

ORIGINAL ARTICLE

pISSN: 1907-3062 / eISSN: 2407-2230

Nephroprotective effect of soy (*Glycine Max (L.) Merr.*) flour against diazinon-induced renal toxicity in rats

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ABSTRACT

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Date of first submission, June 9, 2020

Date of final revised submission,

December 8, 2020

Date of acceptance, December 10,

2020

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Cite this article as: Wisudanti DD, Normasari R, Herdiana F. Nephroprotective effect of soy (*Glycine Max (L.) Merr.*) flour against diazinon-induced renal toxicity in rats. Univ Med 2020;39:192-8. doi: 10.18051/UnivMed.2020.v39.192-198

BACKGROUND

Diazinon is a widely used pesticide to eradicate agricultural pests globally with the potential to cause harmful effects on humans by generating free radicals and depleting endogenous antioxidants. Soy flour possesses high antioxidant effect. This study evaluated the nephroprotective effect of soy flour on diazinon-induced nephrotoxicity in rats.

METHODS

An experimental laboratory study was carried out involving 30 male Wistar rats were randomly divided into five groups of 6 rats. The normal control (Kn) and the negative control (K-) groups received normal saline, the treatment groups (K1, K2, K3) received 10%, 15%, and 20% soy flour in distilled water, respectively, on day 1 to day 28. Subsequently, for five days, all groups received diazinon 40 mg/kg, except for the normal control group. Blood urea nitrogen (BUN) and creatinine levels were measured at the end of the study. The Kruskal Wallis test with the post hoc Mann Whitney U test were used to analyze the data.

RESULTS

Blood urea nitrogen and creatinine levels were lower in all treatment groups (K1, K2, K3) compared to the negative control group (K-), while the K3 level was the lowest (24.28 ± 1.56 mg/dL and 0.93 ± 0.14 mg/dL). There was a significant decrease in BUN and creatinine levels in the experimental rats of the 15% and 20% soy flour groups (K2, K3) compared to the negative control group (K-).

CONCLUSION

Administration of soy flour (*Glycine max (L.) Merr.*) had a nephroprotective effect in preventing increases in BUN and creatinine levels of diazinon-induced nephrotoxic male Wistar rats.

Keywords: Antioxidant, kidney, pesticide, functional food, BUN, creatinine, rats



INTRODUCTION

Organophosphate pesticides are a type of pesticide that is widely used in Indonesia and is favored by farmers because it decomposes faster than the other groups of pesticides, and has a fast and significant power in repelling pests.⁽¹⁾ One of the most widely used organophosphates is diazinon. It has been known that diazinon can be decomposed into other derivative forms, leaves residues in the environment,⁽²⁾ and has a high level of toxicity.⁽³⁾ Diazinon was reported to be able to inhibit acetylcholinesterase activity and other organic functions in animals.^(4,5) The kidney is one of the organs affected because of its role in the excretion of diazinon metabolites, as evidenced by several animal studies that found an alteration in blood urea nitrogen (BUN) and creatinine levels, as well as histopathological changes in rat kidneys.^(4,5) In addition to reactive oxygen species (ROS) accumulation, diazinon can cause deoxyribonucleic acid (DNA) and cell damage through the apoptotic pathway.⁽⁶⁾

Continuous exposure to diazinon can affect kidney function,⁽⁷⁾ therefore a substance that can inhibit ROS activity and cell apoptosis in the kidney is needed. Isoflavone is a class of flavonoid that has been known as an antioxidant that can inhibit cell oxidation reactions.⁽⁸⁾ Soy (*Glycine max* (L.) Merr.) has a high isoflavone content, which has been proven to reduce the concentration of ROS and malondialdehyde (MDA), as well as to activate superoxide dismutase (SOD) and glutathione peroxidase (GPx) in several organs.⁽⁹⁾ In addition, a previous study has proven that the soy isoflavones, namely daidzein and genistein, also have anti-apoptotic properties.⁽¹⁰⁾

There was also a study showing that bread made from 10% soy-flour-enriched wheat flour had a better effect on the kidney of rats than bread without the addition of soy flour.⁽¹¹⁾ In this study, rats were not induced by substances that could damage the kidney, so a little addition of soy flour could have a good effect on the kidney.

Another study on the effect of soy flour on the liver has been conducted in rats induced by carbon tetrachloride (CCl₄),⁽¹²⁾ but only its effect on the liver was studied. The results showed a significant decrease in liver enzyme markers, but the value was still higher than in the normal group. The investigators used a wide dose range between treatment groups and a relatively short time of soy flour administration (7 days). Therefore, a longer administration period with a narrower dose range needs to be evaluated and assessed based on kidney function examination. The present study was aimed at evaluating the protective effect of soy flour against diazinon nephrotoxicity in rats.

