Zagazig J. Pharm. Sci., December 2004
Vol. 13, No. 2, pp. 1-5

ISSN 1110-5089

# PROTECTIVE EFFECT OF DIETARY SOYBEAN IN PARAQUAT-INDUCED OXIDATIVE STRESS IN RATS

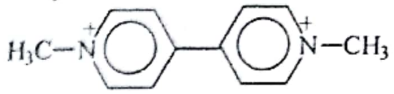
Mohamed Abd El-Hady Attia Sharaf
Biochemistry Department - Zagazig University Hospitals – Zagazig - Egypt

## ABSTRACT

Dietary soybean has been reported to possess many of health benefits. Soybeans were found to contain potential antioxidants. The effect of soybean on paraquat-induced oxidative stress was investigated in rats. Four groups of male Albino rats were fed for two weeks the experimental diet which contained casein as control group and soybean dietary groups. Two groups of the treated rats were supplemented with 0.025% paraquat for induction of oxidative stress. Soybean diet showed significant decrease in serum and hepatic Malondialdelyde (MDA) as an index of lipid peroxidation and oxidative stress. Also there were significant decrease in total serum cholesterol and triacylglycerols. Moreover, soybean diet showed an increase in blood glutathione peroxidase GSH-PX, hepatic glutathione (GSH) and catalase (CAT) levels, besides an inhibition of blood superoxide dismutase (SOD). So, the present data confirmed that, dietary soybean affected the antioxidant status of the body and reduced paraquat induced oxidative stress.

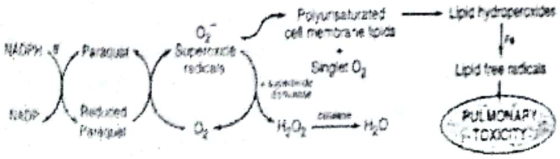
## INTRODUCTION

Paraquat-1,1'-dimethyl-4,4'-bipyridilium dichloride (PQ) is widely used as herbicide.



Several hundred cases of accidental or suicidal fatalities from paraquat poisoning have been reported during the past decade. Pathological changes observed at autopsy are indicative damage to the lungs, liver and kidneys; myocardities is may also present. The most striking pathological changes is a widespread proliferation of fibroblastic cells in the lungs, an effect that is not dependent on the route of administration. The onset of respiratory symptoms and eventual death by respiratory distress may be delayed for several days[1].

Paraquat produces oxidative stress and the biochemical mechanisms for this effect has been proposed, it is believed that paraquat may undergoes single electron cyclic reduction oxidation with subsequent formation of superoxide anion radical ($O_2^-$), through reduction of molecular oxygen in a reaction catalyzed by NADPH oxidase[2].




**Proposed mechanism of paraquat-induced pulmonary toxicity**

Superoxide radical ($O_2^-$) is nonenzymatically transformed to singlet oxygen, which attacks polyunsaturated lipids within cell membranes to form lipid hydroperoxides. These unstable lipid hydroperoxide in the presence of trace amounts of transition metal ions such as iron, are decomposed to give lipid-free radicals which lead to initiation of the chain reactions in lipid-peroxidation cycle. The free radicals produced by paraquat can attack membrane lipid leading to the destruction of alveolar cells, invasion of the space by fibroblasts, loss of pulmonary elasticity, and inefficient gas ($O_2$, $CO_2$) exchange ensue[3].

Paraquat is still used in some 130 countries and considered to be the one of the most specific pulmonary toxicants known. A high mortality rate is encountered in poisonings. The signs and symptoms include lethargy, hypoxia, dyspnea, tachycardia, hyperpnea, adipsia, diarrhea, ataxia, hyperexcitability and convulsions. Necropsy of exposed animals reveals hemorrhagic and edematous lungs, pulmonary fibrosis centrilobular hepatic necrosic and renal tubular necrosis. Lung weights increase significantly despite marked losses in body weight. The same histopathologic picture of pulmonary lesions is observed in mice, rats, dogs and humans[3].

