



## Original article

# Anti-hyperlipidemic effect of crude methanolic extracts of *Glycine max* (soy bean) on high cholesterol diet-fed albino rats

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### Abstract

The cardiovascular diseases (CVD) are showing increasing trend particularly in developing countries. Deranged lipid metabolism is the most common risk factor for development of CVD. Many established drugs are used for the management of dyslipidemia but have side effects; therefore there is need to evaluate medicinal plants for possible lipid lowering activity since they are less toxic. Food substance like Soy bean (*Glycine max*) has been reported to have useful therapeutic effects on heart disease. This study was designed to determine the effect of crude methanol seed extract of *Glycine max* (MEGM) on the serum lipid profiles of *ad-libitum* high-cholesterol-fed male albino wister rats. A total of twenty (25) male rats were used and were randomly assigned into five groups namely A, B, C, D and E of five animals in each group. All rats in groups A-D were given high cholesterol diet, HCD (2000mg/kg) once daily for two weeks. In addition, rats in group A and B received crude methanol seed extract of *Glycine max* once daily at a dose of 400mg/kg and 200mg/kg respectively for two weeks. Group C received atorvastatin 20mg/kg for two weeks and this served as the positive control. Group D served as negative control and received neither the extract nor drug. Group E served as normal control. The biochemical parameters of lipid profile: Total cholesterol (TC), High density lipoprotein (HDL-C), Low density lipoprotein (LDL-C), Very low density lipoprotein (VLDL), and Triglycerides (TG) were assayed. The levels of TC, LDL, TG and VLDL were highly elevated significantly in the affected group (HCD alone) when compared with normal control ( $p < 0.001$ ). Administration of high dose MEGM (400mg/kg), low dose MEGM (200mg/kg) and atorvastatin (20mg/kg) separately in the presence of HCD challenge significantly lowered the elevated levels of TC ( $p < 0.05$ ), LDL ( $p < 0.001$ ), TG ( $p < 0.01$ ) and VLDL ( $p < 0.01$ ) when compared to the affected group. Furthermore and worthy of note, the levels of HDL was significantly reduced in the affected group (HCD alone) in comparison with normal control ( $p < 0.01$ ); however the administration of high dose MEGM (400mg/kg) and low dose MEGM (200mg/kg) separately in the presence of HCD challenge significantly increased the reduced HDL levels ( $p < 0.001$  and  $p < 0.05$ ; respectively) when compared to the affected group. *Glycine max* has anti-hyperlipidemic effect on the hyperlipidemic rats and possesses cholesterol lowering property.

**Key words:** Antihyperlipidemia, Carbimazole, Cholesterol, High cholesterol diet (HCD), Hyperlipidemia, Lipids, Methanol extract, Soy bean, Triglyceride, Wister rats

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Connection between nutrition and health has been probably understood, at least to some extent, among all people of all places and times. For instance; around 400 BC the statement "Let food be your medicine and let medicine be your food" was advised by the father of medicine, Hippocrates, over two millennia ago. In 1968, one of the great minds of this century, twice Noble prize winner, Linus Pauling, coined the term 'Orthomolecular Nutrition'. Orthomolecular is literally, 'pertaining to the right molecule'. Pauling proposed that by giving the body the right molecules (optimum nutrition) most diseases would be eradicated<sup>1</sup>. In addition, foods like soy bean (*Glycine max*) have been reported to have useful therapeutic effects on heart disease<sup>2</sup>.

Hyperlipidemia, a broad term, also called hyperlipoproteinemia or lipemia is the term used to denote raised serum levels of one or more of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), or both TC and TG (combined hyperlipidemia). Dyslipidemia is a broader term that also includes low levels of high-density lipoprotein cholesterol (HDL-C). Hyperlipidemia can also be defined as concentration of lipid in the blood of a fasted (>12 h) patient that exceeds the upper range of normal for that species. These fats are important for our bodies to function but when they are high, they can cause heart disease and stroke<sup>3</sup>. Hyperlipidemia is manifested as hypercholesterolemia and/or hypertriglycerolemia. However, hypercholesterolemia is the most common.