METHODS

Research design

The study of experimental design was conducted in the Physiology and Pharmacology Laboratories, Faculty of Medicine, University of Jember, from October to December 2018.

Animals and treatment

Physically active adult male Wistar rats (*Rattus norvegicus*), around three months of age and weighing 150-300 g, were purchased from "Wistar Farm" in Malang, East Java, Indonesia. The rats were kept under constant conditions (temperature of 25 ± 2 °C and humidity of $60 \pm 5\%$) and were given rodent feed and water ad libitum. A total of 30 rats were involved in this study. The resource equation method⁽¹³⁾ was used to calculate the sample size, where the number of samples per group was 5 for this study. The final sample size after the addition of an estimated 10% of animal mortality due to diazinon toxicity was 6 animals per group.⁽¹³⁾ After acclimatization to the laboratory conditions, the animals were randomly divided into five groups (six rats in each): normal control group (Kn), negative control group (K-), and treatment groups (K1, K2, K3). On day 1 to day 28, the normal control group (Kn) and the negative control group (K-) received normal saline, and

the treatment groups (K1, K2, K3) received 10%, 15% and 20% soy flour in distilled water, respectively. On day 29 to day 33, all groups were given diazinon 40 mg/kg, except for the normal control group. All treatments were performed using a gastric tube and diazinon was administered to those groups dissolved in corn oil. On the 34th day, the animals were anesthetized, and blood samples were taken out by cardiac puncture to measure BUN and creatinine levels.

Soy flour sample

The soy flour was made from the Baluran variety of *Glycine max* (L.) Merr. and was determined at the Agronomy Laboratory, Faculty of Agriculture, University of Jember. The grinding method of soy flour used in this research was the wet method, carried out at the Agricultural Product Process Engineering Laboratory, Faculty of Agriculture, University of Jember. The grinding process was carried out on soy beans that had been soaked for 3 hours, then boiled for 5 minutes, drained, and sun-dried for 4 hours. The beans were then oven-dried at 50°C for 24 hours and ground to flour using a hammer mill, then sifted through an 80 mesh sieve. The sieving process was repeated twice to obtain an optimal quality of soy flour. Then the soy flour was mixed with distilled water to obtain concentrations of 10%, 15%, and 20%.

Measurement of BUN and creatinine levels

The blood samples were centrifuged for 15 minutes at 3500 rpm to separate serum from blood cells, then BUN and creatinine levels were determined using DiaSys® reagent kits, according to the manufacturer's instruction. BUN level was measured based on the method of Urease-GLDH (Glutamate dehydrogenase) enzymatic UV test, and creatinine level was determined by a non-deproteinized kinetic test using Jaffe's method. The solution was read using an Analyticon Biolyzer 100 that had been set to an incubation time of 2 minutes and a wavelength of 340 nm for measuring BUN level and a

wavelength of 492 nm to determine creatinine level. The BUN and creatinine serum levels were expressed in mg/dL units.⁽⁵⁾

Statistical analysis

Statistical analysis was performed using the SPSS 16 application for Windows software. Descriptive data were expressed as mean \pm standard deviation. In the case the data obtained were not normally distributed, the nonparametric Kruskal Wallis data analysis was used for the comparative test, followed by Post Hoc Mann Whitney U. All data were displayed as the means \pm standard deviation. The p-value <0.05 indicated the significance of the data.

Ethical clearance

All experimental protocols have been approved by the Ethics Committee of the Faculty of Medicine, University of Jember, No.1.268/H25.1.11/KE/2018.

RESULTS

The mean BUN and creatinine levels from the lowest to the highest were the Kn group of 24.09 ± 1.34 mg/dL and 0.87 ± 0.4 mg/dL, K3 24.28 ± 1.56 mg/dL and 0.93 ± 0.14 mg/dL, K2 24.88 ± 1.91 mg/dL and 1.1 ± 0.09 mg/dL, K1 26.45 ± 1.93 mg/dL and 1.27 ± 0.19 mg/dL, and K- 28.77 ± 3.27 mg/dL and 1.31 ± 0.13 mg/dL. The lowest mean BUN and creatinine levels were found in the normal control group (Kn), indicating the smallest damage to kidney function, while the highest mean BUN level was found in the negative control group (K-), indicating the most severe damage of kidney function. The mean BUN and creatinine levels can be seen in Table 1.

