EFFECT OF PROTEINOUS AND NON-PROTEINOUS FRACTIONS ISOLATED FROM *PHASEOLUS VULGARIS* AND *VIGNA SINENSIS* FRUITS ON DIABETIC MICE

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This study is concerned with the preparation of cold and boiled aqueous extracts from the fruits of both *Phaseolus vulgaris* and *Vigna sinensis* plants, then isolating the proteinous compounds from these extracts by cold acetone precipitation method and therefore to separate the non-proteinous fraction. The work included the study of the effect of intraperitoneal administration of cold and boiled aqueous extracts, proteinous acetone precipitates, and non-proteinous materials isolated from these extracts on certain blood biochemical constituents (parameters) using a dose of 77 mg kg^{-1} of body weight in normal and alloxan-induced diabetic mice. The results had been compared with those injected with insulin. The results showed that the boiled crude aqueous extract of *Vigna sinensis* plant and its isolated proteinous precipitate has a significant decrease effects for the level of the glucose and total lipids in the blood. On the other hand, the non-proteinous substance of cold aqueous extract of *Phaseolus vulgaris* fruit has a decrease effect for the level of glucose, cholesterol and total lipids in the blood serum of alloxan-induced diabetic mice, therefore may be used in the treatment of diabetes mellitus after make sure there is no side effects as well as we concluded that these materials mentioned above may be used in the treatment of diabetes mellitus .

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Introduction

It is well known that the incidence of diabetes mellitus is high all over the world, especially in Asia. Different types of oral hypoglycemic agents such as biguanides and sulphonylurea are available along with insulin for the treatment of diabetes mellitus,[1] but have side effects associated with their uses,[2,3] therefore it is increasing interest in herbal remedies because of their effectiveness, minimal side effects and relatively low costs.

Herbal drugs or their extracts are prescribed widely, even when their biological active compounds are unknown. Even the world health organization (WHO) approves the use of plant drugs for different diseases, including diabetes mellitus. Therefore, studies with plant extracts are useful to know their efficacy and mechanism of action and safety. Medicinal plants useful in diabetes were reviewed recently.[4,5]

The plant *Phaseolus vulgaris* is commonly called kidneys-bean, French-bean in English. This plant and *Vigna sinensis* plant (cow-pea in English) belongs to *Fabacea* family.[6,7] These plants is reputed to possess varied medicinal properties.[7,8,9]

In the present study, an attempt has been made to study the hypoglycemic, hypocholesterolemic, hypolipidemic effect of *Phaseolus vulgaris* and *Vigna sinensis* fruits proteinous and non–proteinous aqueous extracts, also proteinous precipitate material on normal and alloxan-induced diabetic male mice.

Material and methods

Plants material

Fruits of *Phaseolus vulgaris and Vigna sinensis* plants were purchased from local of agriculture in Mosul city, cleaned and kept in nylon bags in a deep freeze till the day of experiment.

Preparation of crude extracts

The aqueous crude extract was prepared by freezing and thawing (500 g) of the fruits after cutting to small pieces with liquid nitrogen several times to rupture the cell membrane. Cold distilled water in a ratio of 1:3 w/v, was added and the mixture was then homogenized for 10 minute using a blender. The crude homogenate was stirred for additional two hours on ice bath , then filtered through several layers of shash . Finally the mixture was centrifuged using refrigerated centrifuge for 15 minutes at 33520xg. The filtrate of cold crude extracts after reduction its volume to about third ratio by lyophilization was kept for further investigation(precipitation of the protein) . Total protein concentration was determined for this filtrate of cold crude extract by modified Lowry method.[10] This procedure was repeated using (400 g) of the fruits until after homogenized with a blender step, the mixture was boiled for 30 minutes and then leaved to cool, filtered through several layers of shash, centrifuged and lyophilized as indicated above this filtrate which was produced called boiled aqueous crude extract. At the second time, cold and boiled aqueous extract was prepared also using (300 g) fruits of each plants mentioned before were produced as indicated in all steps which was mentioned above till producing filtrate , then this filtrate were lyophilized until drying and produced a powdered materials, kept in a deep freeze at -20ú c in a tight sample tube until used for the next step, which is the

injection of animals experiment This powdered material called cold or boiled crude aqueous extract material (CCAEM) or (BCAEM) respectively.

