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Effect of Some Fruits, Vegetables and Legumes on the Immunity of Diabetic Rats

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The use of traditional plant for diabetes mellitus is widely practiced in Middle East countries. The aim of this investigation is to evaluate the biological effect of fruits, vegetables and legumes on lowering the level of glucose in blood and enhancement of the immune system. Thirty-two male albino rats (130 – 200 g) each were used in the study, 28 of rats diabetes by single intraperitoneal injection of alloxan at dose level of 150 mg / kg body weight. All rats (normal and diabetic) were kept on casein basal diet for 4 weeks. They were then divided into 5 groups. At the end of experiment the weight gain was calculated. Organs (liver – heart – kidney – spleen – lungs) of each rat were removed rapidly and were weighted separately. Blood samples were used for estimation of fasting serum glucose, serum GPT and GOT, serum lipid profile, kidney function, cellular and humoral immunity. Data showed that serum GOT and GPT levels declined significantly (p<0.01) in all treated groups that fed on 20% powder and extract when compared with diabetic control positive, while feeding rat on basal diet containing 20% vegetables extract, legumes powder and fruits extract revealed more hypoglycemic effect. The best results for immunity were 20% fruits extract and vegetables group.

Introduction

Diabetes mellitus is a group of diseases characterized by a defect in insulin secretion and increased cellular resistance to insulin, resulting in elevated plasma glucose levels, abnormalities carbohydrate and lipid metabolism, characteristic pathologic changes in the nerves and small blood vessels, and aggravation of atherosclerosis (Lee and Nieman, 2003). Since 1932, diabetes mellitus has been among the top 10 leading causes of death in America.

It is a major cause of blindness, renal failure, congenital malformation, and lower extremity amputation. The prevalence of coronary artery disease and peripheral vascular disease is twice as common among persons with diabetes, as compared with those without diabetes (American Diabetes Association, 2014).

In 1997, an estimated 124 million people worldwide had diabetes, 97% of these

having type 2 diabetes, otherwise known as non- insulin –dependent diabetes mellitus (NIDDM) or adult – onset diabetes – type 2 diabetes is most prevalent in the adult population, afflicting approximately 17% of people aged greater than 65years (Winlers, 2006). Obesity is a strong predisposing factor for type 2 diabetes. Berry polyphenols may help prevent obesity by inhibiting digestive enzymes, such as lipase, thereby lowering fat absorption (McDougall, Kulkarni, and Stewart, 2008). Hypoglycemic effect of bilberry is a desirable effect for helping to prevent or control type 2 diabetes, which is a highly prevalent condition caused by insulin resistance and B cell failure (ADA, 2010). Type 2 diabetes is associated with increased oxidative stress, inflammation, and dyslipidemia, and is accompanied by an increased risk of CVD, cancer, and vision loss through cataract and retinopathy (Choi et al., 2008; ADA, 2010).

The use of traditional plant for diabetes mellitus is widely practiced in Middle East countries. The world health organization (WHO) has recommended that this should be encouraged, especially in countries where access to the conventional treatment of diabetes is not adequate (WHO, 1980). The berry has various other reputed health benefits, although most interest has been focused on anthocyanin-related antioxidant effects (Park et al., 2007). There are many studies that have investigated the antioxidant and other health-related effects of anthocyanin -rich berries and extracts or juices. bilberry extract protected cells against oxidative damage., bilberry or anthocyanin extracts of bilberry protects rat liver microsomes against oxidative damage and apolipoprotein B against ultraviolet (UV)-induced oxidative fragmentation (Valentova et al., 2006). The bilberry plant is reputed to possess antidiabetic properties,

and its berries and leaves (as well as those of other Vaccinium species) have been used for centuries to ameliorate the symptoms of diabetes.grape seed extracts were effective in increasing CAT and GPx activation in the liver tissue, but not so effective in controlling the formation of lipid peroxides within the liver tissue (Cravotto et al., 2010).

A number of herbal products are known to facilitate enhancement of the immune system. These include Echinacea, garlic, cats claw, astragolus, licorice and other herbs. Flavonoid– rich and carotenoid – rich herbs may be expected to enhance the immune system (Tyler, 1994).

Materials and Methods

Studied ingredients were prepared prior to inclusion in diets as follows:

Fruits, Vegetables and Legumes were cleaned, sun- dried under vacuum at low temperature) and milled to fine powder then added at 20 % level to diets.

Preparation of plant extracts

The clean fruits, vegetables and legumes were ground using porcelain grinder to pass through sievemesh pores of 1mm diameter and used as powdered dried plants.

Sample (200 g) of fruits + 1000 ml distilled water, 200 g sample of
vegetables + 1000 ml distilled water, 200 g sample of legumes +
condensers then boiled for one hour to obtain the extracts.

The boiled mixture was coded and filtered.

The filterate poured in different petri dishes and dried under vacuum at 70 ^{o} c to dried

powder. The powder has been dissolved in certain amount of distilled water. The amount of solution equivalent to 20 % dried was administered orally using a tube.

Biological investigation

Animals: Thirty-two (32) Sprague – Dawley male albino rats weighting 130 – 200 g each, were used in this study. All rats were fed the control (casein) diet for4 consecutive days. Each rat was housed in an individual stainless steel cage under controlled condition. Diets were introduced to rats in a special non- scattering feeding cup to avoid loss of food and contamination. Tap water was provided to rats by mean of glass tubes projecting through wire cages from inverted bottles supported to one side of the cage.