Hyperlipidemia results from abnormalities in lipid metabolism or plasma lipid transport or a disorder in the synthesis and degradation of plasma lipoproteins<sup>4</sup>. Common secondary causes of hypercholesterolemia are hypothyroidism, pregnancy and kidney failure while common secondary causes of hypertriglyceridemia are diabetes, excess alcohol intake, obesity and certain prescription medications<sup>5</sup>. Nevertheless, there is also a genetic predisposition to hyperlipidemia i.e. it can be hereditary<sup>6</sup>. One has a greater chance of developing hyperlipidemia if age is > 45 years in males and > 55 years in females, which in addition comes with its complications.

The consequence of hyperlipidemia is that with time it can cause atherosclerosis, and thus the risk of coronary artery disease (CAD) and stroke is increased. Hyperlipidemia in general has no apparent symptoms but it can be discovered and diagnosed during routine examination or evaluation for atherosclerotic cardiovascular disease. Patients who have dyslipidemia usually present with established cardiovascular disease which includes: angina pectoris, myocardial infarction (heart attack), stroke, peripheral vascular disease, claudication and transient ischemic attacks.

Different classes of drugs are used to treat hyperlipidemia. These classes differ not only in their mechanism of action but also in the type of lipid reduction and the magnitude of the reduction. Statins, the most common group of antihyperlipidemic drugs lowers cholesterol by interrupting the cholesterol biosynthetic pathway. On the other hand, fibrates decrease fatty acid and triglyceride levels by stimulating the peroxisomal  $\beta$ -oxidation pathway<sup>6</sup>. However, these drugs have different side effects including muscle inflammation and breakdown (muscle wasting).

The incidence of cardiovascular disease is on the increase despite the availability of a range of modern medical treatment. Most drugs used for the management of this condition have side effects; therefore there is a need to evaluate medicinal plants for possible lipid lowering activity since they are less toxic than orthodox drugs and also harmonizes with the body system quite well. *Glycine max* was chosen for this study based on its nutritive value and its worldwide availability. We hope to develop simple, sensitive, rapid and economic methods of preventing and possibly treating hyperlipidemia with a simple and common legume. The aim of this research work was to evaluate the anti-hyperlipidemic effect of crude methanolic seed extract of *Glycine max* (soya bean) in hyperlipidemic rats.

## Materials and methods

### Collection and authentication of seed

Fresh samples of *Glycine max* seeds were purchased from New market in Enugu, Enugu state, Nigeria.

#### *Processing of Glycine max seed powder*

*Glycine max* seeds were steeped in water for 24 hours to loosen the back. After dehulling, the seeds were washed three times with clean water to remove debris and sand and then dried under a shed (below 40°C) until they were completely dried. The dried seeds were milled with an electric blender and finally ground into powder using a hammer mill (500# grinder/Fuyu metal, Linyi fuyu metal product Co. Ltd., China) and thereafter, passed through 52 mm sieve (Turgens and Co., Germany)

#### *Preparation of high-cholesterol diet (HCD)*

A mixture of 75 g of commercially available cholesterol powder and 9 g of sodium deoxycholate (bile salt added to increase bioavailability) was dissolved in coconut oil and made up with the same solvent to 300 ml to give 250 mg/ml.

#### *Preparation of atorvastatin solution*

Ten tablets of 10 mg (i.e. 100mg) atorvastatin obtained from *Pfizer*® Inc., New York, USA were ground to powder, dissolved in distilled water and made up to 50 ml mark in a volumetric flask to give a stock concentration of 2 mg/ml.

#### *Preparation of carbimazole solution*

25 tablets of 5 mg (i.e. 125 mg) carbimazole obtained from *Hovid*® Inc., Malaysia were ground to powder, dissolved in distilled water and made up to 500 ml in a measuring cylinder to give a stock concentration of 0.25 mg/ml.