Precipitation of the proteins

Proteinous materials were separated from cold, boiled aqueous extracts using cold acetone precipitation method.[11] This method was performed by adding cold acetone gradually to extract in a ratio (40:60, v/v) respectively with slow stirring at 0 °C. The mixture was left in a refrigerator for 24 hours and the precipitated protein was isolated by centrifugation for 20 minutes at 33520 x *g* at a refrigerated centrifuge. The proteinous precipitate material (PPM) was dried in a lyophilizer to remove trace amount of aqueous acetone, then kept in a tight sample tube in a freezer for the next step. At the same time, acetone was removed from the remained filtrate after precipitation of proteins by rotary evaporator (<40 °C), the remaining filtrate was dried in a lyophilizer till dryness. The powdered material which was produced called non-proteinous material (NPM). This materials was then kept in a tight sample tube in a freezer for the next step.

Experimental animals

Healthy adult male albino mice weighing 25-35 g were obtained from animal house of the Veterinary Medicine College, University of Mosul and housed under controlled conditions of light (14h light and 10 h dark) and temperature (25±2 °C commercial pelleted food and water were given *adlibitum*.

Determination of the effective dose

The animals were randomly divided to 5 groups (3 mice/group). The mice were fasted for 16 h before the experiment was started. *Group 1* (control , normal) injected intraperitoneal with 1 ml normal saline. *Groups (2-6* , normal) were injected intraperitoneal with different doses (50 , 77, 100, 150) mg kg^{-1} body weight (b.w.), respectively with each of solution in normal saline of the materials of cold crude aqueous extract material (CCAEM), proteinous precipitate material (PPM) of *Vigna sinensis* fruits plant. After two hours of injections blood samples were collected for analysis immediately (serum glucose level) by the orbital sinus puncture under ether anesthesia, using non-heparin zed micro-hematocrit capillary tubes. After determined the effective dose of these materials. This doses then it was used for the injection of normal and alloxan-induced diabetic mice.

Induction of diabetes in mice

Healthy adult male albino mice , weighing 25-35 g were selected and randomly divided into groups of 3 mice per group. They were fasted for 24 h before induction of diabetes. They were intraperitoneal injected with alloxan tetra hydrate which was dissolved in 1 ml sterile physiological saline solution immediately before use at a dose of 180 mg kg^{-1} b.w.[12] The diabetic state was monitored by periodic tests for glucosuria (Tes-Tape-Eli Lilly and Co., USA) and hypoglycemia (colorimetric assay kit, Syrbio, France), mice with blood glucose level more than 180 mg 100 ml^{-1} were considered diabetic and used fore the study. At the end of the period, three alloxan diabetic animals were randomly divided for each group for the present study.

Intraperitoneal injection of the mice

The mice (25-35 g weight) were divided randomly into 28 groups (14 for normal and 14 for diabetic mice) each group containing three mice. *Group one* was kept as control. The *second group* was injected subcutaneously with insulin (10 IU kg^{-1}, neutral insulin injection Act rapid 100 IU ml^{-1}, Novo Nordisk, Denmark), while the other groups were injected intraperitoneal with 77 mg kg^{-1} b.w. of crude (cold, boiled) aqueous extract material (CCAEM, BCAEM), non-proteinous materials (NPM) and proteinous precipitate materials (PPM) of *Phaseolus vulgaris* and *Vigna sinensis* fruits plants. The mice were fasted for 16 h before the experiment was started. After two hours of injection blood samples were collected from each mouse by the orbital sinus puncture under ether anesthesia using non-heparin zed microhematocrit capillary tubes.[13] Blood serum glucose, total cholesterol levels were measured using colorimetric assay kits type (Syrbio, France).[14] Total lipids was determined by colorimetric method.[15]

Statistical analysis

Statistical analysis was performed using one - way analysis of variance with a significance level of $p<0.05$. Further specific group differences were determined using Duncan's test,[16] the results were expressed as mean ± S.E.

