

## **HYPOGLYCEMIC EFFECTS OF ARW AND TREATED BEANS AND PEAS ON DIABTIC RATS AS WELL AS THE CLINICAL STATUS OF LIVER FUNCTION**

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### **ABSTRACT**

This study has been designed with the purpose of elucidating the effect of some legumes (white beans and peas) raw and treated materials as hypoglycemic agents as well as determine their effects on liver and kidneys function .The obtained results showed that feeding on soaked-cooked white beans or soaked-cooked peas to alloxanized diabetic male rats had a powerful hypoglycemic agent, which acted as glucose lowering agent. In addition, diabetic animals showed a general improvement in their clinical status, as reflected by blood parameters (the activities of GOT and GPT as well as plasma protein, albumin and globulin contents) and by body weight gain in which hyperglycemia was significantly reduced. The feeding on 40% dried soaked-cooked beans gave the best results concerning biological studies then the other treatments. In conclusion, the feeding on the studied legumes (white beans and peas) either raw or soaked-cooked materials, improved the disturbed effects of alloxanized diabetes but with different degree on the metabolism of diabetic animals.

### **INTRODUCTION**

Legumes are plants with seed pods that split into two halves. Edible seeds from plants in legume family include beans, peas, lentils and soy bean. Legumes are inexpensive, nutrient-dense sources of plant protein that can be substituted for dietary animal protein (Anderson *et al.*, 1999).While sources of animal protein are often rich in saturated fats, the small quantities of fats in legumes are mostly unsaturated fats. Not only are legumes excellent sources of essential minerals, they are rich in dietary fiber and other phytochemicals that may affect health. It is common that the people sock and cook some legumes seeds such as beans, peas and chickpeas before direct eating. These treatments improved the nutritional value of the proteins which hydrolyzed into easily as polypeptides and amino acids. Also trypsin inhibitors were decreased by the treatments. Soaking and cooking are resulted in the utilization of galacto-sugars which produce flatulence and stomach upset.

The glycemic index is a measure of the potential for carbohydrates in different foods, to raise blood glucose levels. In general, consuming foods with high glycemic index values causes blood glucose levels to raise more rapidly, resulting in greater insulin secretion by pancreas than consuming foods with low glycemic index values. Chronically elevated blood glucose levels and excessive insuiin secretion are thought to play important roles in the development of type 2 diabetes mellitus (T2DM) (Willett *et al.*2002).Legumes generally have low glycemic index values, low glycemic load diets have been associated with reduced risk of developing (T2DM) in several large prospective studies (Salmeroh *et al.*,1997).Obesity is another

important risk factor for type2DM. Numerous clinical trials have shown that the consumption of low glycemic index food delays the return to hunger, decreases subsequent food intake, and increases the sensation of fullness compared to high glycemic index foods (Ludwing, 2003). In the present work, the main aim was to study the hypoglycemic effect of raw and treated beans and peas as well as clinical status of liver and kidneys function which were determined *in vivo* using adult male rat animals model.

## **MATERIALS AND METHODS**

### **Materials**

Samples of white beans (*Phaseolus vulgaris* L.), namely Bolessta and peas (*Pisum sativum* L.) namely Masster B were obtained from Food Legume Research Department, (A.R.C.), Giza, Egypt. Samples were soaked in tap water (1:5w/v) for 12 hr. at room temperature then cooked on a hot plat until it became soft as felt between fingers then were dried in a hot air oven 50C.

### **Animals**

Adult male albino rats (50) weighting between 140-170g raised in the animal house of Agricultural Research Center (ARC) used in the present study. The animals were kept under normal laboratory conditions for one week. The animals were fed on normal basal diet, this diet consisted of casein 15%, corn oil 10%, cellulose 5%, salt mixture 4%, vitamin mixture 1% and starch 65% (Lane-Peter and Pearson, 1971). Rats were allowed free excess of water. The rats were housed in cages (5 rats each cage).

