis, histopathology

Introduction
The indiscriminate use of herbicides and pesticides to increase crop productivity has aroused a great concern among the environmental and health scientists due to their adverse effects in both targets as well as non-target species. The insecticides constitute one group of these pollutants, both synthetic and natural, which contribute to the environmental problems. Toxicity is the degree to which a substance is able to damage an exposed organism. It can refer to the effect on a whole organism, such as an animal, bacterium, or plant as well as the effect on a substructure of the organism, such as a cell (Cytotoxicity) or an organ (Organotoxicity). Central concept of toxicology is that effects are dose dependant; even water can lead to water intoxication when taken in large enough doses, whereas for even a very toxic substance such as snake venom there is a dose level below whish there is no detectable toxic effects. Paraquat is a toxic chemical that is widely used as a herbicide (plant killer), primarily for weed and grass control. Paraquat (PQ) is a non-selective contact herbicide discovered in 1955 and was registered as herbicide in 1962 by ICI laboratories (Revkin, 1983) [11, 12]. Chemically paraquat is 1,11-dimethyl-4,41-bipyridinium dichloride.Paraquat has been reported to be highly toxic to humans and animals with many cases of acute poisoning and death (Hood, 1965) [7]. Paraquat intoxication was reported in Korea while fatality rates for intentional paraquat ingestion were reported to range from 58% in Fiji (Summers, 1980) [16], nearly 80% in Southern Mexico (Bus & Gibson, 1984) [3]. Forensic analysis of fatal intentional poisoning in South Trinidad showed that in 105 deaths from poisoning in 1996-97, paraquat was the causative agent in 80 cases (76%) (Beckie, 2011) [2]. Also paraquat was banned in Samoa for being used as a suicide agent in about 70% of all suicide cases between 1999-2000 (Eubank, *et al*., 2008) [5]. The acceptable daily accidental intake of paraquat ion is 0.004 mg/kg body weight (Ashton & Leahy, 2000). Paraquat produces both histological and functional changes in lungs, kidneys, adrenal glands, liver and myocardium, causing multi-organ failure (Paraquat-Monograph, 2003). Chronic exposure of paraquat can lead to lung damage, kidney failure, heart failure and oesophageal strictures (Ossowska *et al.*, 2006) [10].

Ginger *(Zingiber officinale)*
The ginger (*Zingiber officinale* Rosce, Zingiberaceae) approximately 2-3 cm in length, is one of the more commonly used herbal supplements (Langer *et al.*, 1998) [9]. It is taken by many patients to treat a variety of conditions (Ghayur *et al.*, 2005). Its root has been used for perhaps thousands of years in the far East to treat inflammatory diseases (Shen *et al.*, 2003) [14] and also shown to be effective for pregnancy- induced and postoperative nauseaand vomiting (White, 2007). *Zingiber officinale* is the botanical name for ginger, a tropical herbal plant found in abundance in Asia. It belongs to the family of *Zingiberaceae* (Kemper, 1999) [8]. Jagetia *et al*., (2006) reports that ginger has a protective effect in the radiotherapy treated rats as it decreased both the severity of radiation sickness and mortality at all the exposure doses through scavenging OH, O2 and ABTS radicals in a dose-dependent manner in vitro. There are also a recorded protective effects for ginger extract on the hepato toxic effects of both carbon tetrachloride and acetaminophen as it improved the elevated serum liver enzymes and the protective effect of the extract was confirmed also by histopathological examination of the liver (Yemitan & Izegbu, 2006) [17].

Materials and Methods
The experimental investigation of paraquat and ginger was carried out on body weight, Biochemical and Histological

changes in testis of male mice *Mus musculus*, after estimated oral dose via cannula of paraquat and ginger.

Animals

The present experiment was performed on mature male mice *Mus musculus* weighing 30±5gm. All animals were acclimatized to laboratory conditions *i.e.* at 22±3º C temperature and light and dark photoperiod (14L: 10D h) in the Animal House of Laboratory of Endocrinology, Bioscience Department, Barkatullah University, Bhopal (M.P.). Hygienic conditions were maintained with rice husk bedding in separate polypropylene cages. Animals were fed on standard mice feed and water *ad libitum*.