Induction of diabetes in rats

Alloxan: Pure chemical fine (BDH, obtained from Sigma) was used for inducing diabetes in this study. Untreated rats are referred to as the control negative (control-group), while alloxan treated rats are the control positive (control + group); these are groups 1 and 2 respectively. Diabetes was induced in normal healthy male albino rats via intraperitoneal injection of alloxan 150mg/kg body weight according to the method described by Desai and Bhide, (1985). Six hours after the injection of alloxan, fasting blood samples were obtained by retro-orbital method to estimate fasting serum glucose. Rats having fasting serum glucose more than 200 mg /dLwere considered diabetics NDDG, (1994).

Diets

Basal diet was prepared according to Reeves *et al*., (1993). It consists of 20 % protein, 10 % sucrose, 4.7% corn oil, 2% choline chloride, 1% vitamin mixture, 3.5 % salt

mixture and 5% fibers. The remainder was corn starch up to 100 %.

Experimental diets

Experimental diet was prepared from basal diet plus the powdered fruits (20 %), fruits extract (20 %), powdered vegetables (20 %), vegetables extract (20 %), powdered legumes (20 %), legumes extract (20 %).

Experimental design

The experimental work was done in the Faculty of Science, AL-baha university. Rats were housed in wire cages in room maintained at 25 ± 2 ^{o} C and kept under normal healthy conditions. All were fed on normal basal diet for one week before starting the experiment for acclimatization. After one – week period, the rats were divided into groups each with similar alive body weight and were housed individually in wire cages.

Rat groups

Group 1: Control group (control negative) - 4 rats (normal rats) – in which rats were fed normal – basal diet for 45 days.

Group 2: Control positive group (control +) 4 rats. In which rats were fed normal – basal diet for 45 days. These rats were randomly chosen after injection with alloxan by six hours.

Group 3: Fruits groups (8 hyperglycemic rats) were fed on basal diet containing dried fruits which were:
Grapes *(Vitis vinifera),* Strawberry *(Frogaria chiloensis),* Mulberry *(Morus alba, L.),*Apple *(Malus pumila),*

This group was furtherly subdivided into 2 subgroups:

Group 3a: Powdered fruits – 4 control + rats: In which rats were fed 20 % powder fruits diet for 45 consecutive days.

Group 3b: Fruits extract – 4 control + 4 rats: In which rats were fed normal – basal diet with fruits extract 20 % for 45 days.

Group 4: Vegetables group (8 hyperglycemic rats) were fed on basal diet containing vegetables which were:

Cauliflower *(Brassica oleracea var. botrytis),* Onion *(Allium cepa, L.),* Garlic *(Allium sativum, L.),* Mushroom

This group was furthermore subdivided into 2 sub groups:

Group 4a: Powdered vegetables – 4 rats: In which rats were fed 20 % powdered vegetables diet for 45 consecutive days.

Group 4b: Vegetables extract – 4 rats: In which rats were fed normal –basal diet mixed with vegetables extract 20 % for 45 days.

Group 5: Legumes group (8 hyperglycemic rats) were fed on basal diet containing legumes which were:
Soybeans *(Glycine max, L. Merrill),* Chickpea *(Cicer arietinum),*
Fenugreek *(Trigonella foenum graecum, L.)* sedds, Barley *(Hordeum rulgare, L.).*

This group was furthermore subdivided into 2 subgroups:

Group 5a: Powdered legumes – 4 rats: In which rats were fed 20 % powdered legumes diet for 45 consecutive days.

Group 5b: Legumes extract – 4 rats: In which rats were fed normal –basal diet with 20 % legumes extract for 45 days.

Blood sampling

From all the previously mentioned groups, blood samples were collected after 12 hour fasting at the end of the experiment. Using the retro - orbital method by means of a microcapillary glass heparinized tubes, blood was collected into a dry clean centrifuge tube and left to clot in a water bath (37c)at room temperature for half an hour. The blood was centerifuged for 10 minutes at 3000 r.p.m. to separate the serum.

Serum was carefully aspirated and transferred into clean quit fit plastic tubes and kept frozen at (20c) until the time of analysis. The organs (liver, kidney, heart, lungs and spleen)were removed and washed in saline solution, weighted and kept in formalin solution (10 %,v/v) according to methods described by Drury and Wallington (1980), dried then weighted and relative weight of organs was calculated.

Biological Evaluation

$$\text{Relative weight} = \frac{\text{Organ weight}}{\text{Animal body weight}} \times 100$$

All rats were weighted once weekly. At the end of the experiment, biological evaluation of the different diets was carried out by determination of body weight gain % (BWG %), food efficiency ratio (FER) according to Chapman *et al.*, (1959). Using the following formula

Biochemical analysis

Immunity study: Assessment of both cellular and humoral immunity efficiency was carried out in the Faculty of Science - AL-Baha University. Total serum proteins were determined by using biuret reaction according to Weichselbaum (1946).

