Protective Role of Ginger (Zingiber officinale) Against Toxicity of Paraquat on Liver of Mus Musculus

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ABSTRACT
Paraquat (PQ) is the leading pesticide used to prevent weeds in agricultural areas. PQ is widely used in 130 countries, including Turkey. PQ (1,1-dimethyl 4,4'-bipiridinium) is a non-selective contact herbicide, the contact surface being the leaves of the weed. The acute lethal dose (LD) value of the active ingredient when applied orally is 150 mg kg-1 for rats, 70 mg kg-1 for mice, 25-50 mg kg-1 for dogs, and 262 mg kg-1 for chickens. Paraquat has been shown to be highly toxic by the inhalation route and has been placed in toxicity category (the highest of four levels) for acute inhalation effects. Current study was framed to observe toxicological effect of paraquat (Herbicide) on biochemical and histopathological parameters like Acid phosphatase (ACP), Alkaline phosphatase (ALP), GOT, GPT, Protein and Creatinine of male mice (Mus musculus) and protective role of Ginger (Zingiber officinale) against paraquat toxicity. Our investigations revealed that ginger extract performed a protective role against damage in the liver of mice caused by paraquat. Antioxidant effect of ginger showed improvement in the liver cells, affected by paraquat.

1. Introduction
Paraquat is a non-selective contact herbicide discovered in 1955 and was registered as herbicide in 1962 by ICI laboratories (Paraquat-Monograph, 2003). Chemically paraquat is 1,1-dimethyl-4,4'-bipyrindinium dichloride (Roberts et al., 2002). It is used as an active ingredient in different products for protection of crops and is rapidly absorbed by green plants (Paraquat- Monograph, 2006). The toxic effects of paraquat on plants are due to the production of paraquat free radicals, which, after re-oxidation with oxygen molecules, cause disorder in photosynthesis (Luty et al., 1997). In animals, it is absorbed through different routes and readily reaches all organs and tissues of the body and is not metabolized; instead, it is reduced to an unstable free radical, which is then re-oxidized to form a cation and a superoxide anion. The acceptable daily accidental intake of paraquat ion is 0.004 mg/kg body weight (Ashton and Leahy, 2000). Paraquat produces both histological and functional changes in lungs, kidneys, adrenal glands, liver and myocardium, causing multiorgan failure (Paraquat-Monograph, 2003). Paraquat has several mechanisms in inducing cytotoxicity (Fukushima et al., 2002). In mice, when given in acute toxic dose (50 mg/kg), the animals showed signs of necrosis and inflammation of liver parenchyma (Dragin et al., 2006).

The effects of paraquat at a dose of 20 mg/kg were studied on creatine kinase (CK), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), lactate dehydrogenase (LDH) and gamma glutamyl transferase (γ-GT) activities on liver, kidney and lungs of swiss albino mice. The CK, activity was reported to decrease in liver, while GOT, GPT, LDH and γ-GT activities increased (Dere and Polat, 2001).

The toxic effects of paraquat on liver histology showed centrolobular cholestasis, hepatocellular necrosis and macrophagic infiltration of portal areas; the portal tracts were increased in size due to abundance of collagen stroma, slight infiltration by lymphocytes and leukocytes (Bataller et al., 2000).

Medicinal plants play an important role in pharmacology and medicine for many years. Today, it is estimated that about 80% of the world population relies on botanical preparations as medicine to meet their health needs (Ogbera et al., 2010). Ginger (Zingiber officinale) is an example of such valuable plants which is gaining popularity amongst modern physicians and its underground rhizomes are the medicinally useful part (Mascolo et al., 1989). Numerous studies were carried out on ginger and its pungent constituents, fresh and rhizome. One of the most popular uses of ginger is to relieve the symptoms of nausea and vomiting associated with motion sickness, surgery and pregnancy (Gilani and Rahman, 2005). Ginger extracts showed different pharmacological effects such as anti-platelet, anti-oxidant, anti-tumour, anti-rhinoiridal, anti-hepatotoxicity and anti-arthritic effect (Fisher et al., 1991; Sharma et al., 1994; Kamtchouing et al., 2002). Ginger was found to have hypcholesterolaemic effects and cause decrease in body weight, glucose in blood, serum total cholesterol and serum alkaline phosphatase in adult male rats (Bhandari et al., 2005). The present study was undertaken to investigate the effect of paraquat on liver of mice and the possible protective effect of ginger aqueous extract.