Chemical

Paraquat (Brand Name : Crisquat) were obtained from registered pesticide shop of Bhopal named as Vatika Pest Control, which was manufactured by Rallis Tata Enterprise. While as, ginger was brought from local vegetable shop at Bhopal.

Prepration of dose

6.25 ml of Paraquat was dissolved in 18.75 ml of distill water (net vol.25 ml) and was stored at 4°C as working stock solution. While ginger 250gm was grinded in a juice mixer till liquid extract of sample was obtained. Thereafter, the extract was filtered using Wattman's filter paper and the process was repeated once again. The final extract was maintained at 4°C (Ginger stock solution) throughout the experiment.

Experimental design

The eight weeks old weight 30±5 gm male adult mature mice, *Mus musculus* of Parke's strain were used for the experimental studies. All the animals were divided into four groups of 5each. The dose of Paraquat was finalized after observing various literatures and confirmed through experimental investigation, whereas LD50 of paraquat in mice is 20 mg/kg body weight (European Commission, 2000; Joint FAO/WHO, 2000), whereas, ginger is nontoxic in animal studies (REF).

Group I: The animals of this group were served as control, received balanced diet, water *ad libitum* for 30 days.

Group II: The animals of this group received balanced diet, water *ad libitum and* were fed alternatively with Paraquat (1ml/kg body weight) orally via cannula for 30 days.

Group III: The animals of this group received balanced diet, water *ad libitum* and ginger extract (1ml/kg body weight) along with Paraquat (1 ml/kg body weight) alternatively via cannula for 30 days.

Group IV: The animals of this group received balanced diet, water *ad libitum* and were treated alternatively with ginger extract only, orally via cannula (1 ml/kg body weight) for 30 days.

The initial body weights of all mice were taken out on day first *i.e.* 0 days of the experiment and after completion of experiment *i.e.* 30 days with the help of a laboratory weighing balance and the values were expressed in grams/animals. All animals of each group were sacrificed by cervical dislocations at 31^{Ist} day of experiment. The testis were taken out, washed in normal saline (0.9% Nacl), dried by filter paper then weighed and kept in Bouin's fixative for histological studies and some of the tissues were taken for biochemical estimations.

Biochemical and Enzymological Studies

Freshly removed testis were homogenised then biochemical and enzymological parameters were estimated.

1. ALP and ACP: By Bergmeyer Method (1963).
2. Protein Estimation: By Lowry *et al.*, Method (1951).
3. Creatinine estimation: Alkaline Pirate Method (1945).
4. Histopathological Studies: Eosin & Hematoxylene, Ehrlich (1886).

Results

Body Weight

In present study, it has been observed that the exposure of paraquat induced changes in body weight, It has been observed that the induction of paraquat (1 ml/kg b/w) significantly reduces the body weight on 31^{Ist} day. When provided with Ginger dose the body weight was as same as the control. While in Ginger supplement (1ml/kg) along with paraquat showed slight significant recovery in their body weight shown in (Table.1, Fig.1).

Morphological and Physical changes

In present **s**tudy toxicity of paraquat was observed on male mice *Mus musculus* exhibited disrupted moving behavior, localization into the corners of test chamber and independency (spreading out) in their moving action. In addition to this, loss of equilibrium was also observed. During the 30 days of exposure of paraquat, the mice became active and showed erect movement, while the control mice showed normal movement and normal behavior.

Enzymological studies:

The ACP, ALP, Protein and Creatinine levels were assumed by first calculating the optical density, analysis were done and graphs were prepared in comparison of control Vs treated groups after 30 days of exposure.

It was seen that the levels of ACP and ALP in the testicle regions showed significant increase in their levels after paraquat exposure of 30 days with respect to control group. While in mice supplemented with ginger (1ml/kg) along with paraquat significantly lowered the testicular ACP and ALP levels up to 30 days (Table and Fig 2,3).

Biochemical studies

In connection to this, due to the exposure of paraquat, the protein level in the testis of male mice significantly decreased up to 30 days of exposure compared with control, while in mice treated with ginger (1ml/kg) along with paraquat showed significant increase in the levels of protein up to 30 days (Table.4 and Fig. 4).