#### *Preparation of crude methanol extract*

The powdered seeds (800 g) of *Glycine max* was weighed out and macerated in 4 liters of absolute methanol in a gallon and left for 48 hours. The mixture was intermittently agitated during the extraction process. After 48 hours, the mixture was sieved using muslin cloth and filtered with Whatman No.1 filter paper and filtrate then evaporated to dryness on a rotary evaporator (ByllyCHI Rota vapour<sup>®</sup> model R-215, Switzerland). The residue (20 g) obtained was stored in a refrigerator at 4±2°C until required. This was labeled the crude methanol extract of *Glycine max* (MEGM). There was reconstitution to appropriate concentration with water prior to administration.

#### *Determination of extractive value for crude methanol extract*

The concentration of the extract was determined by measuring out 1 ml of the extract and fraction each and then allowing it to dry completely by

heating. The dry residue is then weighed to obtain the concentration which is expressed in g/ml. The yield afforded methanol crude extract of 100 mg/ml. The appropriate concentration then was calculated for the study.

#### *Experimental animals*

##### *Animal housing management*

Twenty-five apparently healthy adult male albino rats about three (3) months old were used for the research. They were obtained from the animal house of the college of medicine, University of Nigeria Teaching Hospital (UNTH) Enugu. The rats were divided into five (5) groups. Groups: A, B, C, D, and E of five (5) rats per group according to their body weights (150±20g) and each group caged separately in clean steel gauzed cages. They were housed under standard condition of temperature (28±3°C) and a 12 hours light / 12 hours dark cycle at the animal house of the college of medicine, UNTH Enugu. They were allowed to acclimatize for a period of two (2) weeks. The rats were fed with standard pellets (Top feed Nigeria) and clean water *ad libitum*. The cages were cleaned daily and food and water changed daily. All the animals were handled in this study according to Institutional guidelines describing the use of rats and in accordance with the American Physiological Society guiding principles for research involving animals and human beings<sup>7</sup>.

##### *Acute toxicity testing*

A modification of the method described by Lorke<sup>8</sup>, for the determination of LD50 was used. The experiment was carried out in two phases using adult Albino rat (weighing 20–25 g). In the first phase, three different doses of the MEGM were administered to three groups of three animals each. Groups A, B and C received 10, 100 and 1 000 mg/kg body weight of the extracts respectively after an overnight fast. They were observed for a 24 hour period for signs of acute toxicity such as dullness, depression, diarrhea and death. From the results in phase one, the second phase was performed using doses of 1500, 2500 and 3500 mg/kg body weight respectively for three groups of four animals per group. This procedure was done for both the aqueous and methanolic extracts.

##### *Phytochemical test*

Phytochemical test was carried out on crude methanol extract using standard procedure to identify the constituent. Alkaloids and phenols were determined according to the methods of Harbone<sup>9</sup>; tannin was determined using the method of Van-

Burden and Robinson<sup>10</sup>. Saponin was determined using the method of Obadoni and Ochuko<sup>11</sup>; flavinoids were determined according to the method of Boham and Kocipai<sup>12</sup>.

#### Induction of hyperlipidemia

Each experimental rat was administered with high cholesterol diet (HCD) at the dose of 2000 mg/kg through an oral gauge every morning for two weeks.

#### Experimental design

The rats were divided into five (5) groups:-

**Group A:** received 1.35 mg/kg of carbimazole, 2000 mg/kg of HCD and 400 mg/kg (high dose) of crude MEGM.

**Group B:** received 1.35 mg/kg of carbimazole, 2000 mg/kg of HCD and 200 mg/kg (low dose) of crude MEGM.

**Group C:** received 1.35 mg/kg of carbimazole, 2000 mg/kg of HCD and 20 mg/kg of atorvastatin. This served as positive control group.

**Group D:** received 1.35 mg/kg of carbimazole, 2000 mg/kg of HCD with neither extract nor drug. This served as the negative control group.

**Group E:** was given neither (carbimazole nor HCD) and no treatment. This group served as the normal control.

#### Acute and sub acute study and collection of blood from animal

At the expiration of two weeks, fasting blood samples were collected from the axillary vein under chloroform anesthesia. The blood samples collected into plain tubes were centrifuged to separate serum for estimation of lipid profile (Total Cholesterol, HDL cholesterol, LDL Cholesterol, VLDL Cholesterol and triglyceride).

#### Biochemical analysis

##### Measurement of serum lipid profile

The serum lipid parameters were estimated by the enzymatic end point (kit) method.