### **Experimental design**

Rats were feeding on the basal diet for two weeks as adaptation period. After that the rats were divided into two groups. The first group (5rats) was kept as normal control and was continued fed on the basal diet. The second group was injected with recrystalized alloxan (150mg/kg) to induce hyperglycemia according to the method of Lazarow and Palay (1954). After 72 hr of the injection, a blood sample was taken from each rat for the determination of serum glucose to ensure the occurrence of diabetes. The second group (diabetic rats) was divided into nine groups each of five rats.

So the ten groups as follows:

- Group No1 fed on a basal diet (Normal control).
- Group No2 fed on a basal diet (Diabetic control).
- Group No3 fed on 80% basal diet with 20% dried raw beans.
- Group No4 fed on 80% basal diet with 20% dried soaked-cooked beans.
- Group No5 fed on 80% basal diet with 20% dried raw peas.
- Group No6 fed on 80% basal diet with 20% dried soaked-cooked peas.
- Group No7 fed on 60% basal diet with 40% dried raw beans.
- Group No8 fed on 60% basal diet with 40% dried soaked-cooked beans.
- Group No9 fed on 60% basal diet with 40% dried raw peas.
- Group No10 fed on 60% basal diet with 40% dried soaked-cooked peas.

The water and diet were allowed in free excess for rats *ad libitum*. The rats were weighted every week. Both food intake and feed efficiency were also recorded till the end of experimental period during the

experiment blood sample was obtained from each rat from eye vein by capillary tube after fasting for an overnight weakly, and blood glucose level was determined.

At the end of experimental period, rats were killed by decapitation and blood samples were collected from each rat and subjected to centrifugation tube at 3000 rpm to obtain the plasma fraction, which was kept in the deep-freezer for the subsequent investigations. Different organs were removed; washed in 0.9 % NaCl saline solution and weighted. The determinations of blood glucose liver function, kidneys function and lipid pattern of experimental animal in the present studies were carried out by kits.

### **Chemical analysis**

Enzymatic determination of plasma glucose was carried out calorimetrically at 510nm according to the method of **Tinder(1969)**.The protein content of plasma was determined at 550nm according to the method of **Gornal et al.,(1949)**. Glutamate pyruvate amino transferase (GPT) and glutamate oxalacetate amino transferase (GOT) activities were determined calorimetrically at 546nm according to the method of **Reitman and Frankel (1957)**. Determination of alkaline phosphates activity was carried out calorimetrically at 510nm according to the method **Belfield and Goldberg (1971)**. Determination of serum albumin was carried out calorimetrically at 630nm according to **Doumas et al., (1971)**. Statistical analysis was calculated and evaluated according to **Miller and Miller (1992)**.

## **RESULTS AND DISCUSSION**

### **Hypoglycemic effect of beans and peas**

Results which were statistically analyzed and summarized in table (1) presented the blood glucose levels of the ten rat groups, zero time and weekly till the end of the experimental period which was prolonged for six weeks. The average values of blood glucose level of the ten groups before injection with alloxan were nearly the same level and the same level was also noticed for the normal control during the experimental period. After alloxan had been injected, the blood glucose level was raised from an average value of 100.6mg/dl at zero time in normal rats group to maximum value of 227.5mg/dl after 6weeks in alloxanized diabetic rats group.

As alloxan may either increase the entrance rate of glucose into the blood stream from the liver (increased hepatic glucogenolysis or gluconeogenesis) or to decrease rate of removal of glucose from the blood by tissues (decreased storage and utilization).

These influences might be due to the absence of an adequate amount of insulin. The alloxan acts directly promptly and specifically on  $\beta$ -cells of the pancreatic cells. In order to study the effect of two legumes (beans and peas when they were raw and soaked-cooked) on alloxanized diabetic rats, the percentage values of reduction in blood glucose level were calculated as shown in table (1).