Results and Discussion

Precipitation of the protein

Precipitation of total proteins from the crude aqueous extract was accomplished by cold acetone technique[11] but not by saturated ammonium sulphate technique.[17] Since the former can be easily removed by evaporation, besides the fact that the precipitation power of both reagents were similar. Moreover , dialysis of the proteinous fraction to get rid of ammonium sulphate may remove some of the low molecular weight protein or peptides. The amount of total protein in crude aqueous extract was determined[10] and the amount of the precipitated protein, efficiency of the precipitation method were shown in (Table 1). Higher amount of the protein was in the cold crude aqueous extract of *Vigna sinensis* fruits and found to be 1.868 %. Whereas, the lower amount was found in the boiled crude aqueous extract of *Phaseolus vulgaris* fruits which has efficiency of the precipitation of the protein is 86.11 % .

Determination of the effective doses for the plant extracts and proteins in normal mice :

The results in Tables 2 and 3 for crude cold aqueous extract material, proteinous precipitate of *Vigna sinensis* fruits plant shows that a dose 77 mg kg^{-1} b.w. mice give a significant decrease in the level of blood serum glucose more than that for other doses.

Table 1. Amount of total proteins in the crude aqueous extract of *Vigna sinensis* plants, *Phaseolus vulgaris* fruits plants, their percentage and efficiency of acetone method precipitation

Extract name symbol	Protein concn., mg ml^{-1}	Total volume in ml	Total protein in the extract, mg	Percent of the protein in plant, %	Weight of the plant, g	Total amount of the protein precipitated by acetone, mg	Efficiency of the precipitation method, %
CCAEM of *Vigna sinensis* fruits plant	5.75	1625	9343.75	1.868	500	5130	54.9
CCAEM of *Phaseolus vulgaris* fruits plant	4.30	1600	6880	1.376	500	4970	72.24
BCAEM of *Vigna sinensis* fruits plant	5.56	1000	5560	1.39	400	4710	84.7
BCAEM of *Phaseolus vulgaris* fruits plant	4.0	900	3600	0.9	400	3100	86.11

Table 2. Effective dose of cold crude aqueous extract of *Vigna sinensis* fruits plant on glucose level in normal mice.

*Conc. of serum glucose mmol L^{-1}	Control	Dose of cold crude aqueous extract in mg kg^{-1} of body weight			
		50	77	100	150
	5.48±0.12	4.73±0.24	3.67±0.7	4.32±0.11	5.62±0.31
% Change	-	-13.69	-33.03	-21.17	2.55

*Values are mean ± S.E.

Table 3. Effective dose of proteinous precipitate material for cold crude aqueous extract of *Vigna sinensis* fruits plant on glucose level in normal mice.

*Conc. of serum glucose mmol L^{-1}	Control	Dose of cold crude aqueous extract in mg kg^{-1} of body weight			
		50	77	100	150
	5.51±0.7	5.03±0.35	4.28±0.14	4.55±0.29	5.55±0.61
% Change	-	-8.7	-22.3	-17.4	0.7

*Values are Mean ± S.E.

Table 4. Effect of cold, boiled crude aqueous extracts, non-proteinous materials of *Vigna sinensis*, *Phaseolus vulgaris* fruits plants on serum glucose, cholesterol, total lipids levels in normal mice.

Treatment of groups	Glucose, mmol L^{-1}	% Change	Cholesterol mmol L^{-1}	% Change	Total lipids mg 100 mL^{-1}	% Change
Normal (control)	5.68±0.21 d	-	2.38±0.65 e	-	384.56±8.25 b	-
Insulin	1.84±0.72 a	-67.6	1.85±0.461 abc	-22.26	213.16±3.95 a	-44.57
CCAEM of *Vigna sinensis* fruits plant	4.36±0.17 b	-23.2	1.69±0.345 a	-28.99	487±6.817 c	26.63
CCAEM of *Phaseolus vulgaris* fruits plant	6.97±0.21 f	22.71	2.08±0.608 d	-12.6	516.1±9.29 d	34.46
BCAEM of *Vigna sinensis* fruits plant	4.8±0.14 bc	-15.49	2.13±0.553 d	-10.5	484.4±8.78 c	25.96
BCAEM of *Phaseolus vulgaris* fruits plant	4.35±0.16 b	-23.41	1.86±0.461 abc	-21.84	489.1±8.21 c	27.18
CNPM of *Vigna sinensis* fruits plant	6.41±0.51 e	-12.85	1.99±0.216 cd	-16.38	523.26±4.73 de	36.07
CNPM of *Phaseolus vulgaris* fruits plant	5.22±0.20 cd	-8.09	1.68±0.336 a	-29.41	389.13±6.46 b	-1.18
BNPM of *Vigna sinensis* fruits plant	6.47±0.10 f	13.91	1.76±0.964 ab	-26.05	541.13±6.45 e	40.71
BNPM of *Phaseolus vulgaris* fruits plant	6.4±0.15 e	12.67	19±0.65 bc	-20.16	381.93±4.49 b	-0.68