**Triglyceride (TG):** Enzymatic method for triglyceride as described by Fossati and Prencipe<sup>13</sup>.

**Total cholesterol (TC):** Enzymatic method for total cholesterol according to Fredrickson et al.<sup>14</sup>

**High density lipoprotein (HDL):** Precipitation method as described by Albers et al.<sup>15</sup>

**Very low density lipoprotein (VLDL):** VLDL was calculated using the equation.

$$VLDL = \frac{TG}{2.2}$$

**Low density lipoprotein (LDL):** LDL cholesterol was calculated using the Friedewald's equation.

$$LDL = TC - (VLDL + HDL)$$

#### Results

The result of the preliminary phytochemical analysis of soy bean is presented in the table 1.

**Table 1: Preliminary phytochemical analysis**

|                  |     |
|------------------|-----|
| Carbohydrate     | ++  |
| Reducing Sugar   | ++  |
| Alkaloids        | +++ |
| Glycosides       | ++  |
| Saponins         | +++ |
| Tannins          | ++  |
| Flavonoids       | ++  |
| Resins           | -   |
| Proteins         | +++ |
| Oils             | ++  |
| Acidic Compounds | ++  |
| Terpenoids       | ++  |
| Steroids         | +   |

+++ = More intensely present; ++ = Present; + = Present (in trace amount); - = Absent

Serum TC, HDL, LDL, TG and VLDL levels in all groups are shown in table 2. The levels of TC, LDL, TG and VLDL were highly elevated significantly in the affected group (HCD alone) when compared with normal control ( $p < 0.001$  or  $p < 0.01$ ). Administration of high dose MEGM (400 mg/kg), low dose MEGM (200 mg/kg) and atorvastatin (20 mg/kg) separately in the presence of HCD challenge significantly lowered the elevated levels of TC ( $p < 0.05$ ), LDL ( $p < 0.001$ ), TG ( $p < 0.01$ ) and VLDL ( $p < 0.01$ ) when compared to the affected group. Furthermore and worthy of note, the levels of HDL was significantly reduced in the affected group (HCD alone) in comparison with normal control ( $p < 0.01$ ); however the administration of high dose MEGM (400 mg/kg) and low dose MEGM (200 mg/kg) separately in the presence of HCD challenge significantly increased the reduced HDL levels ( $p < 0.001$  and  $p < 0.05$ ; respectively) when compared to the affected group.

**Table 2:** Statistical comparison of serum lipid profile parameters in different experimental animal groups

| Treatment               | Serum TC (mmol/L) | Serum HDL (mmol/L) | Serum LDL (mmol/L) | Serum TG (mmol/L) | Serum VLDL (mmol/L) |
|-------------------------|-------------------|--------------------|--------------------|-------------------|---------------------|
| MEGM (400 mg/kg)        | 2.227 ± 0.120**   | 0.783 ± 0.088***   | 1.273 ± 0.018***   | 0.373 ± 0.043**   | 0.170 ± 0.020**     |
| MEGM (200 mg/kg)        | 2.077 ± 0.043***  | 0.507 ± 0.041*     | 1.383 ± 0.039***   | 0.410 ± 0.032**   | 0.187 ± 0.015**     |
| Atorvastatin (20 mg/kg) | 2.193 ± 0.105 **  | 0.700 ± 0.061**    | 1.277 ± 0.018***   | 0.477 ± 0.107**   | 0.217 ± 0.049**     |
| HCD Alone (2000 mg/kg)  | 2.863 ± 0.101     | 0.273 ± 0.038      | 2.047 ± 0.057      | 1.197 ± 0.280     | 0.543 ± 0.095       |
| Normal Control          | 1.850 ± 0.040***  | 0.503 ± 0.026**    | 1.220 ± 0.015***   | 0.280 ± 0.061**   | 0.127 ± 0.027**     |

Values given as Mean ± SEM. \*\*\*P< 0.001, \*\*P< 0.01 or \*P<0.05 is significant when high dose MEGM (400mg/kg), low dose MEGM (200mg/kg), atorvastatin (positive control) or normal control is compared with HCD alone (negative control).

