# The Potential Protective Effects of Diet Fortified with Mung Bean and Soybean Against Oxidative Stress Caused by High Fat Diet in Rats

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# The Potential Protective Effects of Diet Fortified with Mung Bean and Soybean Against Oxidative Stress Caused by High Fat Diet in Rats

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## Abstract

Hyperlipidemia is correlated with oxidative stress, and previous studies have demonstrated that legumes can protect from chronic diseases associated with oxidative stress. This study was carried out to determine the potential protective effects of diet fortified with Mung bean (*Vigna radiate*) and Soybean (*Glycine max*) against oxidative stress caused by a high-fat diet in rats. Forty-eight mature male albino rats weighing (220 ± 5 g) were used in the study. The rats were randomly separated into six groups (eight rats each): rats were fed a basic diet- normal diet (ND) , high-fat diet (HFD), HFD +10% mung bean (HFDM- 10%) , HFD +10% soybean (HFDS- 10%), HFD +50% mung beans (HFDM- 50%), and HFD +50% soybeans (HFDS- 50%). The period of the experiment is four weeks. Dyslipidemia was noticed in (HFD) group. The TC levels in (HFDM- 10%) and (HFDS- 10%) groups decreased by 33.57% and 23.37%, respectively compared with (HFD) group. Furthermore, HDL- C level of (HFDM- 50%) were 34.19% higher than (HFD) group. Diet supplemented with mung beans and soybean revealed a significant reduction in serum lipid profile in a dose- dependent manner. Liver staining showed that (HFD) caused liver lesions, whereas mung bean and soybean could significantly improve these symptoms. Administration of legumes at a higher dose could significantly elevate the activity of antioxidant enzymes and reduce the level of MDA, compared with the (HFD) group. The antioxidant defense system in legume groups showed marked improvement compared with the (HFD) group. In conclusion, mung beans and soybean can be used as natural antioxidants. Mung bean and soybean were able to reduce serum and liver lipids of rats fed a high-fat diet and improve the antioxidant defense system.

**Keywords:** natural antioxidants, lipid profile, antioxidant enzymes

## Introduction

With the development of people's quality of life and dietary changes, hyperlipidemia is becoming one of the chronic diseases affecting an individual's health. It is characterized by increased total cholesterol, low-density lipoprotein and reduced levels of high-density lipoprotein cholesterol **(Assmann and Gotto, 2004)**. Some studies have confirmed that hyperlipidemia is significantly associated with oxidative stress. Hyperlipidemia is an important condition that promotes an increase in reactive oxygen species (ROS) through several metabolic pathways. An imbalance between oxidation states and antioxidants in the human body leads to an increase in free radicals that are harmful to the body **(Costa et al., 2015)**. Dietary antioxidants can protect the human body from reactive oxygen species by several mechanisms. Increased intake of antioxidants has good health effects such as lowering the incidence of cancer and cardiovascular disease **(Summo et al., 2016)**.

Legumes have many benefits due to the phytochemicals in them. Antioxidant activity and phenolic compounds have been observed in many consumed legumes, and a diet based legume reduces the risk of chronic diseases associated with oxidative stress **(Liu et al., 2013)**. In legumes, phenolic compounds including phenolic acids, flavones, isoflavones, and condensed tannins have been identified and characterized **(Cheng, 2009)**.

The mung bean (Vigna radiata), is a plant species in the legume family, rich in proteins, vitamins, and minerals **(Mubarak, 2005)**. Mung beans are rich in biologically active substances, such as flavonoids, alkaloids and tannins. Among the compounds that have been most studied in recent years are flavonoids for their antioxidant, anticancer, antibacterial, anti-inflammatory, and hypolipidemic effects **(Randhir and Shetty, 2007).**

Recently, soybeans gained attention as a functional food because they contain phytochemicals that have a role in preventing cancer and chronic diseases **(Giri and Mangaraj, 2012)**. Studies conducted on soybeans have shown that it contains many phytochemicals, such as carotenoids, coumarins, flavonoids, lignans, phytic acid, triterpenes, and phenolics, which have a role in cancer prevention **(Chandrasekara and Josheph, 2016).** Soybean phytochemicals possess anti-renal failure, anticancer, anti-aging, anti-obesity, and anti-cholesterolemic activities **(Kim et al., 2021).**

Previous studies have been concerned with studying the antioxidant effects of polyphenols in legumes *in vitro*, while few studies focused on the effects of legumes *in vivo*. The aim of this study, is evaluate the potential protective effects of diet fortified with Mung bean and Soybean against oxidative stress caused by a high-fat diet in rats.

