

**EFFECT OF ETHANOL EXTRACT OF *GLYCINE MAX* (SOY BEAN) ON SERUM LIPID PROFILE OF FAT-FED HYPERLIPIDEMIC RATS**

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**ABSTRACT**

Hypercholesterolemia is one of the important causes of cardiovascular disease related mortality and morbidity and recently it has become a significant issue in public health problem of developing countries. The purpose of the study was to see the effect of *glycine max* (soy bean) on serum lipid profile of experimentally induced hyperlipidemic rats. Hypercholesterolemia was induced by high cholesterol diet containing 1% cholesterol in olive oil. This study was done on 24 long Norwegian rats (*Rattus norvegicus*) weighing about 200-210 gram. For convenience, the study was divided into Experiment I and Experiment II. In experiment I, 12 rats were divided into two groups- Group A (n = 6, control group) fed on laboratory diet and Group B (n=6) fed on laboratory diet and soy bean extract for 35 days. In experiment II, the remaining 12 rats were divided into 2 groups, Group C (n=6) were fed on laboratory diet and high cholesterol diet (HCD) for 35 days and Group D (n=6) were fed laboratory diet and high cholesterol diet on initial 10 days and soy bean extract was added along with high cholesterol diet for next 25 days. Serum TC, TG, LDL-C and HDL-C were measured after 35 days. HCD increased TG, TC and LDL-C significantly in group C. *Glycine max* (soy bean) treated group showed that it decreased plasma TC, TG and LDL-C in experimentally induced hyperlipidemic rats but *Glycine max* (soy bean) did not show any significant lipid lowering effect in normolipidemic rats. The results of this experimental study indicate that *Glycine max* can act as a cholesterol lowering agent and thereby can improve cardiovascular functions.

**Key Words:** *Glycine max*, Soy bean, Dyslipidemia, Hypercholesterolemic rats

**Introduction**

Hypercholesterolemia is a condition characterized by high levels of cholesterol above normal in the blood. Cholesterol is a waxy, fat-like substance that is produced in the body and also obtained from foods that come from animals particularly egg yolks, meat, poultry, fish, and dairy products. Dyslipidemias are common metabolic disorders seen in clinical practice. They are important because they may lead to a number of sequelae, including coronary heart disease (CHD), dermatological manifestation (xanthelasmata and xanthomata), pancreatitis

and (more rarely) neurological and ocular abnormalities.

Recent study showed that hypercholesterolemia is associated with enhanced oxidative stress related to increased lipid peroxidation. Increased generation of oxidized LDL is a major factor in the vascular damage associated with high cholesterol level. Hence, the inhibition of oxidative stress in hypercholesterolemic conditions is considered to be an important option for therapeutic approach and efforts have been made to identify the antioxidant functions of various medicinal plants<sup>1</sup>.

According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated for better understanding of their properties, safety and efficacy<sup>2</sup>.

*Genoderma lucidum* is the most popular medicinal mushroom and its hypolipidemic effect on hypercholesterolemic rats has been observed<sup>3</sup>. *Nigella Sativa* (kalajira) extract also found to reduce the serum cholesterol, TG and LDL-C levels as well as to elevate the serum HDL-C level in hyperlipidemic rats<sup>4</sup>.

Soy bean seeds' effect on glucose levels, lipid profile and histological structure of the Liver were investigated<sup>5</sup>. The soybean (US) or soya bean (UK) is a spice of legume native to East Asia, widely grown for its edible bean which has numerous uses. The plant is classed as an oilseeds rather than a pulse by the UN Food and Agricultural Organization (FAO). Soybeans contain at least 12 different types of isoflavones<sup>6</sup>.

Soy protein refers to the protein which is found in soybeans that is often used to replace animal proteins in an individual's diet. The soybean is a legume that contains no cholesterol and is low in saturated fat. Soybeans are also a good source of fiber, iron, calcium, zinc, and B vitamins<sup>7</sup>.