2. Material and methods
The experimental investigation of paraquat and ginger was carried out on body weight. Biochemical and histological changes in liver 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±3°C temperature and light and dark photoperiod (14L: 10D h). 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.

Preparation 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 5 each. 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.

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 31st day of experiment. The liver 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.

3. Parameter Estimation

Body Weight:

The body weight of mice was weighed initially and also after different intervals that is 15 and 30 days of experiment.

Behavioural Studies:

The behaviour of all experimental mice was observed initially and also recorded throughout the experiment of 15 and 30 days.

Biochemical and Enzymological Studies:

Freshly removed Liver was homogenised then biochemical and enzymological parameters were estimated.

3. Protein Estimation: By Lowry et al., method, (1951).

4. 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 31st 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,Hist.1).

Morphological and physical changes:

In present study 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, GOT, GPT, 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, ALP and GPT in the liver regions showed significant increase in their levels while there was a slight decrease in the GOT, after paraquat exposure of 30 days with respect to control group.While in mice supplemented with Ginger (1ml/kg) along with Paraquat showed significantly slight recovery.(Table and Hist. 2,3,4,5).
Biochemical studies:

In connection to this, due to the exposure of paraquat, the protein level in the liver 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 significantly slight recovery. (Table 6 and Hist. 6). Besides this, Creatinine values were seen to be elevated in the liver after the 30 days of exposure of paraquat as compared with control. While showing a slight decrease in the groups treated with ginger (Table 7 and Hist. 7).

Histopathological study:

Liver

Liver is one of the main organs of the body and is located in the right side of the abdominal cavity. The main role of liver is detoxification of the harmful compounds. Liver contains hepatocytes, a stroma of connective tissue, blood vessels and ducts within the stroma, sinusoids between plates of hepatocytes, a surrounding capsule of fibrous connective tissue & serous cover (Visceral Peritoneum) on the capsule. The classical structure & functional unit of the liver is the liver lobule. Across section of a lobule has the shape of polygon, usually a hexagon & the portal triads are typically found at the angles of the polygon. The central structure of the lobule, transverse its long axis, is the central vein.

The liver of the male mice Mus musculus (exposed to Paraquat for 30 days revealed the congestion of hepatic blood vessel sinusoids & vascular degeneration of hepatic cells with nuclear changes. In some areas the lesion were diffused & the Hepatic cells showed dissociation, disorganisation, mononuclear cell infiltration, enlargement of the veins, increasing in the number of kupffer cells & cytoplasm vacuolization similar results were observed by (Binu Kumar et al., 2010). After dichlorvos exposure characterised by vacuolization of hepatic cells, picnosis nucleus. In addition, liver dysfunction may be due to disorder in the control of intra cellular calcium homeostasis & also may be toxic material reached to the liver via the gastro intestinal tract blood supply.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>Treated with Paraquat</th>
<th>Paraquat+Ginger</th>
<th>Ginger</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial weight</td>
<td>28.66±1.6</td>
<td>28.96±1.2</td>
<td>28.10±1.7</td>
<td>28.72±1.5</td>
</tr>
<tr>
<td>After 30 days</td>
<td>30.23±0.94</td>
<td>24.30±0.87*</td>
<td>26.33±0.44</td>
<td>31.03±0.44</td>
</tr>
</tbody>
</table>

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

Table 2: Hepatic Glutamate Pyruvate Transaminase (IU/gm) of male mice Mus musculus after the exposure of Paraquat and Control upto 30 days.

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>After 30 days</td>
<td>5.79±0.23</td>
<td>9.14±0.45***</td>
<td>7.10±0.37**</td>
<td>5.02±0.20</td>
</tr>
</tbody>
</table>
Table 3: Hepatic Glutamate Oxaloacetate transaminase (IU/gm) of male mice *Mus musculus* after the exposure of Paraquat and Control upto 30 days.