## Materials and Methods

### Materials:

The Kits for total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL- C), high- density lipoprotein cholesterol (HDL- C), total antioxidant capacity (T-AOC), catalase (CAT), malondialdehyde (MDA), total superoxide dismutase (SOD), and glutathione peroxidase (GSH- Px), were purchased from El- Gomhoria Company for Trading Drugs, Chemicals and Medical Requirements. Forty-eight albino rats of Sprague-Dawley strain weighing (220 ± 5 g) were purchased from Helwan Farm for Experimental Animals, Cairo, Egypt.

### Methods:

### Experimental animal design:

Mung beans and soybean have been ground into powder and mixed well, and then stored at (4°C). Part of the powder was taken to prepare the extract, and the rest was added to the animal diet. The extraction was made following the descriptions of **(Faller et al., 2012)** with minor modifications.

The basal diet was prepared according to **(Reeves et al., 1993)**, which consists of (20%) protein, (10%) sucrose, (4.7%) corn oil, (2%) choline chloride, (3.5%) salt mixture, (1%) vitamin mixture, and (5%) fibers. The high-fat diet has been prepared according to **(Jiayin et al., 2011)**, which consists of (20%) animal fat + (1%) cholesterol added to the basal diet. 10% and 50% (w/w) mung bean and soybean powder were added into the high-fat diet resulting in HFDM- 10%, HFDS- 10%, HFDM- 50%, and HFDS- 50%, respectively.

Forty-eight male Sprague Dawley rats (220 ± 5 g) were used in the study. All the rats were maintained under standard conditions (22°C ± 2°C), relative humidity (55% ± 0.5%), 12.hr light–dark cycle. After an acclimation period of one week, rats were randomly divided into six groups as follows:

**Group 1:**(ND), rats were fed on basal diet.

**Group 2:**(HFD), rats were fed on high-fat diet.

**Group 3:**(HFDM- 10%), rats were fed on high-fat diet and10% mung bean.

**Group4:**(HFDS- 10%), rats were fed on high-fat diet and10% soybeans.

**Group 5:**(HFDM- 50%), rats were fed on high-fat diet and50% mung bean.

**Group 6:** (HFDS- 50%), rats were fed on high-fat diet and50% soybeans.

The rats of each group were fed with the appropriate diet for 4 weeks. At the end of each week the rat's body weight is measured. At the end of this experiment, rats were prevented from food overnight and sacrificed. For serum preparation rats were anesthetized, and blood samples were collected from the abdominal aorta. The liver was removed, rinsed in saline solution, weighed and stored for the histological study.

**Feeding and Growth Parameters:**

Body weight, liver weight, relative liver weight, and food intake were determined.

Relative liver weight (%) = (liver weight/body weight) × 100

**Biochemical analysis:**

Serum total cholesterol (TC), high density lipoprotein (HDL-C), low-density lipoprotein, triglycerides (TGs) were determined according to the methods of **(Charles et al., 1974)**, **(Lopes et al., 1977)**, **(Okada,1996)**, and **(Fossati and Prencipe, 1982)** respectively. The estimation of glutathione peroxidase (GSH-Px) activity was determined by the procedure of **(Carlberg and Mannervik, 1985)**. Malondialdehyde (MDA), was determined according to (**Draper and Hadly, 1990).** The activities of total superoxide dismutase (SOD), were measured according to **(Nishikimi et al., 1972)**. Liver homogenates were prepared in an ice bath with tissue homogenizers to determine the liver antioxidant enzyme activities, and the supernatants were obtained via centrifugation at 12,000 r/min for 10 min.