The ingestion of commercial paraquat concentrates is invariably fatal and runs a time course of 3 to 4 weeks. The toxic effects of paraquat include initial irritation and burning of mouth and throat, severe gastroenteritis, with esophageal and gastric lesions, abdominal and substernal chest pains, and bloody stools. These effects leads to dyspnea, anoxia, opacity in the lungs seen in chest X-rays. Progressive fibrosis, coma and death also occurred. Moreover, paraquat also induces multiorgan toxicity with necrotic damage to the liver, kidneys, and myocardial muscle, plus extensive hemorrhagic incidents throughout the body[3].

In recent years, the importance of naturally occurring phytochemicals contained in food has been observed. Soybeans are major source of polyphenolic compounds called isoflavones which are hydrolyzed after ingestion to release genistein and diadzein. These isolfavones can be metabolized by bacteria to form isolfavone equol, which, have shown heath protective effects due to their antioxidant properties. Moreover, other compounds with antioxidative capacity in soybeans are tocol, phospholipids, pigments, amino acid and phytic acid.

Dietary soybean has been shown to exhibit hypocholesterolemic[4] and anticarcinogenic effects[5] and to regulate polyunsaturated fatty acid metabolism[6-8].

The amino acid composition of soybean protein, specific soy peptides, soy isoflavones and soy saponins have been proposed as factors responsible for hypocholesterolemic effects of soybean[9].



Scanned with CamScanner

In addition to its hypocholesterolemic effects, the antioxidative activity of soy protein has been investigated. Rats fed a 20% soy been powder were found to have lower concentrations of plasma thiobarbituric acid reactive substances than rats fed a 20% caseion diet. Soybean intake has been reported to inhibit oxidature modification of LDL in vitro[10].

Moreover, soybean diet ameliorated protein urea, hyper-holesterolemia and the increase in serum creatinine and blood urea nitrogen observed in nephrotic rats. The protective effect of 20% soyprotein diet on renal damage in chronic nephropathy was associated with the amelioration in the renal nitrotyrosine formation[11].

The present study was investigated to clarify the antioxidant effect of soybean constituents in paraquat-induced oxidative stress in rats.

## MATERIALS AND METHODS

**Materials:**

Soybean seeds was brought from the Agricutlural research center Giza Egypt. The seeds were dried in water bath at 80°C for 15 min., and grinded into fine powder. Paraquat was purchased from sigma chemical Co (ST. Louis, Mo). All other dietary constituents were purchased from commercial sources.

**Experimental designs:**

Twenty growing 4-weeks old male Albino rats weight (55-65 g) from the Animal House of Faculty of Medicine, Zagazig University were. The rats were allowed free access to water and the experimental diet for 3 days to allow acclimation to these conditions. The rats were housed individually in stainless steel cage with 12h light/dark cycle at a temperature of 22-25°C and relative humidity at 50-55%. These rats were then divided into four groups (5 rats in each).

Group I: Control rats fed on control diet containing casein as a nitrogen source.

Group II: Rats fed on control diet containing casein and paraquat (0.025%).

Group III: Rats fed on soybean powder which contain soy protein (25%) as nitrogen sources.

Group IV: Rats fed soy bean powder (25%) and paraquat (0.025%).

The composition of the experimental diets are shown in table (I) according to[12] and the treatment was carried out for 2 weeks.

**Table (1):** Composition of the experimental diets.

| | Casein G I | Casein + paraquat G II | Soybean G III | Soybean + paraquat G IV |
|---|---|---|---|---|
| Casein | 20 | 20 | - | - |
| Soybean | - | - | 25 | 25 |
| Methionine | - | - | 0.33 | 0.33 |
| Corn oil | 5 | 5 | 5 | 5 |
| Corn starch | 43.67 | 43.67 | 39.45 | 39.43 |
| Sucrose | 21.83 | 21.83 | 20.72 | 20.72 |
| Cellulose | 5 | 5 | 5 | 5 |
| Vitamin mix | 1 | 1 | 1 | 1 |
| Mineral mix | 3.5 | 3.5 | 3.5 | 3.5 |
| Paraquat | - | 0.025 | - | 0.025 |

The first day of treatment, every rat had been given 8 gm of the experimental diet and then increased by 0.5 gm each day. All rats drank water and libitum.