*Values are mean ± S.E., different letters vertically mean significant at $p<0.05$, each group include (3) mice, CCAEM, BCAEM, CNPM, BNPM symbols referred as in method

Table 5. Effect of cold, boiled crude aqueous extracts, non-proteinous materials of *Vigna sinensis*, *Phaseolus vulgaris* fruits plants on serum glucose, cholesterol, total lipids levels in alloxan-induced diabetic mice.

Treatment of groups	Glucose mmol L^{-1}	% Change	Cholesterol mmol L^{-1}	% Change	Total lipids mg 100 mL^{-1}	% Change
Normal (control)	5.68±0.21 b	-	2.38±0.65 c	-	384.56±8.25 cd	-
Control (diabetic)	18.17±0.11 fg	219	2.86±0.1 d	20.1	552.9±13.5 f	43.7
Insulin	2.4±0.77 a	-87.15	2.18±0.78 bc	-23.4	352.26±4.07 b	-36.28
CCAEM of *Vigna sinensis* fruits plant	17.67±0.24 f	-5.48	1.65±0.29 a	-42.2	379.06±8.97 c	-10
CCAEM of *Phaseolus vulgaris* fruits plant	19.35±0.2 g	3.51	3.01±0.71 d	5.14	533.1±14.3 ef	-3.57
BCAEM of *Vigna sinensis* fruits plant	13.31±0.17 c	-28.79	3.14±0.12 d	9.47	365.53±8.21 bc	-33.88
BCAEM of *Phaseolus vulgaris* fruits plant	16.9±0.16 ef	-9.6	2.24±0.37 bc	-21.8	368.46±14.28 bc	-33.35
CNPM of *Vigna sinensis* fruits plant	15.14±0.35 d	-19.02	2.01±0.1 abc	-29.9	520.7±9.2 e	-5.8
CNPM of *Phaseolus vulgaris* fruits plant	12.6±0.14 c	-32.62	1.99±0.81 ab	-30.4	404.36±5.56 d	-26.86
BNPM of *Vigna sinensis* fruits plant	15.7±0.17 de	-15.88	2.01±0.53 bc	-27.1	633.26±5.17 g	14.55
BNPM of *Phaseolus vulgaris* fruits plant	14.8±0.24 ef	-9.42	2.16±0.57 bc	-24.6	298.8±11.05 a	-22.3

*Values are Mean ± S.E., different letters vertically mean significant at $p<0.05$, each group include (3) mice, CCAEM, BCAEM, CNPM, BNPM symbols referred as in method

Table 6. Effect of proteinous precipitate materials isolated from cold, boiled crude aqueous extracts of *Vigna sinensis*, *Phaseolus vulgaris* fruits plants on serum glucose, cholesterol, total lipids levels in normal mice.

Treatment of groups	Glucose mmol L^{-1}	% Change	Cholesterol mmol L^{-1}	% Change	Total lipids mg 100 mL^{-1}	% Change
Normal (control)	5.68±0.21 d	-	2.38±0.54 d	-	381.3±8.3 c	-
Insulin	1.83±0.97 a	-67.78	1.84±0.44 c	-22.68	2.13.1±3.8 a	-44.11
PPM of CCAE of *Vigna sinensis* fruits plant	4.95±0.17 c	-5.96	1.32±0.67 a	-44.53	223.6±6.2 a	-41.34
PPM of CCAE of *Phaseolus vulgaris* fruits plant	3.6±0.2 b	-36.61	1.77±0.69 c	-25.63	383.2±4.7 c	0.49
PPM of BCAE of *Vigna sinensis* fruits plant	3.95±0.95 b	-30.45	1.58±0.25 b	-33.61	336.8±6.5 b	-11.67
PPM of BCAE of *Phaseolus vulgaris* fruits plant	5.61±0.12 d	-1.23	1.6±0.53 b	-33.77	478.4±5.2 d	25.47