Besides this, Creatinine values were seen to be elevated in the testis after the 30 days of exposure of paraquat as compared with control. While showing a slight decrease in the group treated with ginger (Table.5 and Fig. 5).

Histopathological study

Testis

The testis are located in the scrotum and consists of

convoluted seminiferous tubules enclosed by the tunica albuginea, a fibrous capsule of connective tissue. The seminiferous tubules are lined by seminiferous epithelium. At the base of this epithelium are, at regular intervals, the sertoli (sustenttacular) cells, which have large oval nuclei. The seminiferous epithelium further contains cells undergoing spermatogenesis. The least mature cells (Spermatogonias) are located at the base of the epithelium, more matured cells (spermatogonias, spermatocytes, round spermatids, or elongated spermatids) move towards the lumen, and the most mature cells, the spermatozoa are released at the lumen. In the interstitial space between the seminiferous tubules are leydig (interstitial) cells, which have abundant eosinophilic cytoplasm and blood vessels. In the mice, the testies are freely retractable in to the abdominal cavity.

In our study it was observed that testis of animals treated with paraquat for 30 days exhibited a distinct histological difference when compared with control. In testis of these animals, large numbers of seminiferous tubules appeared irregular with intertubular haemorrhage and most of the spermatogonia were observed with cytoplasmic vacuolization. There was marked decrease in the number of spermatogenic cells and the sperm bundles were absent in most tubules. Examination of testis of animals treated for 30 days with paraquat and ginger extract revealed less prominent histopathological changes when compared with the group treated with Paraquat for the same period. Most of the seminiferous tubules were compact with each other. The spermatogenic layers appeared somewhat normal. Advanced degree of improvement was seen in testis of animals treated for 30 days with paraquat and ginger extract. Most of the seminiferous tubules restored its normal structure and there was no histological change in the testis of mice treated with Ginger extract only.

Table 1: Body weight (gm) of Control and Paraquat treated male mice (*Mus musculus)*

Groups	Control	Treated with Paraquat	Paraquat + Ginger	Ginger
Initial weight	28.66±1.6	28.96±1.2	28.10±1.7	28.72±1.5
After 30 days	30.23±0.94	24.30±0.87*	26.33±0.44	31.03±0.44

Table 2: Testicular Alkaline Phosphatase (IU/gm) of male mice *Mus musculus* after the exposure of Paraquat and Control up to 30 days.

Groups	Control	Treated with Paraquat	Paraquat + Ginger	Ginger
After 30 days	147.33±6.24	282.08±9.61***	195.24±7.29*	139.88±5.82

Table 3: Testicular Acid Phosphaatase (IU/gm) of male mice *Mus musculus* after the exposure of Paraquat and Control up to 30 days.

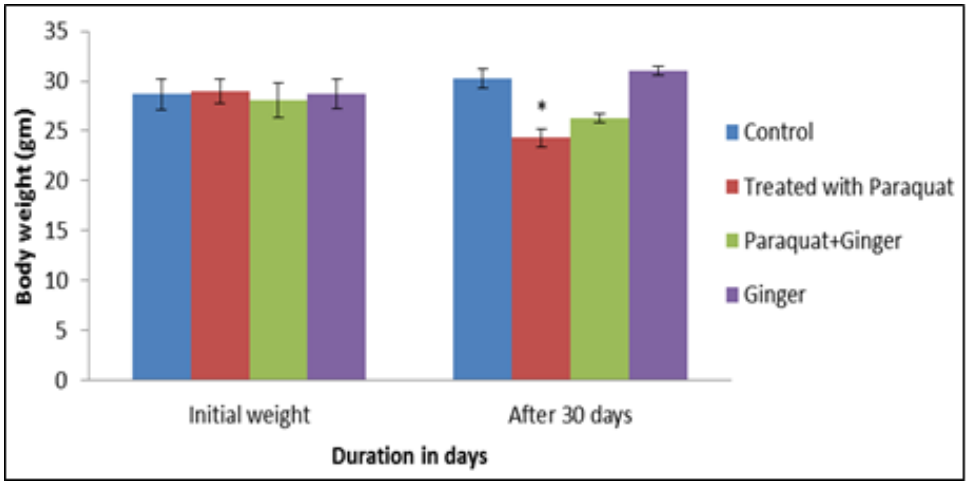
Groups	Control	Treated with Paraquat	Paraquat + Ginger	Ginger
After 30 days	0.984±0.12	2.490±0.18***	1.640±0.15**	0.959±0.10

Table 4: Testicular Protein (mg/gm) of male mice *Mus musculus* after the exposure of Paraquat and Control up to 30 days.