**Scavenging Effect on DPPH Radicals:**

The antioxidant potential of the obtained extracts was measured by using (DPPH) assay as a method described by **(Malenčić et al., 2007).**

**Analysis of total phenols:**

The total phenolic content of the mung bean and soybean were determined using a Folin and Ciocalteu reagent with minor modifications **(Guo et al., 2012)** using gallic acid as the standard. The total phenolic content was expressed as milligram gallic acid equivalents per gram dry legume (mg GAE/g) through the calibration curve of gallic acid.

**Histopathological investigations:**

The livers were collected, fixed, stained with hematoxylin and eosin, and observed under a light microscope **(Shen et al., 2017).**

**Statistical analysis:**

The data were expressed as mean ± standard deviation. The results were analyzed for variance using the GraphPad Prism software (version 6.01; GraphPad Inc.), and statistical significance of differences ($p < .05$) was evaluated using Dunnett's multiple comparisons test **(Snedecor and Cochran, 1980)**.

# Results and Discussion

## Analysis of total phenols:

As shown in figure (1) the total phenolic content of mung bean and soybean ethanol extracts, recorded 2.26 and 1.65 mg GAE/g, respectively. It is reported that ferulic acid and protocatechuic acid are phenolic acids widely existing in plants, which have the functions of antioxidant, inhibiting tyrosinase activity and protecting nerves **(Robbins, 2003 ).**

In general, the polyphenols and flavonoids in legumes are the main antioxidant components, and the content of phenols varies with the variety and origin of legumes. A study conducted by **(Kohen and Nyska, 2002)** showed that dietary legumes are rich in polyphenols with high antioxidant capacity, which have an effect on the activities of antioxidant enzymes in vivo, such as elevated SOD and GSH activities and reduced MDA levels. This agrees with the results of our study that the levels of CAT and SOD in the liver of adult rats fed with HFDM-50% or HFDS-50% were markedly higher than those of rats fed with HFD.

Many researches have confirmed that phenolic compounds have strong antioxidant capacity **(Randhir and Shetty, 2007).** When reacting with oxide substances, these compounds can be used as a donor of hydrogen or electron. The total phenolic content of mung bean was reported to be (8.14 mg GAE/g) and (6.10 mg GAE/g) for soybean **(Yang et al., 2011)**.

Previous studies suggested that Vigna species of legumes are potential sources of antioxidant phenolic and also great sources of strong natural inhibitors for lipase activities **(Sreerama et al., 2012)**. Furthermore, the results of (**Kim et al., 2012)** showed that legumes also have antioxidant activities for their capacities of free radical capture.The higher scavenging capacity might be related to a higher content of natural antioxidants in mung beans, such as flavonoids **(Nithiyanantham et al., 2012).**

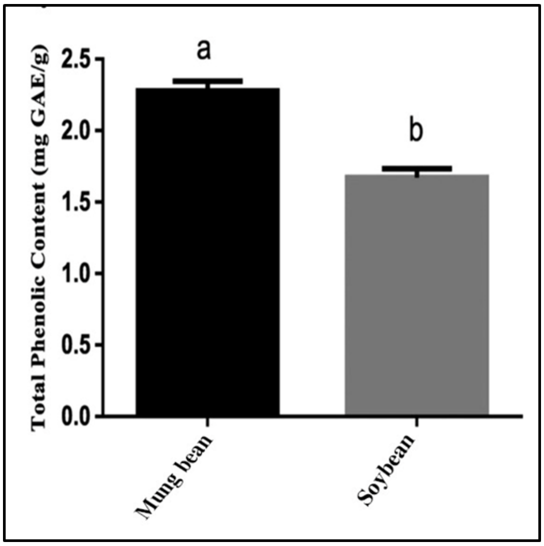


**Figure (1): The total phenolic content of mung bean and soybean ethanol extracts**

There were significant differences in the different expressions of superscripts (a, b), $p < .05$

## Antioxidant activity:

Ethanol extracts exhibited (DPPH) scavenging capacity as shown in figure (2). Antioxidant activity is expressed as percent (DPPH) radical scavenging activity with higher values indicating greater antioxidant activity. The (DPPH) scavenging capacity of mung bean and soybean extracts increased with the increase in concentration. When the concentration of ethanol extracts was 1.0 mg/ml, the (DPPH) scavenging efficiencies of mung beans and soybean were 81.71% and 55.50%, respectively.

