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Short Communication

BIOCHEMICAL EFFECT OF A HIGH DOSE OF THAUMATIN IN WISTAR RATS

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ABSTRACT

Objective: The aim of this work was to investigate the capability of a high dose of thaumatin; a sweet tasting protein, of improving induced protein malnutrition in male Wistar rats.

Methods: For this study, 12 rats were divided into 2 groups and treated orally along with a high-carbohydrate, low-protein diet as follows: water group as a negative control, and thaumatin group at a dose of 464 mg/kg for 3 consecutive w. Blood samples were collected to analyse glucose, triglycerides, total cholesterol, and total protein, and body weight was measured. An oral glucose tolerance test (OGTT) was carried out at the end of the experiment.

Results: Despite the high amount of thaumatin used, only a slight increase in blood glucose occurred and was within the normal range, whereas serum triglycerides and cholesterol decreased significantly unlike control. Body weight had declined in both groups due to a low-protein diet, while total protein and glucose tolerance remained unchanged.

Conclusion: It is found that thaumatin is safe to consume by Wistar rats even at high doses. Besides that high-carbohydrate, low-protein diet caused falling of body weight, it had drawbacks of increased triglycerides and cholesterol levels which can be useful to create animal models of abnormal lipid metabolism without obesity. However, simultaneous ingestion of thaumatin with this diet had altered the outcomes to the best case. In future, it may be possible to use this combination for achieving healthy eating patterns without drug intervention that is needed for obese patients with various dysglycemia or dyslipidemia manifestations and people following regimes for weight reduction.

Keywords: Thaumatin, Sweet-tasting protein, Protein malnutrition, Blood glucose, Lipid profile, Blood protein, Body weight, Wistar rat

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In recent years, industry sectors and consumers are becoming more demanding of sugar substitutes especially natural ones such as thaumatin for their distinctive properties of safety and suitability to diet purposes. Thaumatin is a sweet protein extracted from the fruit of plant *Thaumatococcus daniellii* (Benth.). It is sweeter than sugar by 2000-3000 times on a weight basis and by 100 000 times on a molar basis. It is metabolised as other proteins without an ADI limit [1, 2].

European Union (EU) allows the addition of thaumatin to some food categories as a sweetener and flavour enhancer under European Parliament and Council Directive No. 94/35/EC and 95/2/EC, respectively, within specific limits [3, 4]. The highest maximum level allowed of thaumatin to be added 400 mg/kg in food supplements category in their ready-to-eat form.

Much research has been conducted on this sweetener including its preference at mice [5], structure-sweetness relationship [6], as well as atomic and crystal structure [7, 8], and thermo resistance [9]. However, the biochemical effects of thaumatin and its safety were not adequately studied. Some studies on toxicity, allergy, and biological value of this sweetener were conducted, but most of them are still unpublished [10]. Although a large number of studies have studied many bulk and intense sweeteners for their effects on several blood parameters in healthy and diabetic individuals, thaumatin was not incorporated in anyone of them [11–13].

The aim of this study was to investigate the effect of administrating a very high dose of thaumatin; some 16 000 times the estimated maximum daily consumer intake in 1984 [10], on blood parameters and body weight in Wistar rats together with a high-carbohydrate low-protein diet in order to explore thaumatin's capability of protein compensating for induced protein malnutrition. Furthermore, the selected dose was the maximum which can dissolve in a practical volume of water suitable for oral gavage according to preliminary experiments conducted previously in our laboratory.

Adult male Wistar rats, weighing 170-230 g, were used in this study. The experimental animals were bred in our animal house of Pharmacy College and maintained under controlled laboratory conditions of temperature (20±2 °C) on a 12:12 h light/dark cycle. Experimental protocols were approved by the Institutional Animal Care and Use Committee at Pharmacy College (approval N.5573/ 2014) and performed in accordance with the correspondence Ethical Guidelines.

This study was conducted on 12 rats randomly divided into 2 groups (n=6); (1) control: treated with only water W at an average of 0.91 ml/d, (2) thaumatin T (Naturex, England) at a dose of 464 mg/kg/d. Treatments were given daily by oral gavage (p. o.) for 3 consecutive weeks together with maintaining rats on a high-carbohydrate low-protein (HCLP) diet; 79 and 7% by weight, respectively (local animal diets markets).

For both rat groups, body weight was measured at 4-day intervals using a digital balance (SI-132, Excell). After 7 d, capillary blood samples were withdrawn from tail vein of fasting rats by puncture [14], and blood glucose level (BGL) was determined at the following intervals: 0, 30, 45, 60, 90, 120, 150, and 180 min with Accu-Chek® Active glucometer, Roche, Germany.

Moreover, at the baseline and end of the experiment, overnight fasting rats were subjected to light diethyl ether (Surechem Products LTD, England) anaesthesia then, venous blood samples were collected from the tail vein into dry Eppendorf's-microtubes (Alhayat, Syria) [14]. Subsequently, serum was separated by centrifugation at 3000 rpm for 10-15 min for the determination of fasting triglycerides (TG), total cholesterol (TC), and total protein using appropriate kits from BioSystems S. A. (Spain) with BioSystems A-25 autoanalyzer (Spain). In addition, using glucose strips, fasting BGL was determined, too.