Quantitative estimation of fractions of serum proteins: according to the technique described by Laemmli (1970). Cellular immune response: Separation of lymphocytes: (Boyum, 1968 and Burrels and Wells (1977). Total lymphocyte count (Hudson and Hay, 1980).According to viability cell count (Rai-El-Balahaa *et al*., 1985; Denise *et al*., 1992). Phagocytosis according to (Woldehiwet and Rowan, 1990).

Determination of blood glucose: carried out Tietz (1976) and Yound (1975). triglycerides was carried out according to Jacobs and Van Denmark (1960). HDL according to the method of Fnedewaid (1972) and Gordon and Amer (1977). Determination of VLDL and LDL carried out according to the method of Lee and Nieman (1996). Determination of (GOT) was carried out according to the method of Henry (1974) and Yound (1975). Creatinine: concentration according to Larsen, (1972).Uric acid: was according to Carawy, (1955).Urea: was according to Fawcette and Scott (1960).

Statistical Analysis

Statistical analysis were performed by using computer program statistical package for social science (SPSS), and compared with each other using the suitable tests. All obtained results were tabulated. Statistical analysis has been achieved using IMB-P-C computer by SPSS, program (SPSS, 1998).

Result and Discussion

Food intake (FI), body weight gain (BWG) and food efficiency ratio (FER).

Data of table (1) show FI, BWG % and FER of hyperglycemic rats as affected by feeding on fruits, vegetables and legumes diets. It could be observed tables (1) that due to hyperglycemia loss of weight occurred and negative value of BWG was recorded; BWG % for control (+) group was - 12.73 % this was not due to loss of appetite (FI increase /from (16.9 to 17.1 g/day), but was possibly due to physiological disorders causing lower food efficiency ratio which decreased from 0.055 to -0.400 and lower protein efficiency ratio which declined from 0.458 to -0.332. Similar trends were reported by Ahmed, Reham (2007) which found that diabetic rats showed negative values of FER (.040) and BWG % (-12.69 %); food intake was decreased from 13.99 to 17.1 g/day. Also our findings agree with those of Stoev *et al*., (2000), who found that statistically significant decrease of the body weight for control positive group and increased organs weight. Rats fed on fruits, vegetables and legumes (table 1) showed no more negative values for FER, PER, or BWG % indicating that all plant materials used corrected the mentioned changes; but to different levels. Sometimes the improvement in (PER, FER, BWG %) so pronounced that values were ever better than that of the control (-) group. It could be noticed (table 1) that best PER, FER, and BWG % among fruits, vegetables and legumes groups were nearly the same which recorded for legumes extract diet, while lowest improvement was found for fruits powder diet; other diets revealed intermediate improvement (vegetables extract & powder, legumes powder, fruits extract). But this arrangement holds true for PER, FER or BWG %.

Weight of internal organs

Data of tables (2) show the weight of internal organs of hyperglycemic rats as effected by feeding of fruits, vegetables and legumes.

It could be noted that due to hyperglycemic the weight of heart, kidneys and spleen increased, while that of liver and lungs

189

decreased. These changes were corrected at different degrees when feeding on tested plant parts. Meanwhile all differences between values were non significant. Nevertheless, treatments could be arranged descendingly (the best is first) as follows; Liver: Legumes extract, legumes powder, fruits powder, fruits extract, vegetables extract and vegetables powder; Heart: vegetables powder, fruits powder, fruits extract, vegetables extract, legumes extract and legumes powder; Kidneys: vegetables powder, fruits powder, fruits extract, legumes powder and legumes extract; Spleen: Fruits extract, vegetables powder, vegetables extract, fruits powder, legumes extract and legumes powder; Lungs: legumes powder, legumes extract, This was found by Abo- Shadie (2002) when feeding hyperglycemic rats on 2% garlic diet, and were rat with accordance with El-Sayed (2001) who found a significant increase in liver weight of rats fed diet containing 5% *P. oleracear* leaves, being possibly due to changes in liver content of liver.

Biochemical parameters

Serum glucose

Data presented in table (3) show the serum glucose level of diabetic rat when feeding on fruits, vegetables and legumes: It was revealed that alloxan induced diabetes raised the serum glucose, percent increase was about 78%. This high serum glucose level was recorded even after 28 days of trial diabetic rats on normal basal diet without alloxan injection during the feeding experiment. Certain correction occurred on feeding with test plants. Chemoprevention involves the use of natural substances to reduce the risk of developing DM and its sequels (Ronald, 2001). The results of table (3) indicated that serum glucose for vegetables extract lower than all groups when compared with control positive

significantly (p<0.0 1). Mentioned results in present study that fruits group, vegetables group and legumes group or their extract lower blood glucose level in diabetic rats when they were supplemented to the diet at levels 20 % powdered and extract. The results of table (3) showed that serum glucose was less in the extract than the powder for vegetable and fruit groups, while for legumes serum glucose was lower for the powder. Moreover, Dietary fiber, in particular the soluble fiber fraction plays the important role in controlling glucose concentration in serum and other risk factors associated with diabetes (Giacco *et al*., 2002).The results are in agreement with Fatani *et al*., (2005) they found that oral administration of 50 and 100 mg/kg body weight of red grape seed proanthocyanidins for 72h significantly increased pancreatic glutathione levels and inhibited the increase in lipid peroxidation caused by alloxan (p<0.001). On the other hand, a significant reduction in pancreatic total nitrate content (p<0.001) was observed. Furthermore, GSP caused significant decline in the hyperglycemia induced by alloxan (p<0.001). Also, this is result are agreement with Arola *et al*., (2004). It should be noted that the present work was designed mainly to raise the immunity of diabetics, which are usually subjected to serious disorders other than the diabetes mellitus. The suggested diets, however, revealed pronounced serum glucose lowering effect.