**Biochemical Analysis:**

At the end of the experimental period, the rats were fasting over night, anesthetized with diethyl ether and sacrificed by cervical dislocation and the blood samples were collected in heparinized and non heparinized dry tubes. The heparanized blood was centrifuged at 3500 rpm and the plasma was used for estimation of erythrocytes superoxide diamutase (SOD) and glutathione peroxidase (GSH PX). In saline washed RBCs the SOD was determined using the method of Winteibourn[13]. Glutathione peroxidase GSH-PX was determined according to the method of Paglia and Valentine[14]. We used GSH-PX and SOD Kits which were provided from Randox. The non heperanized blood was allowed for 1h and centrifuged at 3000 rpm for 15 min and the serum obtained was used for estimation of lipid peroxidation as Malondialdelhyde (MDA) using the method as described previously[15] and determination of serum Triacylglycerols and total cholesterol. Triacylglycerols was determined according to the method of Fossati and Principle[16] and total cholesterol was determined according to the method of Deeg and Zeigenohm[17].

Immediately after sacrificing rats, livers were excised plotted free of adhering blood, washed with cold saline. One portion of the liver (500 mg) was homogenized in 1.15% KCl and used for determination of MDA according to the method of uchinyama and Mihara[18]. A second portion of the liver was homogenized in M/15 phosphate buffer pH 7.0 and used for determination of catalase (Maehly and Chance)[19]. The third portion of liver was homogenized in 3% ice cold metaphsphoric acid and used for determination of GSH according to the method of Baulter et al[20]. The protein content of liver was estimated by the method of Lawry et al.[21]

**Statistical analysis:**

The data were checked, entered and analysed by using SPSS version 11.0. Data were expressed as means±SEM. ANOVA tests were used for comparison and the least significant difference (LSD) were calculated and $P<0.05$ was considered significant..

## RESULTS

In view of our data (Table 2), following administration of paraquat (group II), Malondialdehyde (MDA) as an index of lipid peroxidation, was significantly elevated in both serum and liver of this group ($P<0.001$). Soybean supplementation (as in group III and IV) caused a significant decrease in MDA levels compared with casein and paraquat fed group (group II).

Also, there were significant increases in serum total cholesterol and triacylglycerols with paraquat fed rats (group II) and returned to normal levels with soybean supplementation ($P<0.001$). In addition, paraquat fed rats showed elevation of erythrocytes SOD activities which returned to the normal values with soybean feeding (group III, IV).

In addition, there were inhibition and decreases in both hepatic GSH and catalase activities with paraquat fed rats (as in group II). Soybean supplementation could restored to normal, and increased the levels of there two enzymes respectively as shown in our data Table (2) as in group III & IV.



Scanned with CamScanner

**Table (2):** Effect of dietary soybean on serum and hepatic Malondialdehyde and blood total cholesterol, triglycerides, superoxide dismutase, glutathione peroxidase and hepatic glutathione and calalase levels in paraquat-induced oxidative stress in rats (Mean ± SEM).