Soy isoflavones have been reported to lower blood cholesterol levels and to have antioxidant properties. Due to these abilities of soybean isoflavones, they have shown the beneficial effects on prevention and attenuation chronic diseases including cardiovascular diseases<sup>8</sup>, osteoporosis<sup>9</sup> and menopausal symptoms<sup>10</sup>.

So, the aim of this study was to see the effect of soy bean (*Glycine max*) in experimentally induced hypercholesterolemic rats and thereby stimulating researchers to see the effect of soy bean, a safer herbs in hyperlipidemic patients in search of a better and safe solution of hyperlipidemia.

## Materials and Methods

The study was carried out in the Department of Pharmacology and in Animal house, Dhaka Medical College, Dhaka from July 2012 to June 2013.

### *Preparation of Ethanol extract of Glycine max (soy bean)*

This was prepared in the drug laboratory of the Center for Advance Research (CARS), Dhaka University. About 1 kg soy beans were shed dried for 7 days then grinded. 425 gm dried powder of soy bean was suspended in 2 liter of 80% ethanol for 72 hours. Then it was filtered with filter paper and about 590 ml filtrate was obtained. The filtrate was concentrated with the rotary vacuum evaporator. About 30 gm of condensed ethanol extract of *Glycine max* was obtained. Then preparation of suspension was made with 100 mg extract in 20 ml of distilled water to administer orally to the rats. (Around 1 ml of resuspended *Glycine max* extract in water was given per rat per day)

### *Preparation of Laboratory Diet and High Cholesterol Diet*

#### Laboratory Diet

Ingredients for Laboratory diet were purchased from local market which was composed of 60 % corn flour for starch, 20 % fish meal for protein, 10 % wheat flour for fibers, 7 % oil seed cake, 2 % bone meal and 1 % salt.

#### High Cholesterol Diet (HCD)

Cholesterol (manufactured by Loba Chemic) and olive oil were purchased from the local market in Dhaka. Then 10 gm cholesterol was dissolved in olive oil to make it 1 liter solution which was equivalent to 1% cholesterol in olive oil and it was used as high cholesterol diet. Around 1.5 ml solution of High cholesterol diet was given to rats per day to render them hyperlipidemic<sup>11</sup>.

### Reagents

- Lipid profile kit (Randox Laboratories, Ltd, UK)
- Cholesterol liquicolor (Human Gmb H D - 65205 wiesbaden 30ml)
- Triglycerides GPO liquicolor (Human Gmb H D-65204 wiesbaden)
- Precipitant: (Human Gmb H D- 65205 wiesbaden).

**Animals:** The experiments were performed on 24 healthy adult Long Evan's Norwegian (*Rattus norvegicus*) rats weighing between 200-210 gm, age 8-10 weeks and were collected from the Bangladesh Centre for Scientific and Industrial Research (BCSIR) Laboratory. The chosen animals were housed in animal house in Dhaka Medical College and in standard size metallic cages in a well-ventilated room. The rats were allowed to live at room temperature with 12 hours of light and 12 hours of dark schedule. They were fed normal rat diet and given water *ad libitum*. Animals were randomly distributed into four groups of six animals in each. Each cage was labeled for identification of different groups.

**Experimental I:** Effect of *Glycine max* (GM) extract on serum lipid profile in normal rats.

This part of experiment was conducted to demonstrate the effect of extract of *Glycine max* on normal rats. For this purpose a total number of 12 rats were taken and was divided into two groups.

**Group A:** This control group was consisted of 6 rats, which received laboratory diet and distilled water for 35 days.

**Group B:** This group was consisted of 6 rats, which received laboratory diet, distilled water and *Glycine max* (GM) extract at the rate of 30 mg/kg body weight per day orally by gastric intubation for 35 days.

**Experiment II:** Effect of *Glycine max* (GM) extract on serum lipid profile in HCD fed rats:

This part of experiment was conducted to demonstrate the effect of extract of *Glycine max* on HCD (high cholesterol diet) fed rats. For this purpose a total number of 12 rats were taken and divided into two groups.