Finally, at the 22nd day, glucose (Riedel-deHaën, Germany) solution in a dose of 2 g/kg was given orally to overnight fasting rats. Then,

BGL was measured every 30 min up to 2 h [15]. The area under the curve (AUC) of BGL was calculated by the trapezoidal method to represent the glucose tissue utilisation.

Data are expressed as mean±standard deviation (SD). Analysis of data was performed with IBM® SPSS® v.20 using repeated measures analysis of variance (2 groups' × time points) or independent t-test, as appropriate. Differences were considered significant at $p<0.05$.

For **T** group, BGL was similar to that of control throughout the test ($p>0.05$), as shown in table 1. Although BGL had increased gradually and significantly to reach its maximum about 97.8±14.3 mg/dl at 60 min for both groups, thaumatin started rising 15 min earlier than control. The large amount administrated to rats ~1 ml which takes more time to be ingested and absorbed, may have contributed to the delayed rising. In the case of water, there was no an energy source, so the fasting state probably promoted glycogenolysis in skeletal and liver tissues through glucagon secretion which had contributed to slight elevating in BGL. It is known that meals rich in proteins promote glucagon secretion, and that may have cause the early rising in BGL of T group [16]. Since the maximum BGL was not above the physiological range, this increasing had no clinical importance, and we can conclude that thaumatin even in high doses did not affect short-term blood glucose from a clinical view.

Table 1: Levels of blood glucose through 3 h of oral gavage at the 8th day

Group	Blood glucose (mg/dl)*							
	0	30	45	60	90	120	150	180
W	67.8±8.1	77.2±12.7	90.2±13.3††	96.0±13.9††	80.2±11.6†	75.5±7.9†	77.5±10.2†	71.2±6.4
T	66.7±5.9	83.0±10.4†	92.0±10.3††	99.5±15.7††	80.7±11.8†	77.7±7.9†	71.5±13.3	71.8±10.8

Data are expressed as mean±SD, n=6. W: Water, T: Thaumatin, *Blood glucose for T group did not differ significantly from control at all-time points ($p>0.05$), $^{\dagger}p<0.05$, $^{\dagger\dagger}p<0.01$, $^{\dagger\dagger\dagger}p<0.001$ vs. corresponding baseline value

Fig. 1 shows that AUC value for T group did not differ from control ($p>0.05$), meaning that thaumatin did not alter glucose tissue utilisation after 3w. Our findings were in agreement with Figlewicz *et al.* study in 2009, which reported no changing in glucose tolerance where stevia, another natural non-caloric sweetener, had been drunk by rats for 10 w [17]. In previous studies, sucralose and acesulfame potassium in healthy humans, as well as sucralose in diabetic patients, did not affect neither BGL nor glucose tolerance after OGTT [12,18]. People with impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) were approximately 5-10 times likely to develop diabetes within 1 y than normal people [19]. Hence, using alternative sweeteners like thaumatin can contributes to maintain healthy diet and avoid development of diabetes. BGL control can dramatically reduce blood vessel damage in diabetes, and help in avoiding amputations of the lower limbs resulting from diabetic wound and ulcer [20].

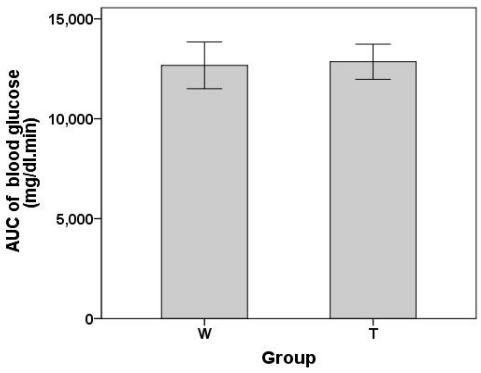

Fig. 1: Area under the curve ($AUC_{0\text{-}120\ min}$) of blood glucose during OGTT test*, Data are expressed as mean±SD, n=6. W: Water, T: Thaumatin, OGTT: Oral glucose tolerance test, *$p>0.05$ vs. control; there were no significant differences

Since BGL value for T group did not change significantly compared to water or over the experimental period (table 2), it can be said that thaumatin did not affect long-term BGL, too. Also, W group BGL did not differ over time, meaning that HCLP di*et al.* one had no effect on long-term BGL. The normal range for total proteins in rats is 6.3-8.6 g/dl [21]. Since the same observation was noticed for total serum protein where its value in T group was comparable to control after 3 w, and there was no change in it over time, it can be concluded that neither HCLP diet nor thaumatin had an effect on serum protein. Furthermore, the diet did not cause a severe malnutrition as no decrease was observed [16].

On the other hand, at the end of experiment, a significant decrease in T group ($p<0.05$) happened in TC level compared to control, while for TG there was an interaction between time and treatment factors ($p<0.001$), where it increased statistically in control group, but decreased in the T one ($p<0.05$) after 3 w.