| | Casein G I | Casein + paraquat G II | Soybean G III | soybean + paraquat G IV |
|---|---|---|---|---|
| 1- Serum MDA µmol/L | 1.76±0.5 | 2.32±0.6[a] | 1.42±0.6[ab] | 1.68±0.4[abc] |
| 2- Liver MDA nmol/g liver | 55.77±5.5 | 81.41±6.3[a] | 51.24±2.5[ab] | 61.43±5.9[abc] |
| 3- Serum total cholesterol mg/dl | 87.37±6.3 | 112.28±8.9[a] | 74.52±3.5[ab] | 100.94±8.7[abc] |
| 4- Serum triacylglycerol mg/dl | 80.62±4.4 | 96.46±3.2[a] | 50.67±5.8[ab] | 81.52±12.2[abc] |
| 5- Plasma SOD u/g.HB | 2100.±27.39 | 2405±13.04[a] | 1993±3.74[ab] | 2110±50.99[abc] |
| 6- Plasma GSH-PX u/g.HB | 61.87±5.7 | 51.34±2.5[a] | 82.59±8.2[ab] | 74.70±5.1[abc] |
| 7- Liver GSH mg/gm liver | 1.44±0.2 | 1.14±0.5[a] | 1.70±0.3[ab] | 1.18±0.4[abc] |
| 8- Liver catalase u/mg protein | 3028.33±28.92 | 2198.16±25.98[a] | 3388±37.4[ab] | 2604.60±51.2[abc] |

**(a) Significant difference from casein control (b) Significant difference from casein + paraquat**
**(c) Significant difference from the coroesponding group fed soybean and soybean with paraquat**
**P<0.001**

## DISCUSSION

Soy foods are recognized to contain potential antioxidants. The antioxidants properties of soybean have been studied by a number of experiments both in vivo and in vitro[22-24]. Therefore the antioxidant properties of soybean provides an additional mechanism by which consumption of soy foods most responsible for many of the suggested health benefits[25]. Also in a series of previous studies, the preventive effect of polyphenols constituents of soybean on paraquat-induced oxidative stress using these variables has been reported[26-28].

The antioxidant activity of soybean itself can potentially be explained by its amino acid composition, mainly soy peptides, L-Arginine which is mostly abundant in soy protein than in Casein[29]. Suetsuna et al.[30], investigated the antioxidative activity of soybean hydrolysate and reported two soy peptides with strong antioxiative activity. In addition, histidine containing peptides from soybean has been reported[31].

Many in vivo studies of the antioxidative effects of soybean peptides and soy aminoacids against paraquat-induced oxidative stress were carrid out. MDA was measured as an indicator of lipid peroxidation and oxidative stress. In our study, there were significant increase in both serum and hepatic MDA levels. In addition, the antioxidant enzymatic defense systems were studied. The paraquat fed rats showed a significant decrease in hepatic catalase, GSH and plasma GSH-PX levels, however erythrocytes SOD was elevated. These toxic effects of paraquat were improved and regulated with soybeans supplementation. It was found that, the inhibition of hepatic catalase activity was due to liberation of superoxide anions[32].

Also the increase in SOD activity might be due to increased absorption of cupper and zinc[33,34]. It well known that both zinc and copper are trace elements which are essential of cell function and act as structural elements in biological macromolecules. Moreover, soybeans have potent hypocholesterolemic activities besides lowering of triacylglycerol levels. The exact mechanisms of regulating cholesterol homeostasis with soybeans was found to be multifactorial and include, up regulating LDL-receptor activity[25], increasing fecal bile acid excretion, ultering the bile acid synthesis and increased hepatic cholesterol secretion[35]. Also soybeans has been suppressed the convertion of linoleic acid to arachidonic acid in rat liver. Also, the phytate content in soybeans played a limited role in the cholesterol-lowering effect[36].

Recently, it was found that, dietary soyprotein isolate and isoflavones modulate hepatic thyroid hormone receptors in rats. The content of thyroid receptors TRbetal protein present in the liver, was markedly increased by dietary soyprotein in both sexes compared with casein. So, the thyroid receptor TRbetal may play a role in mediating the hypocholestero-lemic and lipid lowering actions of soyproteins[37].

In conclusion: consumption of soybean appears to be beneficially affecting and reducing paraquat induced oxidative stress in rats. We can recommend that we should consume whole foods containing naturally occurring phytochemicals such as soybean, in fact to ameliorate the oxidative status due to contamination with some herbicides as paraquat.