(DPPH) scavenging examination is usually used for the assessment of the free radical scavenging of plant extracts for its simple sensitive and reproducible procedures. The plant source of extracts, environmental factors, and the solvent used in the extractions process may explain the differences in the levels of (DPPH) scavenging activities **(Duenas et al., 2004).** In the present study, the (DPPH) scavenging capacity of mung bean ethanol extract was higher than the soybean extract, which consistent with the results of (**Cheng et al., 2009).** The study of **(Nithiyanantham et al., 2012)** confirmed that the higher (DPPH) scavenging capacity in mung beans, may be related to a higher content of natural antioxidants, such as flavonoids.

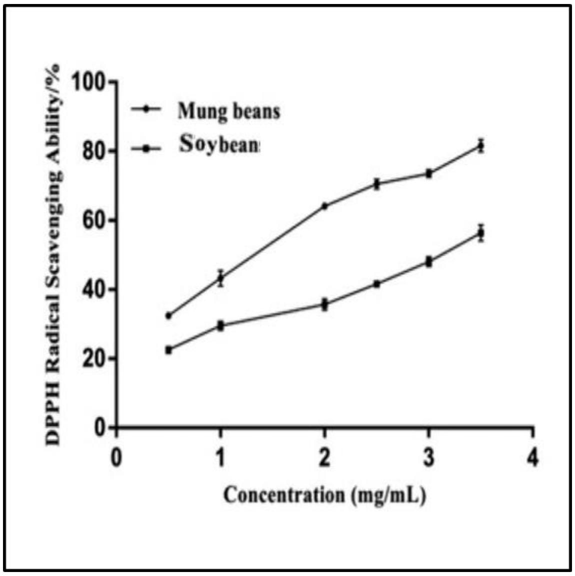


**Figure (2): Scavenging activity of DPPH radicals (%)**

## Effects of legumes on rats' growth:

The changes in the growth of experimental rats after being fed with different diets for 4 weeks are recorded in table (1). Results indicated that the final body weight and relative liver weight of high fat diet group (HFD) recorded 445.5±11.5 g and 4.86±0. 01% respectively, these results were 12.26% and 70.71% higher than rats fed with normal diet (ND) and there was no significant difference in final body weight between legume supplementation groups and (HFD) group. The relative liver weight of rats in (ND), (HFDM-10%), and (HFDM-50%) groups significantly decreased, compared with the (HFD) group. In addition, there were no significant changes in the food intake of each experimental group, which indicates that different diets had no significant effect on the food intake of rats.

**Table (1): Effects of mung bean and soybeans on body weights, relative liver weight (%), and food intake of the experimental groups**

| Group | Initial body weight (g) | Final body weight (g) | Relative liver weight (%) | Food intake (g/day) |
|---|---|---|---|---|
| **ND** | 221.40±2.34[a] | 395.20±6.88[a] | 3.07±0. 01[a] | 25.25±1.33[a] |
| **HFD** | 218.37±3.33[a] | 445.50±11.56[b] | 4.86±0. 01[b] | 23.55±3.50[a] |
| **HFDM-10%** | 222.72±2.63[a] | 441.63±12.12[b] | 4.38±0. 01[c] | 24.43±1.22[a] |
| **HFDS-10%** | 221.43±2.88[a] | 443.54±9.25[b] | 4. 97±0.02[b] | 25.23±1.55[a] |
| **HFDM-50%** | 218.55±2.77[a] | 435.14±8.74[b] | 4.27±0.01[c] | 24.77±1.55[a] |
| **HFDS-50%** | 222.23±3.04[a] | 435.33±12.12[b] | 4.67±0.02[b] | 24.35±1.25[a] |

Values are expressed as mean ± SE. Means with different superscript letters are significantly different at $p \leq 0.05$.