**Group C:** Hyperlipidemic group consisted of 6 rats which received normal laboratory diet, distilled water and HCD (high cholesterol diet) for 35 days

**Group D:** This group was consisted of 6 adult rats and received normal laboratory diet, distilled water and HCD (high cholesterol diet) for 10 days and then *Glycine max* extract (30 mg/kg body weight per rat per day) along with HCD for next 25 days orally by gastric intubation.

### Collection of Blood and Serum

Rats were sacrificed on 36th day, under light anesthesia with chloroform. Approximately 2-3 ml blood from each rat was collected by cardiac puncture in separate clean and dry test tubes with proper identification number. Separated serum, after centrifugation at 4000 rpm for 5 minutes was collected with micropipette and transferred into separately labeled eppendorf tube and then stored at -15°C for biochemical analysis.

### Biochemical Analysis

After collection of all blood specimens, serum total cholesterol (TC), serum high density lipoprotein cholesterol (HDL-C) and serum triglycerides (TG) were measured by Semi-automatic biochemistry analyzer using lipid profile kits. Low density lipoprotein cholesterol (LDL-C) was calculated by Friedwald's formula.

### Statistical Analysis

The data was compared using unpaired t test. Results were expressed as mean( $\pm$ SD) and  $P < 0.05$  was considered statistically significant.

## Results

**Experiment I:** Table I shows the effect of *Glycine max* on serum lipid profile of adult rats. Serum total cholesterol, serum LDL-C, HDL cholesterol and serum triglyceride levels did not show any significant difference between the *Glycine max* treated group (Group B) and the control group (Group A).

**Table-I:** Effect of *Glycine max* on Serum lipid levels of adult rats (A vs B) :

Parameters	Group A (n=6) Mean±SD	Group B (n=6) Mean±SD	P values
Serum T. Cholesterol conc. (mg/dl)	80.67±2.5	78.67±1.21 <sup>ns</sup>	>0.05
Serum TG conc. (mg/dl)	78.33±1.86	76.83±1.94 <sup>ns</sup>	>0.05
Serum LDL-C conc. (mg/dl)	27.33±1.97	26.17±1.72 <sup>ns</sup>	>0.05
Serum HDL-C conc. (mg/dl)	1.67±1.37	31.33±0.82 <sup>ns</sup>	>0.05

*SD* = Standard deviation, Unpaired Student's 't' test was done to see the level of significance. *ns* = not significant ( $p > 0.05$ )

## Experiment II

Table II shows the effect of HCD feeding on serum lipid profile of adult rats. Serum total cholesterol, serum LDL-C, and serum triglyceride levels were raised significantly in HCD group (Group C) when compared with that of control group (Group A). But there was no significant change in serum HDL-C level. Table III shows the effect of *Glycine max* on serum lipid profile of adult rats fed with HCD. Serum total cholesterol, serum LDL-C, and serum triglyceride levels decreased significantly in Group D compared to Group C. But there was no significant change in serum HDL-C level.

**Table-II:** Effect of HCD diet on Serum lipid profile of adult rats (A vs C)

Parameters	Group A (n=6) Mean±SD	Group B (n=6) Mean±SD	P values
Serum T. Cholesterol conc. (mg/dl)	80.67±2.5	128.33±2.94*	>0.05
Serum TG conc. (mg/dl)	78.33±1.86	103.17±2.04*	>0.05
Serum LDL-C conc. (mg/dl)	27.33±1.97	81.17±2.23*	>0.05
Serum HDL-C conc. (mg/dl)	31.67±1.37	31.33±1.21 <sup>ns</sup>	>0.05

*SD* = Standard deviation, Unpaired Student's 't' test was done to see the level of significance .

\* = significant, *ns* = not significant

**Table III:** Effect of *Glycine max* on Serum lipid profile of HCD rats group (C vs D)

Parameters	Group A (n=6) Mean±SD	Group B (n=6) Mean±SD	P values
Serum T. Cholesterol conc. (mg/dl)	128.33±2.94	112.33±7.42*	>0.05
Serum TG conc. (mg/dl)	103.17±2.04	94.50±2.17**	>0.05
Serum LDL-C conc. (mg/dl)	81.17±2.23	73.17±1.47*	>0.05
Serum HDL-C conc. (mg/dl)	31.33±1.21	30.0±1.21 <sup>ns</sup>	>0.05

*SD* = Standard deviation, Unpaired Student's 't' test was done to see the level of significance .