Lipids fractions

Data presented in tables (4) show the level of serum lipids fractions (TC, TG, HDL, LDL, vLDL) and calculated atherogenic index (AF) of hyperglycemic rats. It could be noticed (Tables 4) that along with the increase of serum glucose (Tables 3) in diabetic rats, fasting serum TC, TG, LDL & VLDL were increased, while the level of HDL decreased. This is a usual disorder

recorded for hyperglycemia (Mohamed, Manal, 2006). Nevertheless, the reverse was found when feeding rats on selected fruits, vegetables and legumes. Similar trends were reported by El-Sayed, Mona (2005); Saad, Gehan (2006); Ahmed, Reham (2007); El-Malah, Maysa (2007) and Gadall, Sanaa (2007) working on fruits, legumes, vegetables and herbs. Moreover, El-Adawi (1997) found that the concentration of plasma cholesterol, triglycerides, fatty acids and phospholipids are increased in diabetes mellitus. Diabetic rats exhibited high triglycerides and phospholipids compared to normal rats.

Insulin also affect fat metabolism. Lack of insulin causes extreme atherosclerosis, often leading to heart attack. It causes also a great increase in the amount of stored triglycerides in the liver, leading to a very fatty liver. The reason is the following: the excess of free fatty acids in the blood causes a rapid diffusion of fatty acids into the liver cells (Lee and Niemen, 2003). It could be noticed (Tables 4) that best treatment, which showed lowest TC, TG, LDL and highest HDL were the collection of vegetables powder & fruits extract groups, followed by the collection of legumes powder, vegetables extract and legumes extract groups then came the fruits powder group.

This was also found for the calculated A.I. The present results are confirmed by the findings of Ali *et al*., (2006) they found that raw garlic had a profound effect in reducing the glucose, cholesterol and triglyceride levels. Therefore because hyperlipidemeia is a major etiopathological factor for atherosclerosis, garlic may play an important role in the prevention of atherosclerosis. Our findings agreement with Sauls *et al*., (2006) they found that red wines and grape decrease lipid profile and saturated fatty acids. Fruits vegetable and legumes as well as powders of vegetables and legumes

decreased the LDL levels than that recorded for the control (-) group.

Effect of fruits, vegetables, legumes and herbs groups on the liver function of diabetic rats

Data of tables (5) show the activities of GPT (AST) and GOT (AST) enzymes as influenced by feeding on fruits, vegetables and legumes diets (powders and extracts). It is evident (Table 5) that the activities of GPT and GOT could be nearly arranged the same and ascendingly as follows: Vegetables extract, fruit extract, legumes powder, fruit powder, vegetables powder and legumes extract. Such data indicated that the increase of enzymes activities due to hyperglycemia was corrected by feeding with botanical materials. Some diets (fruits powder) decreased the GPT activity exactly to the level recorded for the control (-) rats (26 u/L), this was also found for GOT in case of legumes powder diet (62.3 u/L).

Some other diets reduced the GPT (fruits extract, vegetables extract and legume powder) and the GOT (vegetables extract) activities to a level which was less than that recorded for the control (-) group. Eidi *et al*., (2006) they found that garlic extract significantly decreased serum glucose, total cholesterol, triglycerides, urea, uric acid, creatinine, GPT, GOT levels.Our findings agreement with Kim *et al*., (2002) they found that leaf *(Brassica juncea)* reduced serum glucose lipid peroxidation, liver and kidney associated with diabetes mellitus. Also, these results agreed with that of Ghamry (2004). The present result agreed also with those that of Nada *et al*., (1997).

Effect of fruits, vegetables and legumes diets groups on the kidneys function of diabetic rats

Diabetic nephropathy is a major

complication of both insulin – dependent and non insulin – dependent diabetes mellitus, as reported by Velasquez *et al.*, (1995), they suggested that hyperglycemia in diabetes is a critical factor in the development of the renal disease. Non protein nitrogen of the blood includes urea, uric acid and creatinine. They are usually of greater clinical interest (Contarow and Schepartz, 1967). Hyperglycemia, thereby caused the disorder of kidneys function as indicated by the increase of serum creatinine, were and uric acid levels (Tables 6). This confirmed the results obtained by others (El-Sayed, 2001; Mohamed, 2006 and Ahmed, 2007).

All botanical material used in present study corrected the disorders of kidneys function to different degrees as compared with control (+) group. In case of urea, however, none of diet reduced the level than that recorded for control (-) rats. On the other hand the control (-) groups which were: Creatinine (vegetables powder & legumes extract), Uric acid (fruits extract, vegetable extract and legumes extract).

Botanical materials of table (6) could be arranged descendingly according to their beneficial effect on kidneys function as follows: Fruit extract, vegetable extract, legume extract and vegetable powder groups.