Table (1): Effects of raw and treated legumes on blood glucose Levels of diabetic rats during the experimental period (6week)

Treatments	zero time		1week		2week		3week		4week		5week		6week	
	mg/dl	%	mg/dl	%	mg/dl	%	mg/dl	%	mg/dl	%	mg/dl	%	mg/dl	%
G 1	95.5	100	97.2	100	98.24	100	100.6	100	102.6	100	99.5	100	100.6	100
G 2	240.1	251.4	242.6	249.6	237.6	241.9	230.2	228.8	234.1	228.2	230	231.9	227.5	226.1
G 3	239.2	250.5	229	235.6	225.7	229.7	213	211.7	211.4	206	198.8	200.5	179.6	178.5
G 4	221.1	231.6	209	215	197.4	200.9	188.2	187	164	159.8	158.6	160	145.8	145
G 5	221.2	231.6	211.6	217.7	197.8	201.3	178	176.9	163.6	159.5	166	167.4	160.4	159.4
G 6	228.2	240	215.8	222	240	207.7	189	187.9	178.3	173.8	168	169.4	152.4	151.5
G 7	221.4	231.8	210.8	216.9	197.8	201.3	183.2	182.1	172.1	167.7	172.8	174.3	167.3	166.3
G 8	224.6	235.2	210.6	216.7	209.6	213.4	187.2	186	171	166.7	157.4	158.7	141.8	141
G 9	226.3	237	208.2	214.2	206.4	210	189.4	188.3	167.1	162.9	166.2	167.6	162.6	161.6
G 10	229.7	240.5	212.6	218.7	188.7	192	179.4	178.3	166	161.8	154.6	156	147.8	146.9
L.S.D at 5%	30.79		25.26		25.60		19.90		25.56		19.90		21.54	

Each value represented the mean of 5 rats

The different treatment of the alloxanized diabetic rats exhibited different effects on the blood glucose level, where the diet with 40% soaked-cooked beans were the most effective as hypoglycemic agents then the other groups where glucose level was reduced at the end of six weeks by 94.2%. Panlasigui *et al.*, (1995) studied the glycemic response in normal subjects to five different legumes. They found that the blood sugar response to all legumes was significantly lowered compared with bread. The glycemic index of white beans was lower than the other legumes. The differences in the glycemic responses among the legumes could be due to differences in amount and kind of dietary fiber, amylase content and the presence of antineutrinos.

Legumes could therefore be added to the list of foods for diabetics. Fung *et al.*, (2002) found that people consuming around 3 servings per day of legumes had a risk reduction T2DM in the order of 20-30% compared with low consumers.

**Effect of beans and peas on body weight gain, food intake and feed efficiency of hyperglycemic rats**

The results illustrated in table (2) indicated that alloxan injection caused significant decreases in the food intake, body weight gain and consequently also a high decrease in feed efficiency. Feeding on raw or soaked-cooked beans and peas for alloxanized diabetic rats resulted significant increase of the food intake and body weight gain.

**Table (2) Effects of raw and treated legumes on body weight gain, food intake and feed efficiency of diabetic experimental animals.**

Treatment	Initial weight	Final weight	Body weight gain		Food intake	Feed efficiency
	g	(6weeks)	g	%	(6weeks)	
G1	156.6	191.4	34.8	22.2	647	0.054
G2	156.4	160.6	4.2	2.68	475	0.009
G3	155.4	167.4	12	7.72	510	0.024
G4	155	180	25	16.12	585	0.043
G5	155.2	171.8	16.6	10.7	580.5	0.029
G6	156	179.4	23.4	15	563.8	0.041
G7	154.8	167.2	11.2	7.23	490	0.023
G8	155.6	192.2	36.6	23.52	618.75	0.059
G9	158.4	183.2	24.8	15.65	555	0.045
G10	155	185.8	30.8	19.87	565	0.055
L.S.D at 5%	12.19	19.75	11.69			

Each value represents the mean of 5 rats.