Groups	Control	Treated with Paraquat	Paraquat + Ginger	Ginger
After 30 days	64.31±2.24	40.26±1.19***	56.04±1.92*	65.84±2.46

Table 5: Testicular Creatinine (mg/gram) level of male mice *Mus musculus* after the exposure of Paraquat and Control up to 30 days.

Groups	Control	Treated with Paraquat	Paraquat + Ginger	Ginger
After 30 days	0.91±0.06	1.26±0.10**	1.02±0.09*	0.92±0.07



Values are mean ± SEM of 5 animals.
*Significant different (P<0.05) from control vs experimental by student's t' test.

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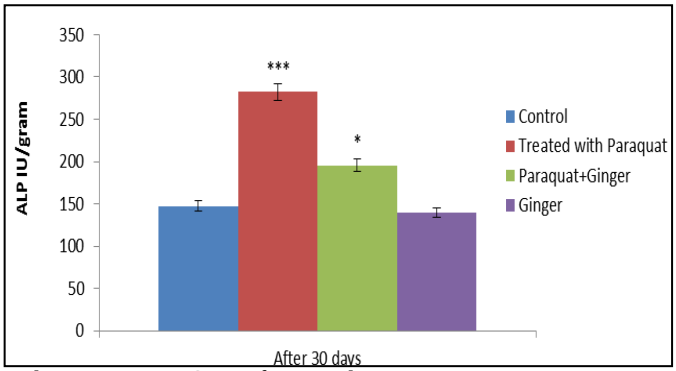

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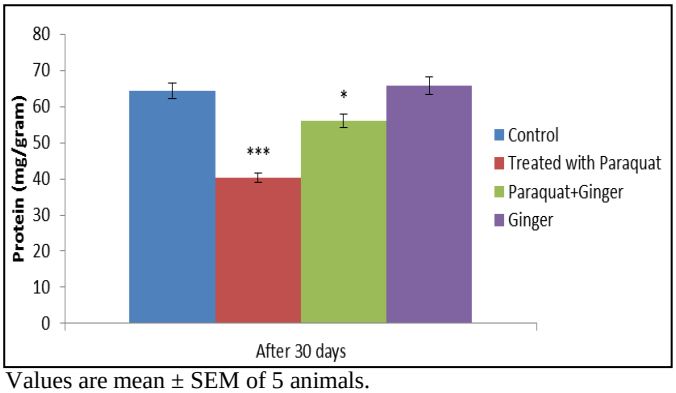
**More Significant different (P<0.05) from control vs experimental by student's 't' test.

Fig 2: Testicular Alkaline Phosphatase (IU/gm) of male mice *Mus musculus* after the exposure of Paraquat and Control upto 30 days



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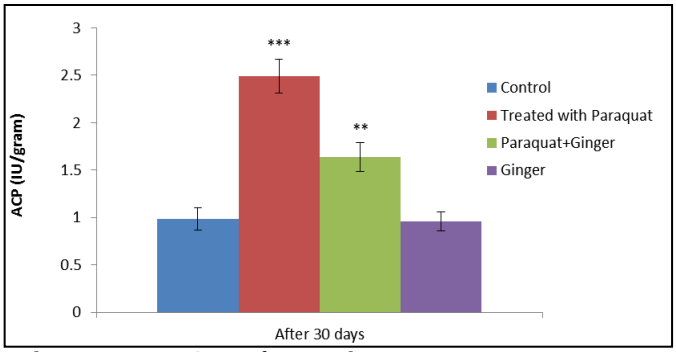

