Theme 3.2; Food and Pharmaceuticals

Phytomodulatory effects of fresh and processed shoots of *Dendrocalamus hamiltonii*: I. Effect on blood glucose and kidney function in Balb/c mice

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Abstract

Bamboo shoot is a vegetable that contains almost all the essential vitamins, amino acids, minerals and other nutrients considered essential for sustaining life and maintaining health. Low fat and high dietary fiber content makes the shoot one of the widely acclaimed nutrient rich food items. Juvenile bamboo shoots after harvest need to be stored in various processed forms until use by consumers due to short shelf life of the shoots and presence of anti-nutrient cyanogenic glycoside. The major processing techniques which are commonly used for enhancing shelf life of the shoots and removal of cyanogenic glycoside are soaking, boiling, salting and fermentation. The purpose of the study was to investigate the effects of aqueous extract of fresh, fermented, brine preserved and boiled shoot of *Dendrocalamus hamiltonii* on blood glucose level and kidney function in Balb/c mice. Results revealed a less fall in fasting blood glucose level in bamboo shoot treated mice as compared to the control group. The lowest fall was observed in the group treated with fermented shoots (107 mg/dl), followed by boiled shoots (84 mg/dl), brine treated shoots (79 mg/dl) and fresh shoots (76 mg/dl). Similarly, serum creatinine level was also increased in all the bamboo shoot treated groups with highest increase in the animals administered with fermented shoots extract. The histopathological studies of renal tissues showed a normal glomeruli and tubules when compared with the control group.

Key words

Bamboo shoots, serum glucose, renal function, histopathology

Introduction

Nutrients and bioactive components in the food influence the function of the body, protect against disease and restore health (Hernandez-Ledesma et al., 2017). Under certain circumstances, diet can be a serious risk factor for a number of diseases but, the degree to which diet influences the balance between healthy and disease states may depend on an individual's genetic makeup. Dietary intervention based on knowledge of nutritional requirement and nutritional status can be used to prevent, mitigate or cure chronic disease. High blood pressure and high level of blood sugar can lead to chronic kidney disease. Similarly, hypoglycemia associated with renal failure is also very common (Arem 1989). An abrupt change in serum creatinine and blood urea nitrogen (BUN) is the most common indicator of kidney injury. If there is an increase or decrease in the values of these markers it indicates dysfunction of kidney. Optimum nutrition, providing all nutrients in both kind and amount, is the cornerstone of good health and the cutting edge of prevention (Krehl 1983). Foods packed with vitamins, minerals, nutrients and bioactive components can lower disease risks and boost the body's immunity.

Bamboo shoot is one such kind of food that contains almost all the essential vitamins, amino acids, minerals and other nutrients considered essential for sustaining life and maintaining health. Low fat and high dietary fiber content further makes the shoot one of the widely acclaimed nutrient rich food items (Miettinen 2003; Nirmala *et al*. 2011, 2014b). In addition, the shoots also have a good profile of minerals such as, potassium, manganese, iron, calcium, chromium, zinc, selenium and phosphorus (Shi and Yang 1992; Nirmala *et al*. 2007). Recent studies have indicated the antioxidant, anticancer, antimicrobial, antidiabetic and cholesterol lowering properties of the shoots (Hiromichi 2007; Park and Jhon 2009, 2010; Woo *et al*. 2012; Tanaka *et al*. 2011, 2013) and all these health outcomes have been attributed to the presence of phenols, phytosterols, dietary fiber and other bioactive compounds in the shoots. Besides its nutritive and therapeutic value, bamboo shoot also contains anti-nutrients such as cyanogenic glycoside hence needs to be processed properly for safe human consumption. However, consumption of improperly processed shoots is associated with food poisoning and may produce symptoms like rapid respiration, drop in blood pressure, dizziness, stomach pains, headache and vomiting convulsion (FSANZ 2004). The major processing techniques which are commonly used for removal of anti-nutrients and also for enhancement of shelf life of shoots are soaking, boiling, salting and fermentation. But along with enhancement of shelf life and palatability, processing often brings about changes in many attributes of shoots including its nutritional, antioxidant and therapeutic values (Bajwa *et al*. 2016a). Hence, the aim of the present study was to investigate the effects of aqueous extract of fresh, fermented, brine preserved and boiled shoots of a popular edible bamboo species, *Dendrocalamus hamiltonii* on blood glucose level and