<table>
<thead>
<tr>
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<th>Ginger</th>
</tr>
</thead>
<tbody>
<tr>
<td>After 30 days</td>
<td>142.85±13.02</td>
<td>140.22±9.32</td>
<td>141.26±10.21</td>
<td>144.15±12.11</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of 5 animals.

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

** More Significant different (P<0.05) from control vs experimental by student’s ‘t’ test.
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</tr>
</thead>
<tbody>
<tr>
<td>After 30 days</td>
<td>5.39±0.22</td>
<td>8.92±0.14***</td>
<td>6.88±0.36**</td>
<td>5.12±0.19</td>
</tr>
</tbody>
</table>

Table 4: Hepatic Acid Phosphatase (IU/gm) of male mice Mus musculus after the exposure of Paraquat and Control upto 30 days.

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

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<th>Treated with Paraquat</th>
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<th>Ginger</th>
</tr>
</thead>
<tbody>
<tr>
<td>After 30 days</td>
<td>6.40±0.52</td>
<td>4.66±0.41***</td>
<td>5.22±0.32**</td>
<td>6.86±0.49</td>
</tr>
</tbody>
</table>

Table 5: Hepatic Alkaline Phosphatase (IU/gm) of male mice Mus musculus after the exposure of Paraquat and Control upto 30 days.

Values are mean ± SEM of 5 animals.
*Significant different (P<0.05) from control vs experimental by student’s ‘t’ test.
**More Significant different (P<0.05) from control vs experimental by student’s ‘t’ test.
Table 6: Hepatic Protein (mg/gram) of male mice *Mus musculus* after the exposure of Paraquat and Control upto 30 days.

<table>
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<tr>
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<th>Ginger</th>
</tr>
</thead>
<tbody>
<tr>
<td>After 30 days</td>
<td>180±7.32</td>
<td>124±5.28***</td>
<td>156±4.72*</td>
<td>186±7.84</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of 5 animals.

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

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

Table 7: Creatinine level (mg/gram) in liver of male mice *Mus musculus* after the exposure of Paraquat and Control upto 30 days.

<table>
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</tr>
</thead>
<tbody>
<tr>
<td>After 30 days</td>
<td>1.002±0.12</td>
<td>2.011±0.22</td>
<td>1.650±0.18</td>
<td>0.821±0.14</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of 5 animals.

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

**More Significant different (P<0.05) from control vs experimental by student’s ‘t’ test.
Transverse Sections of liver (400x)

Fig.1: Control
Fig.2: Paraquat Treated
Fig.3: Ginger + Paraquat
Fig.4: Ginger

Explanation of liver sections:

Fig:- (1) Enlarged view of the normal sections of Control mice Mus musculus showing normal features of hepatocyte structure with prominent and spherical nuclei contained cytoplasmic materials. Small bile duct is surrounded by hepatocyte (H & E 400x).

Fig:- (2) Liver examined after paraquat effect (H & E 400x) up to 30 days showing vacuolated cells with enlarged nuclei and less amount of cytoplasmic materials (arrows). Few pycknotic nuclei are also seen (arrowheads).

Fig:- (3) Liver examined after paraquat and ginger exposure (H & E 400x) upto 30 days showing slight recovery in the areas of necrosis congestion in portal veins and in vacuolated cells.

Fig:- (4) Section of liver examined after ginger exposure of mice Mus musculus showing normal structure of hepatocytes, endothelial lining of the central vein and sinusoidal spaces (H & E 400x).

5. Discussion

Herbicides are used to control organisms considered harmful to us and in agriculture as a clause of chemicals. But it includes harm to human health, water contamination deaths of pollinators, beneficial natural predators, fishes and birds. Other casts include lost of wildlife habitat, soil erosion and the cost of disposal & cleanup of hazardous wastes generated by herbicide manufacturing. Hazards depend on the toxicity of herbicide and the exposure a human will receive in any situation.