It is work mentioning that although lowest correction was found for fruit powder in case of creatinine (from 1.50 in control (+) to 1.33 mg/dl) & urea (from 50 in control (+) to 44.67 mg / dl) and in case of uric acid (from 2.77 in control (+) to 2.28 mg/dl) percent decreases were 11.11 & 4 % which indicates the improvement of health status and kidneys function within 45 days of feeding. Possibly feeding for more period of time may cause much greater improvement.

Immunity Parameters
Cellular immunity

Data of tables (7) show the cellular immunity (phagocytes and lymphocytes) of rats as affected by feeding on fruits, vegetables and legumes diets. Differences between control (-) and control (+) groups as well as by all treatments were insignificant. Nevertheless cellular immunity (Tables 7) could be arranged descendingly based on absolute values (regardless of statistics) of phagocytes as follows: vegetables powder, vegetables extract, fruit extract, fruit powder, legumes powder & legumes extract and according to lymphocytes: Vegetables extract, fruit powder, legumes powder, vegetables powder, fruits extract and legumes extract. Best diet seems to be vegetables extract and the lowest effecting was legumes extract.

Meanwhile compared to control (+) group, least effecting diet (legumes extract) showed about 50 % (49.45%) improvement in cellular immunity (based on lymphocytes); Based on phagocytes this diet showed 7.14 % improvement. Changes of lymphocytes was considerably greater in case of vegetables powder diet, showing about 64 % improvement comparing to control (+) group (Table 8).

Thiery *et al.*, (2006) found that garlic *(Allium sativum)* extract inhibited the adhesion of monocytes to IL – 1 alpha – stimulated endothelial cells. Garlic preparation have also been reported the immune system of patients with AIDS (Abdullah *et al.*, 1989).

Humoral immunity

Alpha, beta and gamma globulins

Data of tables (9) show the levels of

humoral immunity as indicated by determining serum alpha, beta and gamma globulins. It is known that the more the level of globulins, the more immunity will be. For all fractions hyperglycemia resulted in the decrease of values (Tables 9) while the reverse was recorded when the rats showed some recovery which lowered the serum glucose (Table 9). Our findings agree with those of Winlers *et al*., (2006), they found that *Allium sativum* and *Echinacea purpurea* modulate the secretion of multiple cytokines. Lee *et al*., (2006) indicated that *black berry, black raspberry, blue berry, strawberry* extract inhibit growth and stimulate apoptosis of human cancer cells in vitro. Matos *et al*., (2006) found that strawberry contains IgE binding profilim and LTP which lower allergy. Stoev *et al*.,

(2000) indicated that artichoke a protective effect of 5% on humoral immune response (increase of haemagutination).

Thejass and Kuttan (2006) noticed that broccoli enhanced production of cytokines IL – 2 and IFN – gamma. As for the fruits, vegetables and legumes groups best treatment was that of powdered vegetables, followed by vegetables extract, legumes extract, fruits extracts, legumes powder & fruits powder. In case of beta globulin, the best treatment was also the vegetable powder but after that arrangement was different from that of the alpha globulin. Fruit extract group came second to vegetable powder, then fruit extract, vegetable extract, legumes powder and legumes extract.

Table.1 Effect of feeding fruits, vegetables and legumes groups powders & extracts) on food intake, PER, FER, and BWG %) of hyperglycemic rats

Groups Variables	Control (+	Fruits		Vegetables		Legumes	
		Powder 20%	Extract 20%	Powder 20%	Extract 20%	Powder 20%	Extract 20%
Food intake (g)	17.1 ± 0.5^a	16.1 ± 0.3^d	18.1 ± 0.5^b	$25.25 \pm.12^a$	15.6 ± 0.3^d	17.1 ± 0.3^c	$17.7 \pm 0.5b^c$
PER	$-.332 \pm.211^{ab}$	$.104 \pm 053^c$	$.198 \pm.118^{abc}$	$.165 \pm.061^{abc}$	$.257 \pm.076^{abc}$	$.210 \pm.191^{abc}$	$.538 \pm.145^a$
FER	$-.040 \pm.025^{ab}$	$.012 \pm 06^c$	$.024 \pm.014^{abc}$	$.020 \pm.007^{bc}$	$.031 \pm.009^{abc}$	$.025 \pm.023^{abc}$	$.064 \pm.018^a$
Initial weight (g)	149.3 ± 10.2^{ab}	148 ± 5.19^c	150.7 ± 6.8^{bc}	149.3 ± 14.1^{bc}	163.3 ± 8.7^a	151 ± 8.2^{bc}	136.3 ± 5.7^d
Final weight (g)	130.3 ± 39.6^b	$153.7 \pm.5^{ab}$	162.7 ± 10.1^{ab}	163.3 ± 10.4^{ab}	176.7 ± 12.5^a	163 ± 3.5^{ab}	168.3 ± 9.1^{ab}
BWG %)	-12.73 ± 3.3^{ab}	3.85 ± 2.08^c	7.96 ± 4.71^{bc}	9.38 ± 4.53^{bc}	8.21 ± 2.12^{bc}	7.95 ± 7.8^{bc}	23.48 ± 6.67^a

Mean ± SD
*Different letters on the values mean significant differences at P< 0.05.
**Different letters on the values mean significant differences at P< 0.01.