\* = significant, *ns* = not significant

## Discussion

Herbs and spices have been used for generations by humans as food and to treat ailments. Scientific evidence is accumulating that many of these herbs and spices do have medicinal properties that alleviate symptoms or prevent disease. A growing body of researchers have demonstrated that the commonly used herbs and spices such as garlic, black cumin, cloves, cinnamon, thyme, allspices, bay leaves, mustard,

and rosemary, possess antimicrobial properties that, in some cases, can be used therapeutically<sup>12</sup>.

In experiment I, the effect of *Glycine max* was observed on adult long Norwegians rats. Two groups of rats were taken with 6 rats in each group. One group was labeled as the control (Group A) which received standard laboratory diet. Group B which received extract of *Glycine max* at the rate of 30 mg/kg body weight per day along with normal laboratory diet. All conventionally measured indices (serum Cholesterol, serum TG, serum LDL-C, serum HDL-C) of lipid profiles were slightly changed in Group B as compared to those in the control Group A. But changes were not statistically significant. These findings support the findings of another similar study<sup>3</sup>.

In experiment II, the effect of *Glycine max* was observed on serum lipid profile of hyperlipidemic Norwegian rats. On administration of high cholesterol diet, Group C showed significant ( $P < 0.05$ ), increase of serum lipid profiles (TC, TG, LDL-C) when compared to those of controls (Group A). This finding indicates that high cholesterol diet that is used to elevate the serum lipid profile was able to elevate all lipid profile except HDL-C measured in this experiment. These findings support the findings of another similar study<sup>13</sup>.

In Group D, the observation suggested that addition of extract of *Glycine max* at the rate of 30 mg/kg body weight per rat per day with HCD significantly decreased the serum lipid profile ( $P < 0.05$ ). The cause of no effect of *glycine max* on normolipidemic rats could not be explained in this study. It may be due to the underlying mechanism of action which needs to be explored.

It would have been better if we could collect blood sample from Group D rats after 10 days of HCD diet to see the lipid profiles. But then we had to sacrifice the rats for collection of blood. Due to this limitation, we had to start

*Glycine max* extract along with HCD for last 25 days but it does not affect the findings of the study as it was shown in the study that HCD diet render the rats hyperlipidemic. Soy isoflavones are believed to reduce the risk of heart disease by reducing the susceptibility of LDL to oxidation by their antioxidant action<sup>15</sup>. In addition, soy bean stimulates sterol regulatory element binding protein-2 which increase serum cholesterol clearance<sup>15</sup>.

Cholesterol lowering effect of soy bean is due to the ability of soy protein to reduce insulin level which in turn down-regulates the expression of the hepatic transcription factors of sterol regulatory element binding protein (SREBP)-1. The reduction of this factor decreases the expression of several lipogenic enzymes, causing decreased serum and hepatic triglycerides as well as LDL-C and VLDL-C<sup>16</sup>.

Moreover, hypocholesterolemic effect of soy bean may be due to its high content of phytosterols which are similar in structure to cholesterol, so they have the ability to enhance excretion of cholesterol, interfering with cholesterol synthesis and competing for cholesterol acceptor sites in the intestinal walls<sup>17</sup>.

The results and observations of the present study provide a rationale for use of *Glycine max* in the development of a new plant medicine, much needed for the reduction of serum lipid levels (TC, TG, LDL-C). Thus, it could be useful in hyperlipidemic states which lead to conditions such as atherosclerosis, obesity, hypertension, hyperlipidemia, IHD etc. But before establishing ethanol extract of *Glycine max* as therapeutically effective hypolipidemic agent, further studies should be carried out to determine the active principles responsible for hypolipidemic effect and its cellular mechanism of action. Toxicological studies in animal also need to be undertaken as well before any clinical trial for suitability of using in human.

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