kidney function in Balb/c mice. This study would serve as a very important baseline for further studies in developing nutraceuticals and pharmaceutical from the fresh and processed shoots of *D. hamiltonii*.

Material and Methods

Collection of plant material

The juvenile shoots of *D. hamiltonii* were collected from Shillong, Meghalaya. The shoots were harvested two weeks after emergence above the ground. After harvesting, the edible portion of the shoots was extracted gently without causing physical injury. The shoots were transported from Shillong to Botany Department, Panjab University, Chandigarh by air.

Preparation and Processing of plant material

In the laboratory, shoots were washed properly under tap water. After washing the hard basal portion of the shoots was discarded and the culm sheaths were removed carefully until the milky white portion of the shoots were exposed. Weight of the shoots before and after the removal of culm sheaths were also noted down. The shoots were then cut into thin slices and divided into four equal portions and subjected to processing (15 min boiling, 5% brine treatment, fermentation).

Preparation of bamboo shoots extract

For preparation of aqueous extract, 10 g of each sample of bamboo shoot powder was taken and soaked in 100 ml of distilled water in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 120 rpm for 24 h. Extracts were then filtered and dried using a hot air oven at low temperature. The dried crude extract was weighed to calculate the extractive yield and stored in a refrigerator at 4°C in air tight bottles and used for further experiment.

Experimental design

Healthy male Balb/c mice weighing 25-30 g each, procured from Central Animal House, Panjab University, Chandigarh, India, were housed in polypropylene cages, bedded with sterilized rice husk. Animals were maintained at Department of Biophysics, Panjab University, Chandigarh. Mice in all the groups had free access to standard animal pellet diet (Ashirwad Industries Ltd., Ropar, Panjab) and clean tap water throughout the experiment. They were maintained in a 12 h light/dark cycle at 25 ± 2°C. All the experimental protocols were approved by the Institutional Ethics Committee (Panjab University,

Chandigarh, India) and conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals. The animals were randomly divided into 5 groups of 6 animals in each group. Group I served as control; received tap water and feed *ad libitum*. Group II animals received the fresh shoots extract, group III animals administered the extract of fermented shoots; group IV animals were given the extract of brine treated shoots while group V animals received the extract of boiled shoots. Fresh doses were prepared every day in distilled water and administered to the animals at the dose levels of 800 mg/kg, body weight in the dose volume of 1 ml/kg, body weight. The body weights of all the animals were measured once per week throughout the study period by using digital balance. After completion of the treatment period, the animals were kept on fasting overnight and blood was withdrawn from the retro-orbital plexus of the mouse eye with a capillary. Blood (500 µl) was withdrawn in micro centrifuge tubes and incubated in an upright position at 37°C for 3 h to allow clotting. After incubation, the samples were centrifuged at 3000 rpm for 10 min. The serum was carefully aspirated and used for biochemical analysis. Thereafter, animals were sacrificed by cervical dislocation under light ether anesthesia and the kidney tissues were excised out and analyzed for histoarchitecture alterations.

Biochemical estimations

The serum obtained was used for the estimation of glucose, creatinine, urea and blood urea nitrogen (BUN) using commercially available standard kits.

Kidney histology

For histopathological studies, formalin fixed tissues were processed using conventional laboratory procedure. The tissues were dehydrated through ascending grades of alcohol, cleared in benzene and embedded in low melting point paraffin wax. 5µm thick section were cut and then stained with hematoxylin and eosin to investigate histopathological alterations.