Paraquat is a toxic chemical that is widely used as herbicide (plant killer), primarily for weed and grass control. The major routes of exposure to Paraquat are through the skin or from inhalation. It is very harmful to insects, birds, aquatic organisms and human beings. Cells contain enzymes that are necessary to their function. When the integrity of the cell is disrupted, enzymes escape into plasma/serum, were their activity can be measured as a useful index of cell integrity. Modifications in enzyme activity occur by cell death, increase or decrease enzyme production, increased cell membrane permeability or impart circulation. Mice are sensitive to enzymatic and hormone disrupters. Doses of herbicides that are not high enough to kill mice are associated with changes in behaviour and physiology that impart both survival and reproduction. The paraquat is extremely toxic to mice and causes many adverse effects in them. Mice have almost similar genetic makeup like that of Human being. Therefore investigation of the effects of herbicides on mice has a diagnostic significance in evaluation of adverse effect of
Phosphatase (ALP) is a biological energy demand of animals and potential to maintain and repair body tissues. In the event that protein in the diet is not sufficient, amino acids are commonly known. Protein has a critical role as the building blocks of proteins. There are about 20 different amino acids which are called the amino acids. These units are the amino acids which are called the building blocks of proteins. There are about 20 different amino acids which are called the building blocks of proteins. In the event that protein intake 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.

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.

Acid phosphatase (ACP) is a type of enzyme, used to free attached phosphate groups from other molecules during digestion. It is basically phosphomonoesterase. It is stored in lysosomes and functions when these fuse with endosomes which are acidified while they function. Acid phosphates belong to the class of enzymes called hydrolase and they are characterised by their ability to hydrolyse a large variety of organic phosphate esters with formation of alcohol and a phosphate ion alteration in the enzyme is due to adverse effect of xenobiotic on the cells and its organelles. Different forms of acid phosphatase are found in different organs and their serum levels are used as a diagnostic tool for disease in the corresponding organs. Alkaline phosphatase (ALP) is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules including nucleotides, proteins and alkaloids. The process of removing the phosphate group is called dephosphorylation. As the name suggests, alkaline phosphatases are most effective in an alkaline environment. In humans, alkaline phosphatase is present in all tissues throughout the entire body. It is sometimes used synonymously as basic phosphatase.

Liver is the richest source of both GOT & GPT enzymes. Any damage to the Liver cells will result in the increase of both these enzymes (Cole & Bradley, 1973). The increase in quality usually reflects the severity of hepatic damage (Ginsberg, 1970). Any type of hepatocellular or biliary disease can lead to increase in GOT or GPT levels.

In our experiment significant increase were observed in ALP, ACP and GPT activities in the liver of male mice Mus musculus, while there was a slight decrease in GOT after paraquat exposure for 30 days. This increase/decrease was duration dependant. Our results inferred with the findings of Atef, (2005). Who also reported significant elevations of ALP after exposure to Paraquat 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 intake 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).

In our study it was observed that Liver of animals treated with Paraquat for 30 days exhibited a distinct histological difference when compared with control. Liver is one of the main organs of the body and is located in the right side of the abdominal cavity. The main role of liver is detoxification of the harmful compounds. Liver contains hepatosites, a stroma of connective tissue, blood vessels and ducts within the stroma, sinusoids between plates of hepatocytes, a surrounding capsule of fibrous connective tissue & serous cover (Visceral Peritoneum) on the capsule. The classical structure & functional unit of the liver is the liver lobule. Across section of a lobule has the shape of polygon, usually a hexagon & the portal triads are typically found at the angles of the polygon. The central structure of the lobule, transverse its long axis, is the central vein.

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Concerning the effect of ginger, present study indicated that ginger improved the histological and histochemical alterations induced by Paraquat in Liver of mice. Similary, Sakr & Badawy, (2011), reported that ginger extract improved liver damage induced by metiram fungicide in mice. Siddaraju
& Dharmesh, (2007), 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 liver damage induced by Paraquat may be attributed to its antioxidant properties.

6. 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). 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 liver 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 histochemical alterations induced by Paraquat in liver of mice. Thus it is concluded from the present study that the protective effect of ginger extract against liver damage induced by Paraquat may be attributed to its antioxidant properties.

References