Table.2 Effect of feeding fruits, vegetables and legumes groups powders & extracts) on internal organs weight g) of hyperglycemic rats

Groups / Variables	Control (+)	Fruits		Vegetables		Legumes	
		Powder 20%	Extract 20%	Powder 20%	Extract 20%	Powder 20%	Extract 20%
Liver	3.74 ±.310[a]	4.47 ±.3785[a]	4.13 ±.1184[a]	3.98 ±.4849[a]	3.98 ±.525[a]	4.67 ±.252[a]	4.92 ±.589[a]
Heart	.75 ±.185[a]	.75 ±.05[a]	.793 ±.012[a]	.73 ±.057[a]	.79 ±.083[a]	.88 ±.076[a]	.813 ±.023[a]
Kidney	1.3 ± .346[a]	.956 ±.26[a]	1.09 ±.130[a]	.94 ±.196[a]	1.42 ±.45[a]	1.35 ±.132[a]	1.36 ±.152[a]
Spleen	.696 ±.179[a]	.8 ±.1[a]	.583 ±.236[a]	.68 ±.120[a]	0.7 ± 0.1[a]	.98 ±.072[a]	.95 ±.25[a]
Lungs	1.04 ±.092[a]	1.2 ±.410[a]	.89 ±.268[a]	1.18 ±.45[a]	1.09 ±.11	1.33 ±.37[a]	1.3 ± 0.1[a]

Mean ± SD; *Different letters on the values mean significant differences at P< 0.05.
**Different letters on the values mean significant differences at P< 0.01.

Table.3 Effect of feeding fruits, vegetables and legumes groups powders & extracts) on blood glucose level mg / dl) of hyperglycemic rats.

Groups / Variables	Control		Fruits		Vegetables		Legumes	
	(-)	(+)	Powder 20%	Extract 20%	Powder 20%	Extract 20%	Powder 20%	Extract 20%
Serum glucose	84 ± 2[f]	149.3 ± 1.02[a]	130.3 ± 4.51[c]	105.67 ± 4.51[e]	119 ± 4.58[d]	85.66 ± 4.5[f]	107.3 ± 4.04[e]	140.7± 9.02[b]
% decrease of control (+)	-43.7	----	-12.7	-29.2	-20.3	-42.6	-28.1	-5.8
% decrease of control (-)	-	+77.7	+55.1	+25.8	+ 41.7	+2.0	+27.7	+67.5

Mean ± SD; *Different letters on the values mean significant differences at P< 0.05.
**Different letters on the values mean significant differences at P< 0.01.

Table.4 Effect of feeding fruits, vegetables and legumes groups powders & extracts) on fasting serum lipids profile of hyperglycemic rats.

Groups / Variables mg / dl)	Control		Fruits		vegetables		Legumes	
	(-)	(+)	Powder 20%	Extract 20%	Powder 20%	Extract 20%	Powder 20%	Extract 20%
Cholesterol	$119.3 \pm .52^b$	$146.6 \pm .51^a$	116.3 ± 4.51^b	90.67 ± 3.51^d	90.67 ± 2.52^d	99.7 ± 4.5^{cd}	99 ± 8^{cd}	104.3 ± 4.5^c
Triglyceride	97 ± 3^{bc}	102 ± 4^a	84 ± 6^d	63 ± 2^f	72 ± 3^e	72 ± 4^e	96 ± 3^c	100 ± 5^{ab}
HDL	55.7 ± 2.52^a	36.7 ± 3.51^b	39.7 ± 7.02^{ab}	45.7 ± 6.02^{ab}	45.3 ± 2.52^{ab}	43.3 ± 7.51^{ab}	42.3 ± 2.52^{ab}	44.3 ± 4.51^{ab}
LDL	44.2 ± 2.2^b	89.5 ± 4.3^a	59.8 ± 2.1^b	32.4 ± 4.3^d	30.97 ± 1.1^d	42.0 ± 2.1^b	37.5 ± 4.1^c	40 ± 2^c
VLDL	19.4 ± 2^a	20.4 ± 3^a	16.8 ± 2.2^{ab}	12.6 ± 3.3^c	14.4 ± 3.21^{bc}	14.4 ± 5.3^{bc}	19.2 ± 2.1^a	20 ± 2^a

Mean ± SD; *Different letters on the values mean significant differences at P< 0.05.
**Different letters on the values mean significant differences at P< 0.01.

Table.5 Effect of feeding fruits, vegetables and legumes groups powders & extracts) on liver function of hyperglycemic rats.

Groups / Variables U / L)	Control		Fruits		Vegetables		Legumes	
	(-)	(+)	Powder 20%	Extract 20%	Powder 20%	Extract 20%	Powder 20%	Extract 20%
Liver Enzymes								
GPT	26 ± 2^{bc}	83.3 ± 4.51^a	26 ± 4^{bc}	24.3 ± 5.51^{bc}	35.3 ± 4.51^b	22.67 ± 7.51^c	25.3 ± 3.51^{bc}	35.6 ± 3.51^b
GOT	62.3 ± 2.52^d	150.3 ± 2.52^a	65.3 ± 4.51^d	73.6 ± 3.51^c	75.3 ± 3.51^c	57.6 ± 3.52^d	62.33 ± 2.5^d	109.6 ± 7.51^b

Mean ± SD; *Different letters on the values mean significant differences at P< 0.05.
**Different letters on the values mean significant differences at P< 0.01.