## Effects of legumes on serum lipid profile:

As shown in figure (3), dyslipidemia was noticed in (HFD) group. The serum levels of TC, TG, and LDL- C increased markedly in the (HFD) group compared with the (ND) group, and there was a significant reduction in the level of HDL- C. The TC levels in (HFDM- 10%) and (HFDS- 10%) groups decreased by 33.57% and 23.37%, respectively compared with (HFD) group. The HDL- C level of (HFDM- 50%), were 34.19% higher than (HFD) group.

Moreover, the LDL- C level was reduced by 32.28% ($p$ < .05). Diet supplemented with mung beans and soybean revealed a significant reduction in serum lipid profile in a dose- dependent manner. the levels of TC and LDL- C in the higher dose, did go lower, and bigger than the HDL- C level. Interestingly, a higher dose of legumes did not have an effect on the levels of TG, though they were lower than the HFD ones.

Many studies have shown that increasing levels of TC, LDL- C, and TG in serum could increase the occurrence of atherosclerosis and cardiovascular disease. On the contrary, high levels of HDL-C can reduce this risk **(Assmann and Gotto, 2004**). In this study, increased levels of TG and TC in serum were noticed in the (HFD) group, which agrees with **(Xiao et al., 2010).**

However, the elevated levels in LDL-C and TC and reduction in the level of HDL-C could be significantly inhibited in the legume groups, suggesting that mung beans and soybean could be good factors for blood lipid reduction. In a study conducted by **(Yeap et al., 2015),** it was found that the fermented mung beans significantly decreased the levels of TC, TG, and LDL in the serum of mice compared with non-fermented mung beans. This may be due that γ- aminobutyric acid present in fermented mung bean may participate in the reduction the lipid in hypercholesterolemic mice.

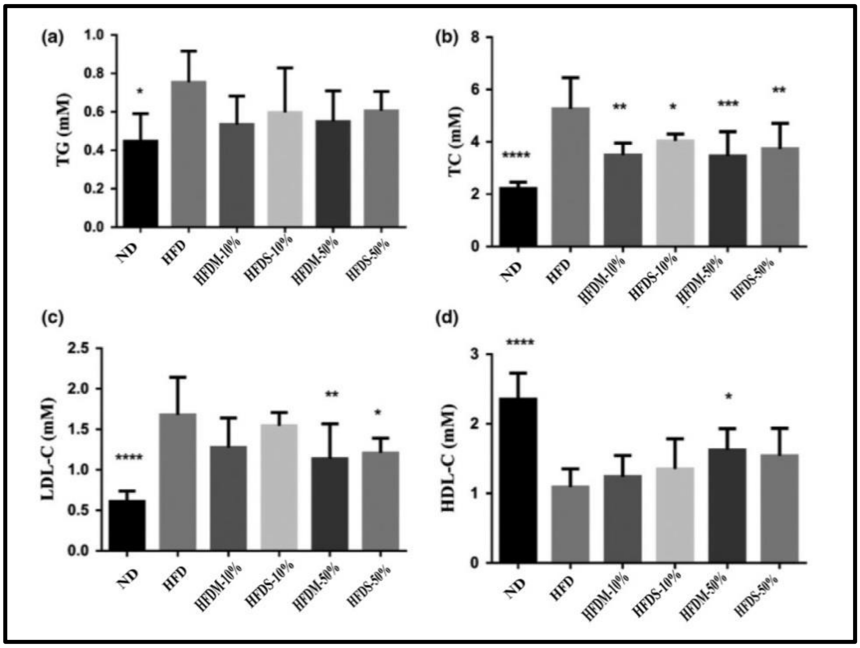


**Figure (3): Effects of mung bean and soybeans on serum lipids of the experimental groups**

Results are expressed as mean ± SD ,***p < .001, **p < .01, and *p < .05 versus HFD