## Discussion

Recent researches of the health benefits of soy foods and soya bean containing several bioactive compounds have received significant attention to support the health improvements or health risks observed clinically or *in vitro* experiments in animals or human. In this particular study, high cholesterol diet caused an elevation in the serum concentrations of total cholesterol, triglyceride and LDL cholesterol, however both high and low doses of the methanolic extract of *Glycine max* (MEGM) showed a significant reduction in serum concentrations of total cholesterol (TC), triglycerides (TG) and low density lipoprotein cholesterol (LDL-C), with a significant increase in high density lipoprotein cholesterol (HDL-C), dependent on the serum cholesterol levels.

The understanding of the impact of thyroid hormones on lipid metabolism has been considerably improved. Thyroid hormone plays an important role in the regulation of lipid metabolism. Thyroid hormone deficiency represents a well-known cause of hypercholesterolemia in hypothyroid patients<sup>16</sup>. Therefore, carbimazole (an anti-thyroid hormone drug) was used in this study to cause a thyroid hormone deficiency which enables hypercholesterolemia to develop faster in the male albino wister rats. Administration of high cholesterol diet coupled with the antithyroid hormone drug, carbimazole caused a marked rise in the serum concentration of total cholesterol, triglyceride and LDL cholesterol, and significantly reduced the HDL cholesterol. Atorvastatin, as expected, significantly lowered the serum concentrations of TC, LDL and triglycerides, with an increase in HDL. The extract showed a very similar effect on the lipid profile with the lower extract concentration decreasing the total cholesterol more than the drug, and the higher concentration increasing the HDL a little higher than the

drug, which seems to be responsible for the slightly higher total cholesterol concentration. Also, the high extract concentration group showed equal effect on the LDL as the drug. Although the mechanisms of this action by soy bean has not been fully elucidated or not fully understood. However, the plausible explanation for the anti-hyperlipidemic action of MEGM could be attributed to the singula or combined hypolipidemic properties of one or more phytochemicals present in soybeans, such as isoflavones, peptides, flavonoids, phytic acid, soy lipids, soyasaponins, vitamins and more<sup>17</sup> which are believed to be responsible for the observed changes.

Soy isoflavones also called phytoestrogens<sup>18</sup>, have a wide range of biochemical activities that probably act synergistically with the soy peptides to mediate favorable effects on lipid metabolism. Some of these include: potent inhibition of protein tyrosine kinase, endothelium dependent relaxation of arteries, action to regulate the expression of peroxisome proliferator-activator receptor gamma (PPAR-γ) and a variety of effects on insulin sensitivity<sup>18</sup>. Some researchers suggest that alteration in the ratio of serum glucagon to serum insulin may affect cholesterol metabolism<sup>17</sup>. They also have antioxidant activities and may decrease digestion and absorption of cholesterol through a direct inhibitory action on the digestive enzymes<sup>20</sup>. Setchell suggests that soy estrogens may contribute to the cholesterol-lowering effect of *Glycine max* since the administration of oral estrogens decreases serum cholesterol and LDL cholesterol concentrations<sup>21</sup>. This was supported by Anthony et al<sup>22</sup>. Soy peptides have also been suggested to play a major role in the anti-hyperlipidemic effects of *Glycine max*. According to Carroll, the amino acid composition of soy affects serum cholesterol concentrations; increases in arginine are accompanied by decreases in serum cholesterol concentrations<sup>23</sup>.

The hypocholesterolemic effects of soy soya saponins have long been recognized<sup>24</sup>. Two mechanisms by which saponins can affect cholesterol metabolism were suggested. Firstly, some saponins form insoluble complexes with cholesterol and inhibit intestinal absorption of both endogenous and exogenous cholesterol. Secondly, saponins can interfere with the enterohepatic circulation of bile acids by forming mixed micelles, thereby effectively blocking the re-absorption of bile acids from the terminal ileum<sup>25</sup>.