Table.6 Effect of feeding fruits, vegetables and legumes groups powders & extracts) on kidneys function of hyperglycemic rats

Groups	*Control(+)*		Fruits		Vegetables		Legumes	
Variables (mg/dl)	(-)	(+)	Powder 20%	Extract 20%	Powder 20%	Extract 20%	Powder 20%	Extract 20%
Creatinine	.82 ±.03[bc]	1.5 ± 0.4[a]	1.33 ±.251[ab]	.84 ±.035[bc]	.78 ±.035[c]	.92 ±.025[bc]	.96 ±.03[bc]	.85 ±.045[bc]
Urea	26 ± 4[d]	50 ± 3[a]	44.67 ± 5.50[b]	26 ± 3[d]	28.67 ± 4.51[cd]	29.67 ± 2.51[c]	28.3 ± 4.5[cd]	31.67 ± 2.52[c]
Uric acid	2.23 ± 25[ab]	2.77 ±.075[a]	2.28 ±.03[ab]	1.92 ±.025[b]	2.67 ±.45[a]	1.33 ±.252[c]	2.17 ±.025[ab]	2.53 ±.152[ab]

Mean ± SD; *Different letters on the values mean significant differences at P< 0.05.
**Different letters on the values mean significant differences at P< 0.01.

Table.7 Effect of feeding   herbs groups powders & extracts)   on cellular immunity phagocytes & lymphocytes) of hyperglycemic rats

Groups	*Control*		Fruits		Vegetables		Legumes	
Variables	(-)	(+)	Powder 20%	Extract 20%	Powder 20%	Extract 20%	Powder 20%	Extract 20%
Phagocytes	75 ± 9[a]	70 ± 7[a]	76 ± 12[a]	77 ± 9[a]	80 ± 18[a]	79 ± 8[a]	76 ± 20[a]	75 ± 13[a]
Lymphocytes	1.33 ± 12[ab]	0.91 ±.03[b]	1.51 ±.22[a]	1.37 ±.32[ab]	1.49 ±.08[a]	1.73 ±.018[a]	1.50 ±.009[a]	1.36±.19[ab]

Mean ± SD; *Different letters on the values mean significant differences at P< 0.05.
**Different letters on the values mean significant differences at P< 0.01.

Table.8 Effect of feeding   herbs groups (powders & extracts)   on cellular immunity
(phagocytes & lymphocytes) of hyperglycemic rats (% increase of control +)

Groups Variables	Control (+)	Fruits		Vegetables		Legumes	
		Powder 20%	Extract 20%	Powder 20%	Extract 20%	Powder 20%	Extract 20%
% Phagocytes of control (+)	-----	+ 8.57	+ 10.00	+ 14.29	+ 12.86	+ 8.57	+ 7.14
% Lymphocytes of control (+)	----	+ 65.93	+ 50.55	+ 63.74	90.11+	64.84+	+ 49.45

% of control " + "). Mean ± SD

Table.9 Effect of feeding fruits, vegetables and legumes groups powders & extracts) on
humoral immunity of hyperglycemic rats (Globulins fractions)

Groups Variables g / dl)	Control (+)		Fruits		Vegetables		Legumes	
	(-)	(+)	Powder 20%	Extract 20%	Powder 20%	Extract 20%	Powder 20%	Extract 20%
Globulins Alpha	1.27 ±.014^{ab}	1.15 ± .027^{abc}	1.02 ±.009^{c}	1.19 ± 0.1^{abc}	1.33 ±.08^{a}	1.26 ±.13^{ab}	1.09 ± 0.07^{bc}	1.25 ± 0.1^{ab}
Beta	1.41 ± 0.1^{a}	1.21 ± 0.1^{ab}	1.33 ±.12^{ab}	1.29 ±.1^{ab}	1.34 ±.07^{ab}	1.23 ±.08^{ab}	1.16 ±.13^{ab}	1.09 ±.04^{b}
Gamma	1.85 ±.04^{d}	1.67 ±.11^{e}	2.02 ±.06^{c}	1.95 ±.04^{c}	2.41 ± 0.1^{a}	2.18 ±.07^{b}	1.84 ±.05^{d}	1.75 ±.09de

Mean ± SD
*Different letters on the values mean significant differences at P< 0.05.
**Different letters on the values mean significant differences at P< 0.01.

Table.10 Effect of feeding fruits, vegetables and legumes groups powders & extracts) on humoral immunity of hyperglycemic rats (Globulins fractions) % increase of control   (+)

Groups Variables	*Control* (+)	Fruits		Vegetables		Legumes	
		Powder 20%	Extract 20%	Powder 20%	Extract 20%	Powder 20%	Extract 20%
GLOBULINS: % ALPHA of control (+)	----	- 11.30	+ 3.48	+ 15.65	+ 9.57	- 5.22	+ 8.70
% BETA of control (+)	----	+ 9.92	+ 6.61	+ 10.74	+ 1.65	- 4.13	- 9.92
% GAMMA of control (+)	----	+ 20.96	+ 16.77	+ 44.31	+ 30.54	+ 10.18	+ 4.79

% of control " + ") ;Mean ± SD
*Different letters on the values mean significant differences at P< 0.05.
**Different letters on the values mean significant differences at P< 0.01.