## REFERENCES

1. Goodman L.S., Hardman J.G., Limberd L.L. and Gilman A.G.: Goodman and Gilman's The Pharmacological Basis of Therapeutics. 10th ed.; New York, McGrow-Hill (2001) p.1895-1897.
2. Bus, J.S., Cagen, S.Z., Olgaard, M. and Gibson, J.E.; **Toxicol. Appl. Pharmacol.**; 35, 501-513 (1976).
3. Klaassen, C.D. and Watkins, J.B.; Essentials of toxicology; New York, McGrow-Hill, (2003) p. 344.



Scanned with CamScanner

4. Carroll, K.K. and Kurosawa, E.M.; **J. Nutr.**, 125: 594 S – 597 S (1995).
5. Messina, M.; **Nutr. Cancer**; 21: 133-131 (1994).
6. Madani, S., Lopez, S., Blond, J.P., Prost, J. and Belleville, J.; **J. Nutr.**; 128: 1084-1091 (1998).
7. Koba, K., Wakamatsu, K., Obata, K. and Sugans, M.; **Lipid**; 28: 457-464 (1993).
8. Chol, Y.S., Goto, S., Ikeda, I. & Sugano, M.; **Br. J. Nutr.**; 61: 531-543 (1989).
9. Potter, S.M.; **J. Nutr.**; 125: 606 S – 611 S (1995).
10. Madani, S., Prost, J. and Belleville, J.; **Nutrition**; 16: 368-375 (2000).
11. Tovar, A.R., Pedraza-Chaverri, J., Barrera, D., et al.; **Life Sci.**, Jan 9; 74(8): 987-99 (2004).
12. Aoki, H., Takenaka, A., Gtaka, Y. and Igarashi, K.; **J. Nutr.**; 132: 2258-2262 (2002).
13. Winterbourn, C.C., Hawkins, R.E., Brain, M. and Carell, R.W.; **J. Lab. Clin. Med.**, 85: 337-341 (1975).
14. Paglia, D.E. and Valentine, W.N.; **J. Lab. Clin. Med.**; 70: 158-169 (1967).
15. Yoshioko, T.N., Kawada, T., Shimoda, M. and Mori.; **Am. J. Obst. Cynecol.**; 135: 372-376 (1979).
16. Fossati, P., Principle, L. ; **Clin. Chem.**; 28: 2077 (1982).
17. Deeg, R. and Ziegenohm, J.; **Clin. Chem.**; 28: 1574 (1982).
18. Uchiyama, M. and Mihara, M.; **An. Biochemistry**; 86: 271-278 (1978).
19. Maehly, A.C. and Chance, B.; The assay of catalase and peroxides in: "Methods of Biochemical analysis" Glick, D (edr): vol 1, interscience publishers, Inc, New York, PP. 357-424 (1954).
20. Beutler, E., Duron, O. and Kelly, B.M.; **J. Lab. Chin. Med.**; 61: 882-888 (1963).
21. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.; **J. Biol. Chem.**; 193: 265-275 (1951).
22. Tikkanen, J.J., Wahala, K., Ojala, S. et al.; **Proc. Natl. Acad. Sci.**; 95: 3106-3110 (1998).
23. Wei, H., Bowen, R., Cai, Q. et al.; **Proc. Soc. Exp. Biol. Med.**; 208: 124-130 (1995).
24. Jenkins, D.J., Kendall, C.W., Garsetti, M. et al.; **Metabolism**; 49: 537-543 (2000).
25. Setchell, K.D.R., Cassidy, A.; **J. Nutr.**; 129: 758 S – 767 S (suppl) (1999).
26. Ando, N., Igarashi, K., Takenaka, A. and Hara, Y.; **Foods Sci. Technol.**, Res; 6: 146-149 (2000).
27. Igarashi, K., Kimara, Y. and Takenaka, A.; **Biosc. Biotechnol. Biochem.**; 64: 1600-1607 (2000).
28. Tsuchiya, T., Suzuki, O. and Igarashi, K.; **Biosci. Biotechnol. Biochem.**; 60: 765-768 (1996).
29. Wallner, S., Hermetter, A., Mayer, B. and Wascher, T.C.; **Eur. J. Clin invest.**; 31: 98-102 (2001).
30. Suetsuna, K.; **J. Jpn. Soc. Nutr. Food. Sci.**; 52: 225-228 (1999).
31. Chen, H.M., Muramoto, K., Yamauchi, F., Fujimoto, K. and Noklhara, K.; **J. Agric. Food Chem.**; 46: 49-53 (1998).
32. Kono, Y. and Fridovich, I.; **J. Biol. Chem.**; 257: 2751-2754 (1982).
33. Kakkar, R., Mantha, S.V., Radhi, J. and Prasad, K.; **Clinical Science**; 94: 623-632 (1998).
34. Craft, N.E. and Failla, M.L.; **Am. J. Physiol.**; 244: E 122-E128 (1983).
35. Duane, W.; **J. Lipid. Res.**; 38: 1120-1128 (1997).
36. Huang, Y.S., Koba, K., Liu, J.W., Bobik, E.Jr., Mills, D.E. and Sugano, M.; **Biosci. Biotechnol. Biochem. Jan.**; 67 (1): 15-22 (2003).
37. PapademeTriou, S.A., Xiao, C.W., L'Abbe, M.R., Gilani, G.S., Cooke, G.M. and Curran, I.H.; **J. Nutri.**, Apr; 134 (4): 743-9 (2004).