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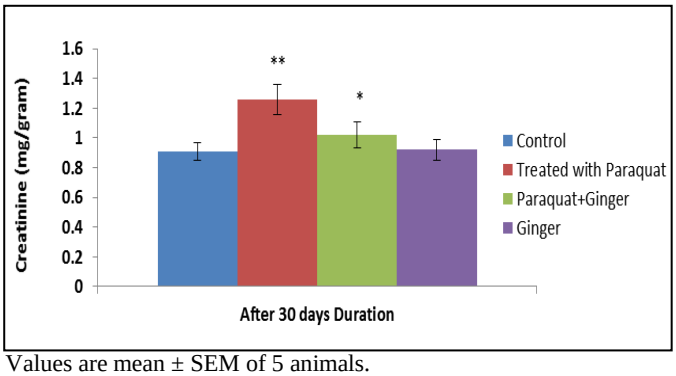

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Fig 5: Testicular Creatinine (mg/gram) level of male mice *Mus musculus* after the exposure of Paraquat and Control upto 30 days.

Testicular Sections Pixel 400x (H & E)

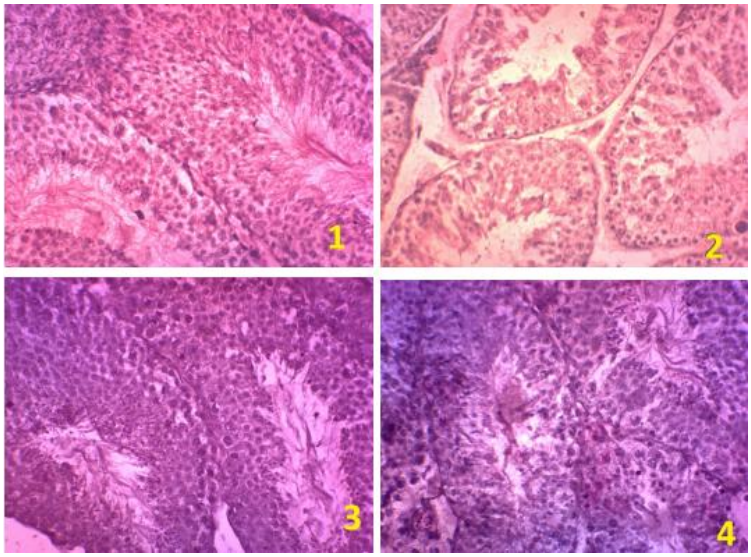

Fig 6: Histopathological Observation

Explanation of testicular transverse sections

Fig.6(1): Testicular transverse section of control male *Mus musculus* showing well defined structure of seminiferous tubules with different stages of spermatogenic cells i.e. Spermatogonial cells, spermatocytes and spermatids. Sertoli cells are also clearly visible and lumens are filled with large amount of spermatozoa (H & E 400x).

Fig. 6(2): Testicular transverse section of animals treated with paraquat for 30 days showing maximum hypertrophy in seminiferous tubules. Spermatogenic cells are less in number and in atrophied condition. Severe atrophied conditions are noticed in leydig cells. Lumens are completely devoid of sperms (H & E 400x).

Fig. 6(3): The photomicrograph of testis of male mice *Mus musculus* treated with paraquat and ginger extract for 30 days showing recovery in most of the seminiferous tubules, Intersititial cells and sperm bundles are present in most tubules (H & E 400x).

Fig. 6(4): The photomicrograph of testis of male mice *Mus musculus* treated with ginger extractfor 30 days showing normal seminiferous tubules and intersititial or Leydig cells and sperm bundles are normal in shape and size (H & E 400x).

Discussion

Present investigation was carried out with a novel technique of toxicant exposure to mice. The exposure method allowed immediate internal exposure of Paraquat to the vicinity of reproductive system. Since we have used extremely low concentrations, it is suggestive to use this type of a method to investigate mechanism of toxicant exposure. However, our exposure in this experiment was around LD50 value for Paraquat exposure of this method. Therefore it is clear that the method used will help in understanding of the toxicant effect on target tissue.

In our experiment significant increase were observed in ALP and ACP activities in the testis of male mice *Mus musculus* after paraquat exposure for 30 days. This increase was duration dependant. Our results inferred with the findings of Atef, (2005) [1] who also reported significant elevations of ALP after exposure to cadmium and attributed the increase to liver dysfunction.