Table.11 Effect of feeding fruits, vegetables and legumes groups (powders& extracts) on some humoral immunity indices of hyperglycemic rats (total protein " TP ", albumin "ALB", total globulin "GLOB" and A/B ratio)

Groups Variables	*Control*		Fruits		Vegetables		Legumes	
	Control (-)	*Control* (+)	Powder 20%	Extract 20%	Powder 20%	Extract 20%	Powder 20%	Extract 20%
TP g / dl)	$6.58 \pm .017^c$	$6.05 \pm .07^c$	$6.70 \pm .007^c$	$6.79 \pm .12^c$	$7.95 \pm .15^a$	$6.98 \pm .01^b$	6.5 ± 0.3^c	$6.48 \pm .09^c$
ALB g / dl)	$2.32 \pm .09^a$	$2.02 \pm .004^b$	$2.33 \pm .08^a$	$2.36 \pm .08^a$	$2.36 \pm .15^a$	2.31 ± 0.1^a	2.41 ± 0.1^a	$2.39 \pm .08^a$
GLOB g / dl)	$4.53 \pm .009^e$	$4.03 \pm .014^d$	$4.54 \pm .011^{cd}$	4.43 ± 0.06^c	$5.08 \pm .03^a$	$4.67 \pm .04^b$	$4.09 \pm .02^f$	$4.09 \pm .07^f$
A / B g / dl)	0.512	0.501	0.533	0.533	0.465	0.495	0.589	0.584

Mean ± SD**;** *Different letters on the values mean significant differences at P< 0.05.
**Different letters on the values mean significant differences at P< 0.01.
A / B: Albumin to globulin ratio.

Table.12 Effect of feeding fruits, vegetables and legumes groups powders & extracts) on some humoral immunity indices of hyperglycemic rats total protein " TP ", albumin " ALB ", total globulin " GLOB " and A/B ratio)
% increase of control (+)

Groups	Control		Fruits		vegetables		Legumes	
Variables	Control (-)	Control (+)	Powder 20%	Extract 20%	Powder 20%	Extract 20%	Powder 20%	Extract 20%
TP (g / dl)	+ 13.2	----	+ 10.74	+ 12.23	+ 31.41	+ 15.37	+ 7.44	+ 7.11
ALB (g / dl)	+ 14.9	----	+ 15.35	+ 16.83	+ 16.83	+ 14.36	+ 19.31	+ 18.32
GLOB (g / dl)	+ 12.4	----	+ 12.66	+ 9.93	+ 26.06	+ 15.88	+ 1.49	+ 1.49
A / B g / dl)	+ 2.19	----	+ 6.39	+ 6.39	- 7.19	- 1.20	+ 17.57	+ 16.57

Mean ± SD
*Different letters on the values mean significant differences at P< 0.05.
**Different letters on the values mean significant differences at P< 0.01.
A / B: Albumin to globulin ratio**.**

Arrangement in case of gamma globulin was more or less similar to thus of alpha globulin as vegetables powder, was the best treatment, followed by vegetables extract, fruit powder, fruit extract, legumes powder & legumes extract. Last two groups showed also lease values considering alpha globulin.

Serum total protein (TP), albumin (ALB), globulin (Glob) and albumin / globulin ratio (A / B)

Total protein (TP)

It is evident (Tables 11) that due to hyperglycemia, TP was decreased; difference however was insignificant. On the contrary TP increased when feeding rats diets with added botanical materials. For fruits, vegetables and legumes diet, vegetable powder treatment showed highest

TP, followed by vegetable extract, fruits extract, fruits powder, legumes powder and legumes extract diet. Overall, the best treatment was recorded for vegetable powder, vegetable extract, fruits extract, fruits powder, legumes powder and legumes extract. Although legumes extract showed the lowest correction, improvement of TP was by 7.44 %

Albumin (ALB)

ALB decreased by hyperglycemia and increased by different treatments. ALB of fruits, vegetables & legumes groups was highest for legumes powder, followed by legumes extract, fruits extract, vegetables powder, fruits powder and vegetables extract. With some exceptions arrangement of groups was nearly revealed compared to that of the TP.

Serum globulin (GLOB)

Total globulins (Tables 11) declined by hyperglycemia and improved by different feeding treatments, following nearly the same arrangement as that of gamma globulin (Tables 11). Highest improvement of T. globulins in fruits, vegetables and legumes groups for vegetables powder treatment, followed by vegetables extract, fruits powder, fruits extract, legumes powder & legumes extract.

A / B ratio

A / B ratio did not show the arrangement recorded for TP, ALB, or T. globulins (GLOB). Anyhow, A / B ratio decreased by hyperglycemia, while was increased when feeding diabetic rats on tested plants. For fruits/ vegetables/ legumes treatments the best group was recorded for legumes powder one, followed by legumes extract, fruits powder, fruits extract, vegetables extract & vegetables powder. Considering all tested plants the best treatment was that of legumes powder followed by legumes extract, fruits powder, fruits extract, vegetables extract, and vegetables powder. The last treatment caused a decreased in A / B ratio by 7.19 %, while best group (legumes powder) showed 16.57% improvement (increase).

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