**Received: Oct. 3, 2004**
**Accepted : Dec. 2, 2004**

Scanned with CamScanner

Zagazig J. Pharm. Sci., December 2004
Vol. 13, No. 2, pp. 1-5

# التأثير الواقى لحبوب فول الصويا من التوتر المؤكسد والمستحدث بمادة الباراكوات فى جرذان التجارب

د. محمد عبد الهادى عطية شرف

قسم الكيمياء الحيوية – مستشفيات جامعة الزقازيق – الزقازيق – مصر

تحتوي حبوب الصويا علي كثير من الفوائد الصحية، فهي تحتوي علي كثير من المواد الفعالة والمضادة للأكسدة.

ويهدف هذا البحث علي دراسة تأثير التغذية بفول الصويا كمضاد للأكسدة مقارنة بالكازين على التوتر المؤكسد الناجم من مضادات الأعشاب وهو الباركوات Paraquat فى ذكور الجرذان البيضاء.

وقد شملت الدراسة ٢٠ من ذكور الجرذان البيضاء، حيث قسمت إلى أربعة مجموعات:

* مجموعتان تتغذيان علي مطحون فول الصويا إلا أن واحدة تتغذى على الصويا فقط والأخري على الصويا مضافاً إليها مضاد العشب Paraquat باركوات بتركيز ٠,٠٢٥% لمدة أسبوعين.
* والمجموعتان الأخريان تتغذيان علي الكازين كمصدر للنيتروجين إلا أن واحدة تتغدي على الكازين فقط والأخري علي الكازين مع مضاد العشب Paraquat بتركيز ٠,٠٢٥%.

وقد أوضحت النتائج أن مطحون فول الصويا قلل من مستوي أكسدة الدهون والمتمثل في انخفاض مستوي المالون داي الدهيد (MDA) كمعيار للأكسدة في كلٍ من الكبد والدم مع انخفاض نسبة كلٍ من الدهون الثلاثية والكوليسترول في الدم إنخفاضاً جوهرياً. كما انخفض أيضا نشاط إنزيم سوبر أوكسيد ديسميوتيز SOD بالدم بينما زاد نشاط كلٍ من الإنزيم المؤكسد للجلوتاثيون PX (GSH-PX) والجلوتاثيون بالكبد (GSH).

تشير هذه النتائج إلى تأثير فول الصويا كمضاد للأكسدة الناجمة عن استعمال بعض مضادات الأعشاب مثل مادة الباركوات Paraquat.

Scanned with CamScanner