The effect of HCLP diet used was clear in control group where both of serum TG and TC had increased up to 38 and 20%, respectively. Despite this diet is unsuitable for weight loss because of its negative effects on both TG and TC, it may be applicable in next years to create animal models of abnormal lipid metabolism. The high intake of carbohydrates seems to be converted to glycogen and the excess amount to fat in adipose tissues [16].

In T group the opposite had occurred. The decreasing in serum TC and to a higher degree in TG in T group means that a lipolysis process may be stimulated during those 3 w, by thaumatin itself or a synergism effect with the diet used. The high dose of thaumatin (464 mg/kg) as a protein, can promote glucagon secretion which has catabolic effects included TG break down to glycerol and free fatty acids (FFA). It is unlikely that thaumatin caused these effects through activation of the thyroid gland by its hydrolysates according to a previous WHO report [10]. However, other endocrine glands like adrenal cortex may be involved indirectly through cortisol [16].

Table 2: Initial and final rat venous blood and serum parameters

Group	Period	W	T
Blood glucose (mg/dl)	Baseline	115.8±24.9	104.0±10.7
	3rd week	104.3±17.3	109.4±10.8
Serum TG (mg/dl)	Baseline	101.7±29.8	122.7±31.0
	3rd week	140.7±35.5†	93.0±26.5†
Serum TC (mg/dl)	Baseline	54.4±11.5	53.8±9.9
	3rd week	65.7±14.5††	48.4±12.8*
Serum total protein (g/dl)	Baseline	6.67±0.32	6.95±0.46
	3rd week	7.25±0.47	7.13±0.13

Data are expressed as mean±SD, n=6. W: Water, T: Thaumatin, TG: Triglycerides, TC: Total cholesterol, $^{*}p<0.05$ vs. control. $^{\dagger}p<0.05$, $^{\dagger\dagger}p<0.01$ vs. corresponding baseline value.

As presented in (fig. 2), body weight for T group did not differ significantly from control (p>0.05) at all intervals. Additionally, in both W and T groups, body weight had decreased during the study period. This is highly possible because of the low-protein diet used which made the body decompose skeletal muscle proteins in order to synthesise necessary proteins especially essential ones [16]. However, the pattern of body weight declining was a little different between groups. Declining was insignificant comparing to baseline value in T group until the 21st day (p<0.01), meaning that body weight changed slowly. Whereas for control, changing was little quicker and significant starting from the 5th day (p<0.05).

Although the percentage of weight loss was not statically different, it was slightly lower in T group where it reached-9.5±6.1 and-8.4±5.3% for W and T, respectively. We have assumed that thaumatin as a protein, may have compensated partly for the low-protein intake and reduce degradation of structural proteins. Therefore, it had an anabolic effect [16]. However, it lacks histidine in its structure which is an essential amino acid in rats, and that can explain weight loss but at a slower rate [10][22]. Perhaps, the studied thaumatin's dose was not high enough to overcome the low protein intake clearly. Besides introducing thaumatin together with HCLP diet had reduced body weight at a smaller rate which is healthier for the body, it decreased TG and TC which maintained loosen weight and protected from cardiovascular diseases [16].

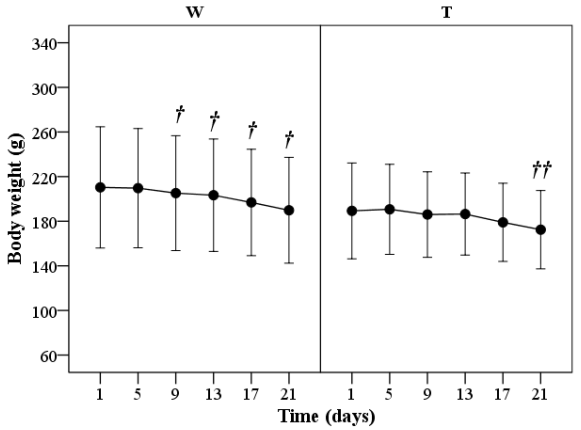

Fig. 2: Body weight changes during 3 w, Data are expressed as mean±SD, n=6. W: Water, T: Thaumatin, †p<0.05, ††p<0.01 vs. corresponding baseline value

This experiment had demonstrated the fact that thaumatin is safe at extreme doses in Wistar rats. Combining between HCLP diet and thaumatin had showed that it is useful for weight loss, blood glucose control, and lowering cholesterol and triglycerides levels simultaneously. What is offered by this combining is fundamental for managing diabetes in obese people and delay or reduce its serious complications like retinopathy which is the leading cause of vision impairment and blindness, and cardiovascular risk which still represents the major cause of mortality in diabetes [23, 24]. Simply, it is just suitable for weight management and, in the future, these findings can have important applications in humans with dysglycemia, dyslipidemia, obesity, and diabetes. Further studies are required to elucidate the specific mechanisms involved in thaumatin's effects and confirm these findings in animals and humans especially diabetic ones. We recommend performing other studies on this interesting protein through incorporating it in different diet regimens as a food component not additive, studying glycemic index of HCLP diet to standardise its most suitable carbohydrates to proteins ratio for patients with different metabolic disorders. In coming years, HCLP di*et al.* one can serve for inducing animal models of abnormal lipid metabolism without obesity.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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