Statistical analysis was performed using the Kruskal Wallis test, and showed that the levels of BUN ($p=0.014$) and creatinine ($p=0.01$) in the five groups were significantly different. The test was followed by the Mann Whitney U post hoc test as shown in Table 2. The normal control group (Kn) showed significantly lower

Table 1. Distribution of mean BUN and creatinine levels by treatment groups after intervention

	Treatment groups					p value
	Kn (n=6)	K(-) (n=6)	K1 (n=6)	K2 (n=6)	K3 (n=6)	
BUN (mg/dL)	24.07 ± 1.34	28.76 ± 3.27	26.44 ± 1.93	24.88 ± 1.90	24.28 ± 1.56	0.014
Creatinine (mg/dL)	0.86 ± 0.39	1.30 ± 0.13	1.26 ± 0.18	1.10 ± 0.09	0.93 ± 0.14	0.011

Data presented as mean ± SD; BUN : blood urea nitrogen; Kn: control; K (-): negative control; K1: 10% soy flour; K2: 15% soy flour; K3: 20% soy flour

BUN and creatinine levels ($p < 0.05$) compared to the negative control group (K-). The levels of BUN and creatinine in the 15% and 20% soy flour supplementation groups (K2 and K3) decreased significantly when compared to the negative control group ($p < 0.05$). Between K2 and K3, there was a significant difference in creatinine level, but not in BUN level. This study showed that giving 20% soy flour was more effective in reducing creatinine level.

DISCUSSION

In our study, the levels of BUN and creatinine in the normal control group were significantly different from the negative control group. This indicates that the 40 mg/kg diazinon induction for five days was able to increase the mean BUN and creatinine levels. Diazinon can increase BUN and creatinine serum levels because it has a pro-oxidant effect that induces oxidative stress and lipid peroxidation in the kidney. The result of this research is in line with several previous studies,^(4,5,14) which proved that giving diazinon orally for several days in vivo can significantly increase the levels of BUN and creatinine. Shah and Iqbal proved that giving

diazinon 15 mg/kg orally for 8 weeks in mice can significantly increase BUN and creatinine levels.⁽¹⁴⁾ Other similar studies proved that giving diazinon 20 mg/kg orally for 2 weeks in experimental rabbits can significantly increase BUN and creatinine levels.⁽⁴⁾ Diazinon 40 mg/kg orally given for 5 days increased BUN and creatinine levels significantly.⁽⁵⁾ The results indicated that diazinon exposure could decrease renal glutathione, cause the depletion of antioxidant enzymes and the excessive production of oxidants.

Diazinon is absorbed from the gastrointestinal tract and metabolized rapidly. Diazinon induces biochemical and histopathological changes in several organs.^(4,5) One of the targeted organs is the kidney, which plays a major role in excretion. Diazinon causes formation of ROS and induces oxidative stress.⁽¹⁴⁾ As mitochondria play an important role in apoptosis, mitochondrial dysfunction by oxidative stress will release cytochrome C and activate caspase that leads to apoptotic cells.⁽¹⁰⁾ Diazinon exposure may cause toxic effects through inhibition of acetylcholinesterase (AChE) in the the nervous system.⁽¹⁵⁾ This inhibition causes ACh to remain at its receptors, which increase the

Table 2. The results of Mann Whitney U test of BUN and creatinine level

Treatment Groups	Kn		K-		K1		K2		K3	
	BUN	creatinine								
Kn			0.009	0.036	0.059	0.090	0.401	0.389	0.751	0.674
K-	0.009	0.036			0.175	0.523	0.012	0.008	0.012	0.009
K1	0.059	0.090	0.175	0.523			0.402	0.159	0.076	0.020
K2	0.401	0.389	0.012	0.008	0.402	0.159			0.462	0.033
K3	0.751	0.674	0.012	0.009	0.076	0.020	0.462	0.033		

Kn: control; K (-): negative control; K1: 10% soy flour; K2: 15% soy flour; K3: 20% soy flour

production of NO free radicals. The accumulated NO will be bound by GSH to GSNO, thereby reducing GSH levels. Decreasing levels of GSH result in free radicals such as hydroxyl radicals (OH \cdot), superoxide anion radicals (O $_2^{\cdot-}$), and peroxy radicals (ROO) that cannot be neutralized. These free radicals react with unsaturated fatty acids or polyunsaturated fatty acids (PUFAs) which are found in many cell membranes, resulting in lipid peroxidation.⁽¹⁶⁾

Diazinon can also interfere with the mitochondrial respiration pathway resulting in disruption of adenosine triphosphate (ATP) production in the electron transfer pathway in the mitochondria by inducing the BH3-only protein that activates allosteric Bax and Bak, which interferes with mitochondrial permeability.⁽¹⁷⁾ Impaired mitochondrial membrane permeability releases proapoptotic factors such as cytochrome c, second mitochondria-derived activator of caspase (SMAC), and apoptosis inducing factor (AIF), which can lead to kidney cell degeneration and decreased kidney function.⁽¹⁸⁾

Our study showed that soy flour supplementation at concentrations of 10%, 15%, and 20% was able to prevent the increase in mean BUN and creatinine levels in male Wistar rats induced by diazinon. However, BUN and creatinine levels of the 15% and 20% soy flour groups decreased significantly compared to the negative controls. This result was in accordance with a previous study on the nephroprotective effect of soy flour on the histopathological change in rat kidney induced by diazinon.⁽¹⁹⁾ The aforementioned study proved that soy flour supplementation at a dose of 1 g/mL, 1.5 g/mL and 2 g/mL for 28 days had a significantly lower histopathological score compared to the group without diazinon. The group on 2 g/mL soy flour had the lowest renal histopathological score, approaching the normal control group score.