*Values are Mean ± S.E., different letters vertically mean significant at $p<0.05$, each group include (3) mice , PPM of CCAE , PPM of BCAE, symbols referred as in method

Table 7. Effect of proteinous precipitate materials isolated from cold , boiled crude aqueous extracts of *Vigna sinensis*, *Phaseolus vulgaris* fruits plants on serum glucose , cholesterol , total lipids levels in alloxan-induced diabetic mice.

Treatment of groups	Glucose mmol L^{-1}	% Change	Cholesterol mmol L^{-1}	% Change	Total lipids mg 100 mL^{-1}	% Change
Normal (control)	5.68±0.21 b	-	2.38±0.54 b	-	381.3±8.3 b	-
Control (diabetic)	18.18±0.13 g	22	2.86±0.92 c	22.1	555.13±13.7 d	45.6
Insulin	2.42±0.63 a	-87.03	2.29±0.5 ab	-19.19	353.23±3.17 a	36.1
PPM of CCAE of *Vigna sinensis* fruits plant	14.46±0.31 c	-22.63	2.39±0.56 b	-16.7	410.1±9.31 bc	-25.82
PPM of CCAE of *Phaseolus vulgaris* fruits plant	8.66±0.35 c	-53.67	2.55±0.43 b	-10.87	594.9±4.73 e	7.62
PPM of BCAE of *Vigna sinensis* fruits plant	11.27±0.29 d	-39.73	2.52±0.6 b	-12.07	342.36±6.51 a	-38.07
PPM of BCAE of *Phaseolus vulgaris* fruits plant	15.86±0.17 e	-15.15	2.12±0.48 a	-26	424.16±6.64 c	-23.27

*Values are mean ± S.E., different letters vertically mean significant at $p<0.05$, each group include (3) mice , PPM of CCAE , PPM of BCAE, symbols referred as in method

This dose is more effective for decreasing the level of serum glucose in normal mice as an effective dose for normal and alloxan induced diabetic mice by intraperitoneal injection. The effective doses of this plant was also taken for *Phaseolus vulgaris* fruits plant.

Effect of aqueous extract , non-proteinous materials , proteins on some biochemical parameters on normal and alloxan-induced diabetic mice

The results of cold, boiled crude aqueous extracts, non-proteinous and proteinous precipitate materials for *Vigna sinensis*, *Phaseolus vulgaris* fruits plants on the level of glucose, cholesterol and total lipids blood serum in male normal and alloxan –induced diabetic mice were listed in Tables 4, 5, 6 and 7.

Effect of crude aqueous extract (cold, boiled) , non-proteinous materials of *Vigna sinensis, Phaseolus vulgaris* fruits plants on serum glucose level in normal and alloxan-induced diabetic mice.

Results depicted from Tables 4 and 5 indicated that treatment with cold, boiled crude aqueous extracts of *Vigna sinensis* fruits plant and boiled aqueous extract of *Phaseolus vulgaris* at a dose 77 mg kg^{-1} of b.w. intraperitoneal a significant decrease in the level of serum glucose on normal mice compared to their control group. Also this dose injection with boiled aqueous extract, cold and boiled non-proteinous materials of *Vigna sinensis* produced a significant decrease which is (28.79 %, 19.02 % and 15.88 %) respectively in the level of serum glucose in alloxan-induced diabetic mice.

