

## Biological Evaluation of the Purified Alpha Amylase Inhibitor from Kidney Bean (Giza 133)

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**T**HE BIOLOGICAL evaluation of the purified alpha amylase inhibitor of kidney bean (Giza 133) by using rats was carried out. Results showed that all rats gained weight at different levels. Rats in control groups gained more weight (64g) and faster than those with inhibitor. The results indicated that using 200 UI/100g diet was economically recommended. Diets supplemented with 200 UI increased BV, NPU by (16 %), and reduced starch hydrolysis. Glucose absorption in blood and serum insulin in all digestion hours, with little starch hydrolysis after 4 hr digestion noticed. The results also proved that using alpha amylase inhibitor in diet caused higher level of NEFA, reduction in the rate of forming fatty substances in subcutaneous layers in rats and consequently growing rate was reduced.

**Keywords:** Alpha-amylase inhibitor, Kidney bean Giza 133.

The effect of adding different levels of inhibitors from different sources to standard diet degestion showed that increasing the undigested starch in the rat's faeces is parallel to the amount of inhibitors. Results suggested that addition of alpha amylase inhibitors from different sources to diet without any treatment gave much better results especially when preparing diabetic or body weight control food (Jaffe & Vagalette, 1968).

The supplementation of starch-free and starch containing diets with an extracted alpha-amylase inhibitor of red kidney beans (*Phaseolus vulgaris*) indicated that its amount was equivalent to that supplied by 11 and 82g of kidney bean flour per rat per day. The inhibition effect was reduced in case of starch free diet while it had no effect on starchy diet. These results showed that excess dietary supplemented with red kidney bean alpha-amylase inhibitor (free of trypsin inhibitor and phytohemagglutinin activity) did not affect the hydrolysis of dietary starch as measured by the ability of dietary starch to provide energy for the growing rat (Savaianb *et al.*, 1977).

Human pancreatic alpha-amylase inhibitor had great pharmaceutical interest as a tool for the regulation of internal blood sugar levels to treat metabolic diseases like diabetes mellitus and obesity. In the course of a general screening

program for enzyme inhibitors of microbial origin an alpha-amylase inhibitor had been purified from culture broth of *Streptomyces tendae* (Koller, 1985).

This work was aimed to study the biological evaluation of the purified kidney bean Giza 133 alpha amylase inhibitor (*in vivo*), trying to understand the specific interference between the inhibitor and starch-free.

### Material and Methods

#### Materials

Alpha amylase inhibitor of kidney bean seeds Giza 133 (285 UI/mg protein) was prepared according to Mahmoud (2006). Human pancreatic and salivary alpha-amylase were obtained from Sigma Company, England. Young rats from House Animal's Laboratory in National Research Centre were used.

#### Methods

The biological evaluation of the alpha-amylase inhibitors was determined according to the methods described by Savaiano *et al.* (1977).

The experiment was conducted for 15 days using 25 young female rats (65±1.3g) and were divided into five treatment groups according to units inhibitor used, *i.e.*, (100, 200, 300 and 400 UI/100g diets). All the rats were ad-libitum to diet in all periods.

The rats were fed daily and each received, a meal composed of corn yellow starch 60 %, soybean 20 %, fattening concentration 10 %, bran 5 %, vitamins 0.25 %, bones powder 1.5 %, salts 0.5 %, mollas 2.75 % and 150 mg N, with the above different levels of inhibitor.

The growth data from each group were analyzed statistically by analysis of variance (Snedecor & Cochran, 1967), and the dietary treatment mean growth rates were compared using Duncan's multiple range test (Duncan, 1955) at the 5% level of probability.

Urine and faeces were collected separately during all periods and analyzed for nitrogen. The biological value (BV), true digestibility (TD) and net protein utilization (NPU) of the samples were determined according to Mitchell (1924) and modified by Eggum (1973).

As follow:

$$BV = 100 \times \frac{N \text{ intake} - (\text{fecal N} - \text{metabolic N}) - (\text{urinary N} - \text{endogen N})}{N \text{ intake} - (\text{fecal N} - \text{metabolic N})}$$

$$TD = 100 \times \frac{N \text{ intake} - (\text{fecal N} - \text{metabolic N})}{N \text{ intake}}$$

$$NPU = TD \times BV$$

*Determination of starch in faeces*

Starch in faeces was determined according to the method described in AOAC (1990).