## Histopathological examination of liver tissues:

Figure (4) showed that the hepatocytes in the (ND) group retained normal morphology including central veins, clear outline of hepatic impellers, and almost no fat droplets (Figure 4-a). However, in the (HFD) group, the structure of hepatic lobules was disordered, the number of lipid droplets in the cells increased, the volume of lipid droplets became bigger, and a small number of hepatocytes had steatosis. The lipid droplets in the hepatocytes of all legume groups were significantly reduced compared with the (HFD) group, and their volumes were decreased. All these above indicated that mung bean and soybean had certain effects in assisting the prevention of fatty liver.

The results of a study conducted by **(Abulnaja and Rabey, 2015 )** confirmed that liver and heart tissues of rats fed with 2% cholesterol had necrosis of hepatocytes and cytoplasmic blebbing. This study in consistent with the results of our study which suggested that hypercholesterolemia was associated with pathological changes in vital organs. A Supplementation diet with mung beans and soybean could protect the liver and other tissues, and high doses of legumes can return to their normal state. Moreover, high fat diet may cause the production of reactive oxygen species (ROS) **(Wang., 2015).**

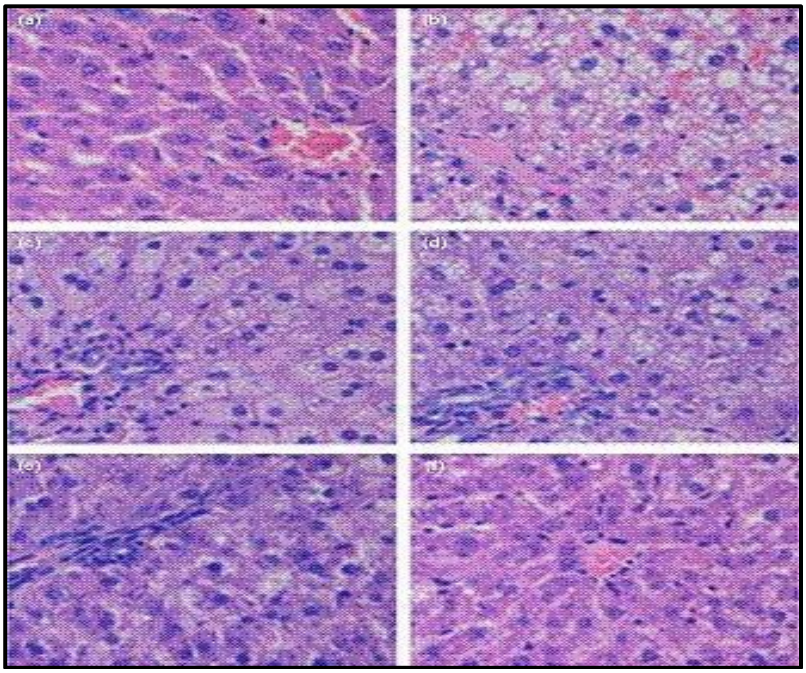

**Figure (4): Histopathological examination of liver tissues of the experimental groups**

**a: ND; b: HFD; c: HFDM- 10%; d: HFDS- 10%; e: HFDM- 50%; and f: HFDS- 50%**

## Effects of legumes on antioxidant status:

Antioxidant enzymes and lipid peroxidation were evaluated in serum and in the liver. It was observed in figure (5), that a high-fat diet caused disorder in the levels of MDA and the activities of the antioxidant enzymes. However, the supplemented diet with mung beans and soybean could relieve these disorders.

Administration of legumes at a higher dose could significantly elevate the enzyme activities and reduce the level of MDA, compared with the (HFD) group. In (HFDM- 50%) group, the activities of serum SOD, CAT, and GSH- Px were elevated by 32.09%, 86.13%, and 100.40%, respectively, and serum MDA level decreased by 31.86% ($p < .05$).