It means that they helped the rats to overcome the impaired body function caused by alloxan injection. The gain in body weight at the end of experimental period for the normal control was 34.8g, while the hyperglycemic control.

Showed 4.2g. Feeding on 40% soaked-cooked beans (G8) showed the highest gain in body weight (36.6g/6weeks). It could be noticed that gain in body weight of G3, G4, G5, G6, G7, G9 and G10 showed significant increases 12, 25, 16.6, 23.4, 11.2, 24.8 and 30.8g/6weeks, respectively. Concerning food intake, the values for normal control (G1) was 647g and 475g for hyperglycemic control (G2), while other groups (G3, G4, G5, G6, G7, G8, G9, and G10) were 510, 585, 580.5, 563.8, 490, 618.75, 555, and 565g/6weeks, respectively. It means that feeding on 40% raw beans and 40% soaked-cooked beans gave the lowest and highest food intake respectively relative to control. The data observed that the feed efficiency value of normal diet was 0.054g/g but the average value of the animal hyperglycemic control was 0.009g/g. The feed efficiency value of the diabetic rats fed on 40% soaked-cooked beans (G8) had the highest value 0.059g/g and the feed efficiency value of the diabetic rats fed on 40% soaked-cooked peas (G10) was 0.055g/g, so G8 and G10 showed insignificant differences compared to normal control. The other groups were lower than normal control. It can suggest that the improvements of the feeding on treated diets in blood glucose levels of diabetic rats were paralleled with the weight gain and feed efficiency which improved by legumes feeding (treatment diets). The obtained results are in agreement with those reported by Metwali *et al.*, (1994) who found that body weight gain, total food intake and feed efficiency ratio were decreased by feeding of dietary fiber compared with control (casein). Also (Warren *et al.*, 2003) found less food intake at lunch when a high-fiber breakfast was consumed earlier in the day.

**Effect of beans or peas on organs of hyperglycemic rats**

Organs weight and organ weight / body weight ratio of rats fed normal and different treatments of beans or peas were showed in table (3). The organ weight was increased with the increases in body weight of all experimental animals.

**Table (3): Effects of raw and treated legumes on the weight of liver, kidney, heart and brain of diabetic experimental animals.**

Treatment	Final body weight (g)	Liver weight (g)	Liver /body weight	Kidney weight (g)	Kidney / body weight	Heart weight(g)	Heart /body weight	Brain weight	brain / body weight
G1	191.4	5.31	2.77	1.28	0.67	0.54	0.28	1.48	0.77
G2	160.6	7.51	4.68	1.41	0.88	0.67	0.42	1.43	0.89
G3	167.4	7.12	4.25	1.33	0.79	0.59	0.35	1.50	0.89
G4	180	6.36	3.53	1.29	0.72	0.59	0.33	1.50	0.83
G5	171.8	6.46	3.76	1.29	0.75	0.57	0.33	1.42	0.83
G6	179.4	5.79	3.23	1.3	0.72	0.58	0.32	1.49	0.83
G7	167.2	6.19	3.7	1.23	0.74	0.56	0.33	1.45	0.87
G8	192.2	5.92	3.08	1.28	0.67	0.63	0.33	1.51	0.79
G9	183.2	6.28	3.43	1.32	0.72	0.62	0.34	1.47	0.80
G10	185.8	5.84	3.14	1.29	0.69	0.60	0.32	1.45	0.78
L.S.D at 5%	19.75	1.368		0.283		0.1762		0.2214	

Each value represents the mean of 5 rats.

The average values of liver weight were ranged between 5.79 and 7.12g which was 5.31g for normal control but of diabetic control was 7.51g. In addition, liver / body weight ratio was 2.77 for normal control and 4.68 for hyperglycemic control. While the liver weight to body weight were 4.25, 3.53, 3.76, 3.23, 3.7, 3.08, 3.43 and 3.14 for G3, G4, G5, G6, G7, G8, G9 and G10 respectively. All groups showed insignificant differences.