Proteins are the building blocks which are essential constituents of animal food. Proteins are composed of small units. These units are the amino acids which are called the building blocks of proteins. There are about 20 different amino acids which are commonly known. Protein has a critical physiological function and is primarily used in the body to build, maintain and repair body tissues. In the event that protein in-take is greater than that of required by the body for its primary function, excessive protein is converted to energy for immediate use or stored in the body as fat.

Besides this, our studies have shown that the protein levels were significantly decreased due to the exposure of paraquat on male mice *Mus musculus* for 30 days of experiment as compared to control. It might be due to catabolism of protein. As the results were observed by (Prakash, 2001). He reported decrease in the levels of protein in the ovary and uterus. The changes in the levels of protein with treatment suggest either an increased catabolism of bio molecules to meet the enhanced energy demand of animals under stress or their reduced synthesis due to impaired tissue function (Ivanova, 1977). Creatinine is the catabolic product of the creatinine phosphate, which is used by the skeleton muscle. The daily production depends on muscular mass and it is excreted out of the body entirely by the kidneys. Our study showed that creatinine level was increased in male mice *(Mus musculus)* after the exposure of paraquat for 30 days as compared to control. Urea level can be increased by many other factors such as dehydration, anti diuretic drugs and diet, while creatinine is more specific to the kidney, since kidney damage is the only significant factor that increases serum creatinine level (Garba *et al*., 2007) [6].

In our study it was observed that testis of animals treated with Paraquat for 30 days exhibited a distinct histological difference when compared with control. In testis of these animals, large numbers of seminiferous tubules appeared irregular with intertubular haemorrhage and most of the spermatogonia were observed with cytoplasmic vacuolization. There was marked decrease in the number of spermatogenic cells and the sperm bundles were absent in most tubules. Examination of testis of animals treated for 30 days with Paraquat and ginger extract revealed less prominent histopathological changes when compared with the group treated with Paraquat for the same period. Most of the seminiferous tubules were compact with each other. The spermatogenic layers appeared somewhat normal. Advanced degree of improvement was seen in testis of animals treated for 30 days with Paraquat and ginger extract. Most of the seminiferous tubules restored its normal structure. Many investigation on the effect of different doses of herbicides on different organs in different animals have been done. The studies have shown that Paraquat have changes on histopathology in rats and especially in testicle and ovary atrophy (Deepananda and De Silva, 2013) [4].

Concerning the effect of ginger, present study indicated that ginger improved the histological and histochemical alterations induced by Paraquat in testis of mice. Similarly, Sakr and Badawy (2011) [13] reported that ginger extract improved testicular damage induced by metiram fungicide in mice. Siddaraju and Dharmesh (2007) [15] reported that ginger extracts exhibited free radical scavenging, inhibited lipid peroxidation, DNA protection and reduced power abilities indicating strong antioxidant properties. It was reported that the mechanism of protection of ginger is related to its antioxidant properties. It is concluded from the present study that the protective effect of ginger extract against testicular damage induced by Paraquat may be attributed to its antioxidant properties.

Conclusion

Chemically Paraquat is 1, 11-dimethyl-4, 41-bipyridinium dichloride. It is a widely used and effective herbicide with a broad spectrum of activity. Paraquat has been reported to be highly toxic to humans and animals with many cases of acute poisoning and death (Hood, 1965) [7]. It is useful to us in one way but like all herbicides it enters to the body of organisms' including humans & produce harmful effects. The present investigation of the effect of herbicides on Mice has a diagnostic significance in evaluation of adverse effects on human health.

Through this experiment we concluded that paraquat has induced changes in bio-chemical parameters & histopathological alterations. In the testis of the male mice (*Mus musculus*) these changes are duration dependent. The changes caused by paraquat either affecting directly on the organ by modulating the enzymes as well as causing biochemical changes. On the other hand concerning the effect of ginger, present study indicated that ginger improved the histological and histo-chemical alterations induced by Paraquat in testis of mice. Thus it is concluded from the present study that the protective effect of ginger extract against testicular damage induced by Paraquat may be attributed to its antioxidant properties.

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