The decreasing effect of these extracts in agreement with other studies.[18,19,20] This decrease may be due to these extracts may be contain an active materials which activate β-cells of pancreas to secretion insulin or may be containing materials acts as insulin to increase glycolysis in peripheral tissues or decrease gluconeogensis.[21]

Effect of crude aqueous extract (cold, boiled) , non-proteinous materials of *Vigna sinensis Phaseolus vulgaris* fruits plant on serum cholesterol level in normal, and alloxan-induced diabetic mice

Treatment with extracts and non-proteinous (cold, boiled) for fruits of each plants *Vigna sinensis*, *Phaseolus vulgaris* which was indicated in Tables 4 and 5 at a dose 77 mg kg^{-1} b.w. were give a significant decrease in the level of serum cholesterol on normal and diabetic mice compared to their control group, except the boiled crude aqueous extract of *Vigna sinensis* and cold for *Phaseolus vulgaris*. At the same time, boiled aqueous extract of *Phaseolus vulgaris* produce a significant decrease in cholesterol level in normal and diabetic mice and resembles the decrease of insulin and return the level of cholesterol for diabetic mice to their normal level compared to normal control mice group. This decrease may be due to the presence of saponin which lower blood cholesterol and increase rejecting of bile acids and neutral lipids out of the body[22] or may be due to inhibit hydroxyl methyl glutaryl-CoA reductase enzyme which is responsible for the biosynthesis of cholesterol.[23]

Effect of crude aqueous extract (cold, boiled) , non-proteinous materials of *Vigna sinensis, Phaseolus vulgaris* fruits plants on serum total lipids level in normal and alloxan-induced diabetic mice

Results for cold, boiled aqueous extracts of *Vigna sinensis*, *Phaseolus vulgaris*, non-proteinous (cold, boiled) for fruits of *Phaseolus vulgaris* and cold for *Vigna sinensis* showed a significant decrease in total lipids level when injected in diabetic mice. These results are in agreement with the results indicated for decreasing of total lipids level in alloxan-induced diabetic rats for cold and boiled aqueous extract of *Melia azedarach* and *Lactuca serriola* leaves.[19] These extracts may be containing compounds which acts similar to the action of insulin, this insulin inhibit lipolysis of lipids during its effect on glucagon and catecholamine hormones which are increase lipolysis.[24]

Effect of proteinous precipitate materials for *Vigna sinensis, Phaseolus vulgaris* fruits plants on serum glucose, cholesterol and total lipids level in normal and alloxan-induced diabetic mice

Treatment with the proteinous precipitate materials for cold, boiled crude aqueous extracts of *Vigna sinensis, Phaseolus vulgaris* fruits plants at a dose 77 mg kg^{-1} b.w. intraperitoneal in male normal and diabetic mice were indicated in Tables 6 and 7. The results were showed a significant decrease in the level of serum glucose at different ratio, the proteinous precipitate material which was isolated from cold aqueous extract of *Phaseolus vulgaris* was showed a higher decrease percent (36.63 %) for normal mice and a decrease (53.6 %) for diabetic mice. These results in agreement with the results for cold and boiled aqueous extract of *Oleo europae* leaves for chickens.[25] This decrease may be due to activate beta cells of pancreas to secretion of insulin hormone or may be these proteinous precipitate act to increase biosynthesis of liver glycogen from glucose as the action of *Momordica charantica* fruit extract when given to mice, this extract showed a decrease of glucose level in normal and streptozotocin-induced diabetic mice.[26]

The decrease effect of the proteinous precipitate materials of cold and boiled of each plants used in diabetic mice which is similar to a decrease of insulin and which are reached to normal level value of cholesterol may be due to occurrence changes in the flora of intestine who have the role to increase the excreted bile acids and then decreased serum cholesterol level.[27] On the other hand, a significant decease effect were produced by cold, boiled proteinous materials of *Vigna sinensis* fruits plant, but the *Phaseolus vulgaris* proteinous (cold, boiled) material showed a significant increase for the level of serum total lipids on normal and diabetic mice. This increase may be due to possess these precipitate an effect similar to glucagon or epinephrine hormones in action which were activate lipolysis of lipids during the activation of lipase enzyme which increase lipolysis in adipose tissues[28]. It was concluded that boiled crude aqueous extract material and its proteinous precipitate material which was isolated from it for *Vigna sinensis* fruits plant possess a decreasing effect for glucose and total lipids in blood, also cold non-proteinous material which due to *Phaseolus vulgaris* fruits plant possess a hypoglycemia, hypocholesterolemia and hypolipidemia effect which are used for the treatment of diabetes mellitus after sure there is no side effect.

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