The values of kidneys weight were 1.28g and 1.41g for normal control and diabetic control, respectively. The average values in the other groups were ranged between 1.23 and 1.33g. Also kidneys / body weight ratio was 0.67 for normal control and 0.88 for diabetic control. While the other groups were ranged between 0.67 and 0.79. The values of heart weight were 0.54 and 0.67 for normal and diabetic control, respectively. But hypoglycemic diets groups (G3, G4, G5, G6, G7, G8, G9 and G10) had weights of 0.59, 0.59, 0.57, 0.58, 0.56, 0.63, 0.62 and 0.6g, respectively. The heart weight to body weight showed insignificant differences. The average values of brain weight were ranged between 1.42 to 1.52g which was 1.48g for normal control. Also the brain / body weight showed insignificant differences for all groups. The obtained results are in the line with those obtained by Khlon *et al.*, (1993) who found that the liver weight was increased with the increase in body weight of rats Abdel Rahim *et al.*, (1995) found that the organs weight and organ / body weight ratio were changed in which some ratios were increased but others were decrease relative to control. Also some of them were not alter at value of control animals.

#### **Effect of beans and peas on liver function of normal and hyperglycemic rats:**

Result in table (4) and (5) show the effects of raw bean, raw peas, soaked-cooked beans and soaked-cooked peas on plasma total protein, albumin and globulin with their ratio as well as activities of alkaline phosphates (ALP), glutamate-oxalacetate transaminases (GOT) and glutamate-pyruvate transaminases (GPT) with their ratio, as liver function of normal and hyperglycemic rats. The plasma protein level in normal control rats (100%) was reduced to 74.5% in the alloxanized rats after 6 weeks. Also, albumin level in control rats (100%) was reduced to 67.85% in the hyperglycemic control rats these were paralleled with globulin contents which reduced to 76.7% of normal control. When the hyperglycemic rats fed beans and peas, plasma protein, albumin, and globulin showed slight changes which were around the normal value. GPT activity was significantly stimulated by alloxan injection. This activity of alloxanized diabetic rats was nearly double that of the normal control. However, the effect of alloxan injection on GOT activity was less than of GPT. The activity of ALP in normal control rats (100%) was increased to 226.85% in hyperglycemic control rats after 6 weeks. On the other hands, the feeding with this legume to alloxanized diabetic rats was characterized by an improvement in protein level, albumin, and globulin and by a significant inhibition in GOT, GPT and ALP activities. The feeding on 40% soaked-cooked beans was characterized by the highest improvement.

**Table (4): Effects of raw and treated legumes on protein, albumin and globulin contents of diabetic experimental animals.**

Each value represents the mean of 5 rats.

**Table (5): Effects of raw and treated legumes on alkaline phosphates and transaminases activities as liver function of diabetic experimental animals**

Treatment	ALP		GOT		GPT		GOT/GPT
	u/l	%	u/l	%	u/l	%	
G1	127	100	41.86	100	20.48	100	2.04
G2	288.1	226.85	58.8	140.47	40.58	198.14	1.44
G3	178.5	140.55	56.2	134.26	34.92	170.5	1.6
G4	163.6	128.82	49.6	118.5	31.72	154.88	1.56
G5	180	141.73	53.4	127.57	34.08	166.4	1.57
G6	157.3	123.86	51.6	123.27	31.16	152.15	1.66
G7	175.5	138.19	52.4	125.18	31.4	153.32	1.67
G8	155.1	122.13	45.8	109.41	27.08	132.23	1.69
G9	171.9	135.35	50.4	120.4	30.28	147.85	1.66
G10	158.6	124.88	47.8	114.19	29.06	141.89	1.64
L.S.D at5%	34.50		9.631		8.838		

Each value represents the mean of 5 rats.