Phytosterols have long been known to reduce intestinal cholesterol absorption, leading to decreased blood LDL-cholesterol levels and lower cardiovascular disease risk. They are not systemically absorbed, and so are thought to act primarily in the intestinal lumen. As cholesterol analogs phytosterols compete for cholesterol in absorptive micelles resulting in reduced solubility of cholesterol<sup>26</sup>. A number of studies have proposed a phytosterol / phytostanol action on cholesterol esterification and lipoprotein assembly (ACAT, apoB), cholesterol internalization (NPC1L1, ANXA2), cholesterol synthesis (HMGCoA reductase, C-24-reductase) and removal of apoB100-containing lipoproteins (LDLr)<sup>27</sup>.

Phytate (IP6), though a major source of phosphorus in soy, is a strong chelator of important minerals such as calcium, magnesium, iron, and zinc, and can contribute to mineral deficiencies in people, as well as decreasing enzyme activity<sup>28</sup>. However, recent studies demonstrate that this "anti-nutrient" effect of IP6 is only manifested when large quantities of IP6 are consumed<sup>29</sup>. This could explain why the lower dose of the MEGM lowered the total cholesterol more than the higher dose. However, the higher dose caused a greater increase in HDL to compensate for the higher lipid content.

HDL increase as seen in the extract group may, however, be attributed to increase in the amount of ApoA1 level in the liver which is a main component protein of HDL<sup>30,31</sup>. Also, inhibition of endothelial lipase (EL) by MEGM could be the cause of the elevated level of HDL observed in this study<sup>32</sup>. Endothelial lipase is an enzyme that degrades and metabolizes HDL with decreased phospholipids.

The result of this study indicates that soya bean is effective in preventing increases in serum lipid concentrations and consequently preventing atherosclerosis and its associated coronary heart disease since it is generally accepted that elevated serum total cholesterol, elevated triglyceride, high LDL and low HDL are positively correlated to the

risk of coronary heart disease. This goes a long way to support the FDA claim that "25g of soy protein a day, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease"<sup>33</sup>. Similar results were also observed in a study conducted by Ikenna et al<sup>34</sup>, where administration of soymilk under high cholesterol diet challenge in rats significantly attenuated renal injury and prevented dyslipidemia in the animals<sup>34</sup>. The observed protection by soymilk could be as a result of the hypocholesterolemic effect and the antioxidant activity of soy. This may be due to individual (singular) or combined actions of one or more phytochemicals present in soy; such as isoflavones, phytic acid, soya saponin, phytosterol<sup>34</sup>.

### Limitation

Cardiac markers such as: troponin, LDH, myoglobin, CRP, AST, etc should have been measured to truly ascertain soy's true cardio protection; however the scope of our work did not permit it.

### Conclusion

In conclusion, observation from this study indicates that soya bean is effective in preventing increase in serum lipid concentrations and consequently preventing atherosclerosis and its associated coronary heart disease.

### Recommendations

Clearly, the observed health effects from soy or soy-based foods (other than those attributed to nutrients like protein) are not solely from the actions of certain individual or types of compounds, but rather are due to the mixed effects of different compounds. Additive, synergistic, and/or antagonistic effects of different soy components combine to provide a final effect of soy foods and these effects may also be altered by phytochemicals from other non-soy foods in the meal. In order to fully understand the mechanism of health effects of soy, we recommend that it is important to identify the genuine bioactive compounds in soy. Also, the mechanisms behind the hypocholesterolemic effects such as changes in cholesterol and bile acids absorption and reabsorption, and whether they are excreted in the feces should be investigated further. More studies should be carried out on soy bean to further reveal its anti-hyperlipidemic properties and cardio-protective properties in the prevention of atherosclerosis. Daily dose of exercise and a healthy balanced meal should become high priority amongst the populace. This is where supplementation and nutritional awareness can play a vital role in prevention of some disorders such as hyperlipidemia and CVD. Awareness should be

spread among people especially those who lead or are bound to sedentary life style. Also if possible, supplementation with soy (*Glycine max*) with at least moderate physical activity can guarantee effective prevention and improvement in cardiovascular disease. It is important to enrich our diet with anti-oxidants rich food such as soy milk to protect against many chronic diseases. Further characterization and purification of the phytochemicals in soy for pharmaceutical benefits should be done.

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