Starch in faeces = the weight of glucose obtained X 0.925

*Determination of blood glucose, serum insulin and NEFA*

Blood glucose, serum insulin and NEFA were determined in rats blood according to Puls & Keup (1973).

*Determination of amylase activity*

Alpha-amylase activity was determined according to the method described by Bernfeld (1955).

*Alpha-amylase inhibitor assay*

The alpha-amylase inhibitor activity was determined using dinitrosalicylic acid (DNS) method described from Bernfeld (1955) and modified by Ishimoto & Chrispeels (1996).

**Results and Discussion***Effect of kidney bean alpha-amylase inhibitor on rat's growth rate*

The effect of kidney bean alpha-amylase inhibitor on rats growth rate which were provided food ad-libitum formulated so that adequate levels of vitamins, minerals and starch accompanied with four levels of inhibitor units, *i.e.*, (100, 200, 300 and 400 UI/100 gram diets) were tested and shown in Fig. 1.

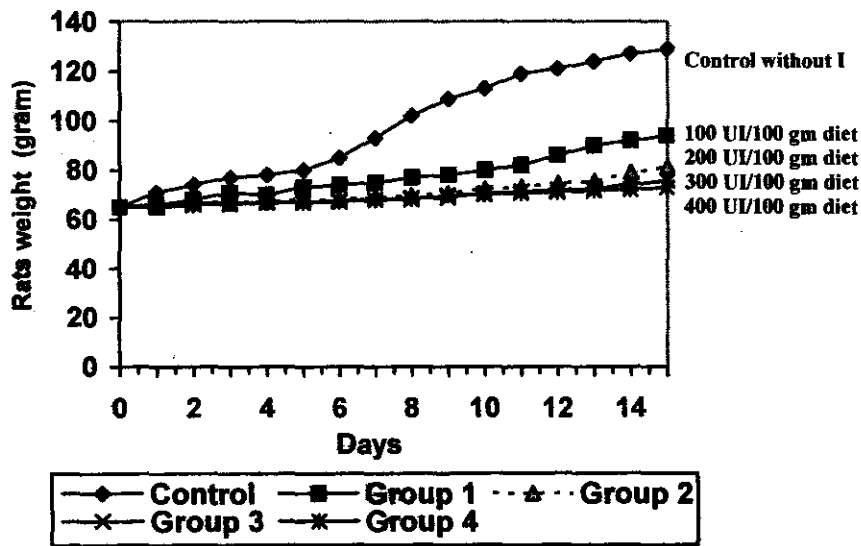


Fig.1. Kidney bean alpha-amylase inhibitor on rats growth rate.

The growth data of rats (Fig. 1) fed on starch diets containing different levels of kidney bean alpha-amylase inhibitor showed that all groups of rats gained weight at different rates. Rats in control groups gained weight (64g) faster than those in other groups with inhibitor. All rats in groups (1, 2, 3 and 4) with different levels of inhibitor, *i.e.*, (100, 200, 300 and 400 UI/100g diets) gained little weight compared with each other and also with control sample being 29, 16.3, 10.7 and 8.0 grams, respectively which in agreement with Savaiano *et al.* (1977).

Results indicated that the digestion of starch was reduced by the addition of inhibitor resulting in decreasing glucose absorption. It was concluded that these results reduced calorie availability, which would result in a reduced growth rate. It was also recommended that using 200 UI/100 g diets was more economically, regarding that there was inconsistency in the obtained results with higher inhibitor levels.

#### *Alpha-amylase inhibitor and its effect on digested and undigested starch in faeces*

The physiological effect of alpha-amylase inhibitor on digested and undigested starch in faeces of the experimental rats *in vivo* is shown in Table 1.

**TABLE 1.** Alpha-amylase inhibitor and its effect on digested and undigested starch in rats faeces.

Units inhibitor in the diet	Starch in the diet (%)	Undigested starch (%)	Digested starch (%)
Control without inhibitor	60	1.2	58.8
100 UI	60	23.9	36.1
200 UI	60	52.3	7.7
300 UI	60	54.8	5.2
400 UI	60	55.0	5.0

Results in Table 1 show that the digestion of starch in control sample was almost completed 58.8 % with both salivary and pancreatic alpha-amylase to maltose then glucose, which was emphasized by the presence of small amounts of starch in faeces 1.2 %. Additions of alpha-amylase inhibitor in the diet reduce the rate of starch digestion, and the undigested starch increased in the faeces.