The present results are in a good agreement with the finding obtained by Venkateswary *et al.*, (1993). They proved that there was a marked decrease in the serum protein content of alloxanized diabetic rats when compared with that of the normal control animals, whereas the ingestion of hypoglycemic agents of medical plants could correct this metabolic

Treatments	Protein		Albumin		Globulin		Albumin/ Globulin
	g/dl	%	g/dl	%	g/dl	%	
G1	6.8	100	4.23	100	2.57	100	1.65
G2	4.84	74.5	2.87	67.85	1.97	76.7	1.46
G3	5.59	82.2	3.26	77.07	2.33	90.66	1.4
G4	5.81	85.4	3.53	83.45	2.28	88.7	1.55
G5	5.37	79	3.30	78	2.07	80.5	1.59
G6	5.71	84	3.31	78.25	2.4	93.4	1.38
G7	5.51	81	3.45	81.56	2.06	80.2	1.67
G8	6.12	90	3.64	86.05	2.48	96.5	1.47
G9	5.44	80	3.35	79.2	2.09	81.3	1.6
G10	5.58	82	3.41	80.61	2.17	84.4	1.57
L.S.D at 5%	0.6009		0.4372		0.1637		

disturbance significantly. Increasing the excretion of nitrogen in urine in alloxanized diabetic animals (Abdel-Rahim *et al.*, 1995), and increase the activity of transaminases as evident in the present study stems from the second amino group of urea, with enters the urea cycle arriving in the form of aspartate (Murray *et al.*, 2006).

In connection, the present results indicated that feeding with legumes like beans and peas rich in dietary fiber and proteins especially the



processing beans and peas. Had hypoglycemic effects and improved liver and kidneys function which accompanied with improvement in general clinical status of diabetic rats.

Generally, it can be concluded that the treatments with legumes by feeding of diabetic rats improved the metabolism of diabetic rats. This means that legumes can use as food stuff for treatment of diabetes.

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التأثير الخافض لسكر الدم للفاصوليا البيضاء و البسلة المعاملة و الغير معاملة.  
في الفئران المصابة بمرض السكر بالإضافة لحالة نشاط الكبد لهذة الفئران  
إبتسام عبد المنعم<sup>(1)</sup>، نادية طه صالح<sup>(2)</sup> و إيمان رشاد محمد<sup>(2)</sup>.  
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صممت هذه الدراسة لتقييم بعض البقوليات مثل الفاصوليا و البسلة كحبوب  
(بدون معاملة أو بعد معاملتها) كخافضات لسكر الدم بالإضافة لتقدير تأثيراتها على  
وظائف الكبد لهذة الفئران.  
و قد أظهرت نتائج هذه الدراسة أن تغذية الفئران المصابة بمرض السكر علي أي من  
الفاصوليا البيضاء و البسلة سواء المنقوعة المطبوخة منها أو الغير معاملة انخفاض في  
مستوي سكر الدم في الفئران المصابة. كما أن التغذية بهذة البقوليات أظهر تحسن  
واضح في مكونات الدم مثل نشاط الانزيمات الناقلة للأمين (GPT, GOT) كذلك  
محتوي البلازما من البروتين و الالبيومين و الجلوبيولين بالإضافة لتحسين نمو الجسم لهذة  
الفئران عند تغذيتها علي هذة البقوليات سواء المعاملة منها أو الغير معاملة . و هنا يجب  
التتوية بأن التغذية علي مستوي ٤٠% فاصوليا معاملة أعطي أفضل تحسن مقارنة  
بغيرها.  
و من هنا يمكن إجمال نتائج هذه الدراسة في أن التغذية علي أي من البقوليات بدون  
معاملة أو بعد معاملتها قد أظهر تحسن واضح كعلاج لمرض سكر الدم و لكن بدرجات  
مختلفة.