In (HFDS- 50%) group, the activities of serum GSH- Px, SOD, and T- AOC increased by 110.99%, 28.56%, and 45.64%, respectively, and serum MDA level decreased by 28.44%. The effects on antioxidant status in livers were noted mainly in liver CAT and T- AOC levels, which were raised by 28.82% and 71.45% for (HFDM- 50%) group and 31.27% and 69.41% for (HFDS- 50%) group, respectively.

The biological effects of (ROS) were controlled in vivo by the defense of antioxidant enzymes. A Supplementation diet with legumes could significantly improve the activities of the main antioxidant enzymes in the serum and liver of (HFD) rats, and then enhance the lipid oxidation caused by the high-fat diet. These results consistent with the researches in animals **(Feillet et al., 2009)** and humans **(Patel et al., 2007)**, which oxidative stress was induced by high fat and high carbohydrate diets. The activities of GSH-Px, CAT, T-AOC, SOD, and MDA levels of the serum and livers of rat groups were used as indicators of oxidative stress after 4 weeks of supplementation diet with legumes. Daily consumption of mung bean and soybean powders could improve the liver antioxidant status as shown by the increased levels of GSH-Px, CAT, and T-AOC. No significant effects were watched in the activities of SOD and MDA under the same conditions.

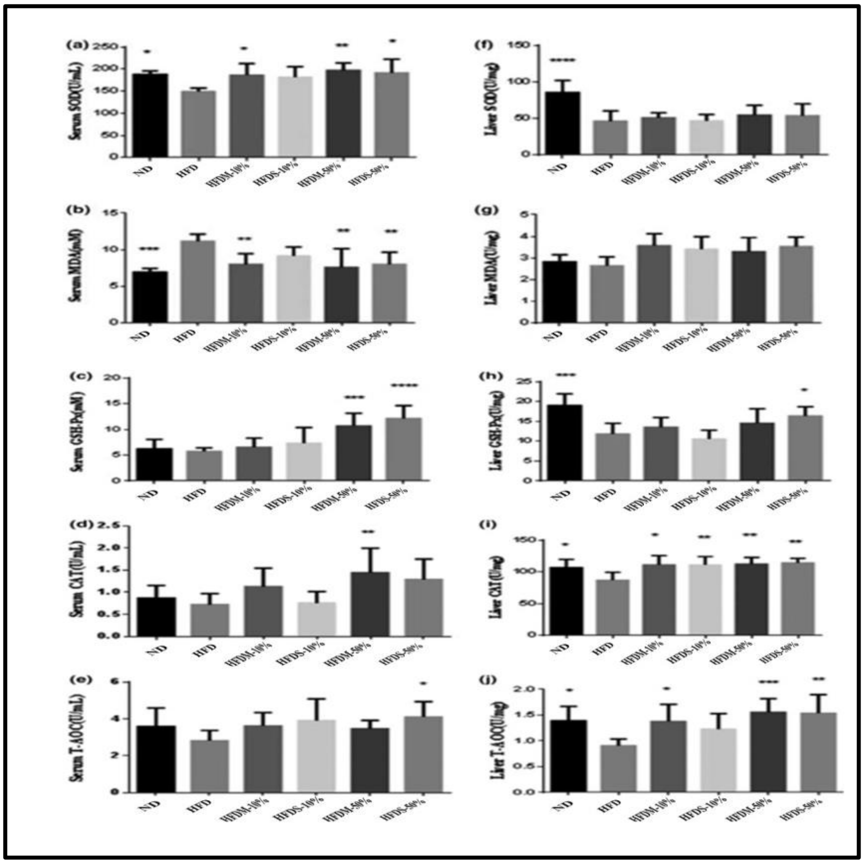


**Figure (5): Effects of mung bean and soybeans on serum and liver tissue antioxidant enzymes of the experimental groups**

Results are expressed as mean ± *SD*, ****p* < .001, ***p* < .01, and **p* < .05 versus HFD

## Conclusion and Recommendations

In conclusion, dietary fortification with 50% of mung bean or soybean can greatly restore the levels of total cholesterol, low density lipoprotein cholesterol, and high density lipoprotein cholesterol in serum. The results suggested that mung bean and soybean can effectively improve oxidative stress injury caused by a high fat diet in rats, and suggested that mung beans and soybean have strong antioxidant activity. The study recommended, raising the nutrition awareness of these types of legumes for their quality and biological effects.