In another study,⁽¹¹⁾ a diet of bread made from a mixture of wheat and soy flour at concentrations of 10%, 20%, 30%, and 40% for

28 days was able to prevent an increase in BUN and creatinine levels compared to bread made from pure wheat flour. In that research, the rats were not exposed to nephrotoxic substances, whereas in the present study, the 10% concentration and the negative control groups were not significantly different, indicating that the 10% concentration was not yet effective in preventing the increase in BUN and creatinine levels.

Decreasing BUN and creatinine levels occur due to the presence of isoflavone compounds, supported by vitamin E and other antioxidant substances such as phenol and linoleic acid found in soy flour.⁽¹¹⁾ These substances can prevent ROS accumulation, lipid peroxidation, and cell apoptosis. Soy flour is one of the types of processed soy containing high isoflavone levels.⁽²⁰⁾ Isoflavone has biochemical activities as antioxidants by suppressing free radical activity and donating electrons and hydrogen atoms to inhibit cell oxidation reactions.⁽⁸⁾ The two main isoflavones in soy are genistein and daidzein, which have antioxidant and anti-apoptotic activities by inhibiting the activity of Bak so that they can inhibit cell apoptotic pathways.⁽¹⁰⁾ Vitamin E has a role in protecting unsaturated fatty acids in cell membranes, and inhibits the lipid autoxidation process by forming relatively stable tocopheroxyl radicals.⁽²¹⁾

In this study, soy flour was given before diazinon induction to determine the effectiveness of soy flour protection against kidney damage before exposure to diazinon. Based on the results of this study, the prevention of increased BUN and creatinine levels occurred in the groups given soy flour. The results proved that the antioxidant and anti-apoptotic contents of soy flour could prevent diazinon-induced increases in BUN and creatinine levels. These results are in line with research on the nephroprotective effects of pomegranate (*Punica granatum*) seed oil⁽²²⁾ and juniper (*Juniperus procera*) leaf extract.⁽²³⁾ These studies showed that the antioxidant-rich plants acted as protective agents

for renal function by preventing increased BUN and serum creatinine levels in experimental animals induced by diazinon in rats and by thioacetamide in mice, respectively.

The administration of soy flour at concentrations of 15% and 20% showed a nephroprotective potential, as evidenced by the BUN and creatinine levels, which were statistically significant compared to the negative control approaching the normal control group. However, 20% soy flour supplementation significantly reduced creatinine level compared to the 15% soy flour group, but not the BUN levels, which were not significantly different between the 15% and 20% soy flour groups. These findings prove that administration of 20% soy flour is the most effective way to prevent the increase in creatinine levels induced by diazinon in rats.

The results show that soy flour intake provides significant benefits for rats with nephrotoxicity induced by diazinon. The research results will have a major impact on improving the nutritional status and health of the community through regular consumption of functional foods such as soy flour. Therefore, further research is needed to identify edible food forms that have a similar effect as potential nephroprotective agents.

The limitations of this study are the uncontrolled external factors that can affect BUN and creatinine levels. These factors include activity and diet. High activity with increased muscle mass will affect creatinine levels in the blood, as a result of the breakdown of phosphocreatine that occurs in skeletal muscles. Meanwhile, urea is excreted as a result of protein metabolism, so that its levels in the blood are influenced by protein consumption and protein catabolism. Overweight or obesity in rats can also affect kidney function, thereby affecting levels of BUN and serum creatinine.⁽²⁴⁾ Therefore, the use of rats in this study should be carried out in a weight range that is not too wide and varied. The difficulty in this study is that there is a rapid increase in rat body weight

during the adaptation period, so that the range of rat body weight is quite wide, namely 150-300 grams.

CONCLUSIONS

This study demonstrated that soy (*Glycine max* (L.) Merr.) flour has a nephroprotective effect on diazinon-induced nephrotoxicity in Wistar rats by preventing the increase in BUN and creatinine serum levels. Apart from that, it was also concluded that 20% of soy flour provided the best overall quality.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

ACKNOWLEDGMENT

The researchers would like to thank the Faculty of Medicine, University of Jember, for the opportunity given in this research.

CONTRIBUTORS

DDW contributed to the basic concept and design of the study. DDW and FH contributed to performing the experiment and data collection. FH contributed to data analysis. DDW, RN, FH contributed to implementation and manuscript development. All authors have read and approved the final manuscript. 

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