The conclusion of these results is in agreement with that mentioned in the above Table 1.

#### *Biological value, true digestibility and net protein utilization $\pm$ SD in rat feeding experiment*

The Biological Value (BV), True Digestibility (TD), and Net Protein Utilization (NPU) in the rat feeding with 100 and 200 unit inhibitor is shown in Table 2.

**TABLE 2. Biological value (BV), true digestibility (TD) and net protein utilization (NPU)  $\pm$ SD\* in the feeding experiment.**

Diet Units Inhibitor/g	BV	TD	NPU
0	62.1 $\pm$ 3.0	82.2 $\pm$ 1.5	53.9 $\pm$ 2.0
100	67.1 $\pm$ 2.8	84.1 $\pm$ 1.1	60.7 $\pm$ 1.5
200	77.4 $\pm$ 1.6	88.4 $\pm$ 3.3	69.6 $\pm$ 2.7

$\pm$ SD\*: Standard Division

Results show that the BV of the protein in diet increased by supplementation of inhibitor. NPU increased approximately by 16 % at 200 UI in comparison to the non-supplemented diet.

The improved protein status of rats by inhibitor feeding are in agreement with data shown in Fig. 1.

*Alpha-amylase inhibitor and its effect on blood glucose, serum insulin and serum non-esterified fatty acid in rats*

The effect of kidney bean alpha-amylase inhibitor on blood glucose, serum insulin and serum non-esterified fatty acid of rats fed on diets containing 200 UI is shown in Table 3.

**TABLE 3. Alpha-amylase inhibitor and its effect on blood glucose concentrate, serum insulin and serum non-esterified fatty acid on normal rats.**

Time in hours	Serum glucose (mg %)		Serum insulin ( $\mu$ U / ml)		NEFA (m mol / L)	
	Starch	Starch + inhibitor	Starch	Starch + inhibitor	Starch	Starch + inhibitor
0	47.2	47.2	27.0	27.0	0.775	0.775
1	63.4	47.4	39.1	26.8	0.770	0.900
2	72.5	48.6	43.0	27.2	0.775	1.200
3	80.1	50.3	48.4	27.5	0.615	0.895
4	74.9	52.0	49.3	28.0	0.535	0.655

Results in Table 3 show that addition of 200 UI in all digestion periods reduce starch hydrolysis and so glucose absorption in blood and serum insulin which is obviously noticed comparing with the control sample without inhibitor.

Although kidney bean alpha-amylase inhibitor has been shown to reduce the rate of starch hydrolysis in rat stomach, hydrolysis of starch however did occur in small amounts (4 hr) due to the uncompleted inhibition of the inhibitor with 93 % purity and which explain the minor increase either in blood glucose of serum insulin between the 200 Unit Inhibitor digestion hours.

It could be concluded that alpha-amylase inhibitor might be used to slow down the rate of starch digestion and attenuate hyperglycemia and serum insulin response to starch digestion (Puls & Keup, 1973; Frerichs *et al.*, 1974 and Puls & Keup, 1974) and to reduce the rate of carbohydrates conversion to adipose and aorta lipids (Keup & Puls, 1974).

Results in Table 3 show also that NEFA in control sample without inhibitor is declined progressively with digestion hours comparing with sample (200 UI), which is enhanced after 2 hr digestion followed by a rapid declining in their amount. These results indicated that the action of alpha-amylase inhibitor presented in the diet caused a higher level in the amount of NEFA, thereby reduced the rate of forming fatty substances in subcutaneous layers in rats and consequently growing rate is reduced.

### Conclusion

It could be concluded that alpha-amylase inhibitor from kidney bean Giza 133 is the economically effective for dietary uses at 200 UI/100g diet. It could be used to slow down the rate of starch digestion and attenuate hyperglycemia and serum insulin response to starch digestion, and reduce the rate of carbohydrates conversion to adipose and aorta lipids.

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