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# التأثيرات الوقائية المحتملة للغذاء المدعم ببقلة الماش وفول الصويا ضد الاجهاد التأكسدي الناتج عن اتباع نظام غذائي مرتفع الدهون في الفئران

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## الملخص العربي

إن فرط شحميات الدم يرتبط بالإجهاد التأكسدي ، وقد أظهرت الدراسات السابقة أن البقوليات يمكن أن تقي من الاصابة بالأمراض المزمنة المرتبطة بالإجهاد التأكسدي. تهدف هذه الدراسة إلى تقييم التأثيرات الوقائية المحتملة للغذاء المدعم ببقلة الماش ، فول الصويا ضد ما يسببه الاجهاد التأكسدي الناتج عن اتباع نظام غذائي عالي الدهون في الفئران. تم استخدام ذكور فئران الالبينو البالغة وزن ( 220 ± 5 جم) وتم تقسيمهم إلى 6 مجموعات ( كل مجموعة =8 فئران) وكانت مدة التجربة 4 أسابيع. المجموعة الأولى تغذت على الغذاء الاساسي، المجموعة الثانية تغذت على نظام غذائي عالي الدهون، المجموعة الثالثة تغذت على نظام غذائي عالي الدهون مع 10% بقلة الماش، المجموعة الرابعة تغذت على نظام غذائي عالي الدهون مع 10% فول الصويا ، المجموعة الخامسة تغذت على نظام غذائي عالي الدهون مع 50% بقلة الماش، المجموعة السادسة تغذت على نظام غذائي عالي الدهون مع 50% فول الصويا . لوحظ اضطراب مستويات دهون الدم في مجموعة الفئران التي تغذت على الغذاء عالي الدهون. انخفضت مستويات الكوليسترول الكلي في مجموعة الفئران التي تغذت على غذاء عالي الدهون مع 10% بقلة الماش ومجموعة الفئران التي تغذت على غذاء عالي الدهون مع 10% فول الصويا بنسبة 33.57٪ و 23.37٪ على التوالي مقارنة بمجموعة الفئران التي تغذت على الغذاء عالي الدهون. علاوة على ذلك، كان مستوى كوليسترول البروتين الدهني مرتفع الكثافة في المجموعة التي تغذت على غذاء عالي الدهون مع 50% بقلة الماش أعلى بنسبة 34.19٪ مقارنة بمجموعة الفئران التي تغذت على الغذاء عالي الدهون. أكدت النتائج أن النظام الغذائي الذي

احتوى على 50٪ من بقلة الماش أوفول الصويا يمكن أن يعيد إلى حد كبير من مستويات الكوليسترول الكلي، الكوليسترول منخفض الكثافة، الكوليسترول الدهني عالي الكثافة في مصل الدم الى المستويات الطبيعية. كما أظهرت النتائج أن النظام الغذائي عالي الدهون تسبب في ضرر كبد الفئران ، في حين أن بقلة الماش و فول الصويا يمكن أن تحسن هذه الأعراض بشكل كبير. كما أظهر نظام الدفاع المضاد للأكسدة في مجموعات البقوليات تحسنًا ملحوظًا مقارنة بمجموعة النظام الغذائي عالي الدهون. كل هذه النتائج أكدت أنه يمكن استخدام بقلة الماش و فول الصويا كمضادات أكسدة طبيعية. فقد كانت بقلة الماش وفول الصويا قادرين على تقليل نسب الدهون في مصل الدم والكبد بشكل كبير وتحسين نظام الدفاع المضاد للأكسدة مقارنة بمجموعة الفئران التي تغذت على نظام غذائي عالي الدهون.

**<u>الكلمات المفتاحية:</u>** مضادات الأكسدة الطبيعية، شحميات الدم، انزيمات مضادات الأكسدة