Statistical analysis

The data obtained from the experiments are expressed as mean ± SD (standard deviation). For statistical analysis, data were subjected to analysis of variance (ANOVA) followed by post-hoc test and values are considered statistically significant at $F < 0.05$.

Results

Glucose

In the normal control mice, serum glucose level was 68 ± 2.92 (mg/dl). Treatment with aqueous extract of fresh and processed shoots at concentration 800 mg/kg, bw (body weight) for six consecutive weeks resulted in a significant ($p < 0.05$) increase in serum glucose level as compared to control mice (Figure 1). Although, glucose level increased, it was still within the standard normal reference range. The highest increase was observed in the group treated with fermented shoots (107 mg/dl), followed by boiled shoots (84 mg/dl), brine treated shoots (79 mg/dl) and fresh shoots (76 mg/dl).

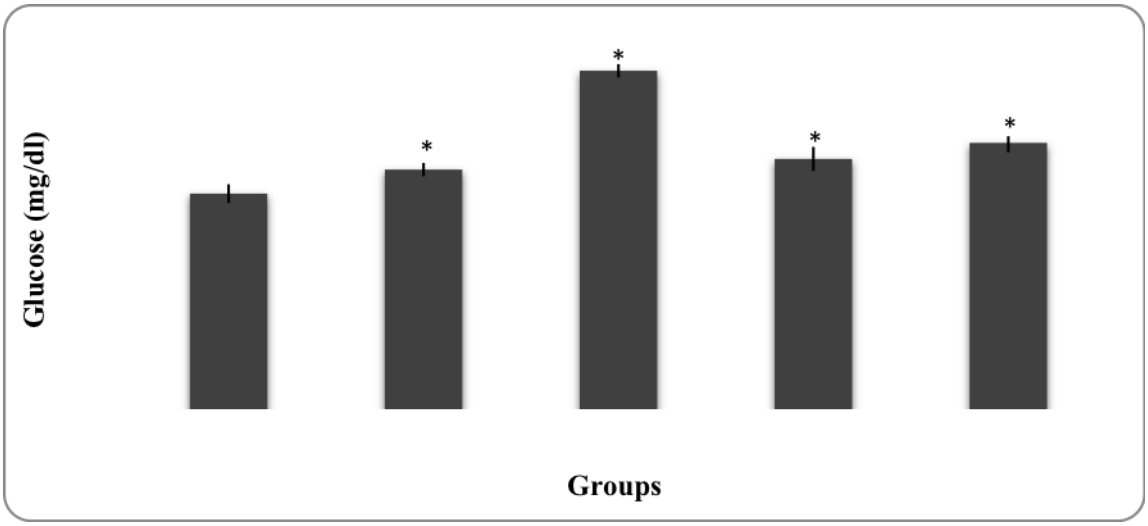

Figure 1. Effect of fresh and processed shoots extract on serum glucose level, Group I: Control, Group II: Fresh shoots; Group III: Fermented shoots; Group IV: Brine treated shoots; Group V: Boiled shoots; Data is analyzed by one-way ANOVA followed by post-hoc test; (*$P < 0.05$) significant as compared to control group

Kidney function test

Kidney function in all the experimental groups were monitored by analyzing the levels of serum creatinine, blood urea and blood urea nitrogen (BUN). In the normal control mice, the creatinine, blood urea and BUN level was 0.306 ± 0.01, 56 ± 1.01 and 24 ± 0.52 (mg/dl) respectively. A significant ($P < 0.05$) increase in the serum creatinine level was observed in all the groups treated with fresh and processed shoots with highest increase (43%) in the animals received fermented shoots extract (Figure 2). Blood urea level increased in the groups treated with brine preserved and boiled shoots by 11% and 12% respectively while, decreased in the animals received fresh (9%)and fermented shoots (12%). Regarding

blood urea nitrogen (BUN), increase was observed in all the groups except the animals treated with fermented shoots, where a slight decrease (4%) was observed (Figure 3).

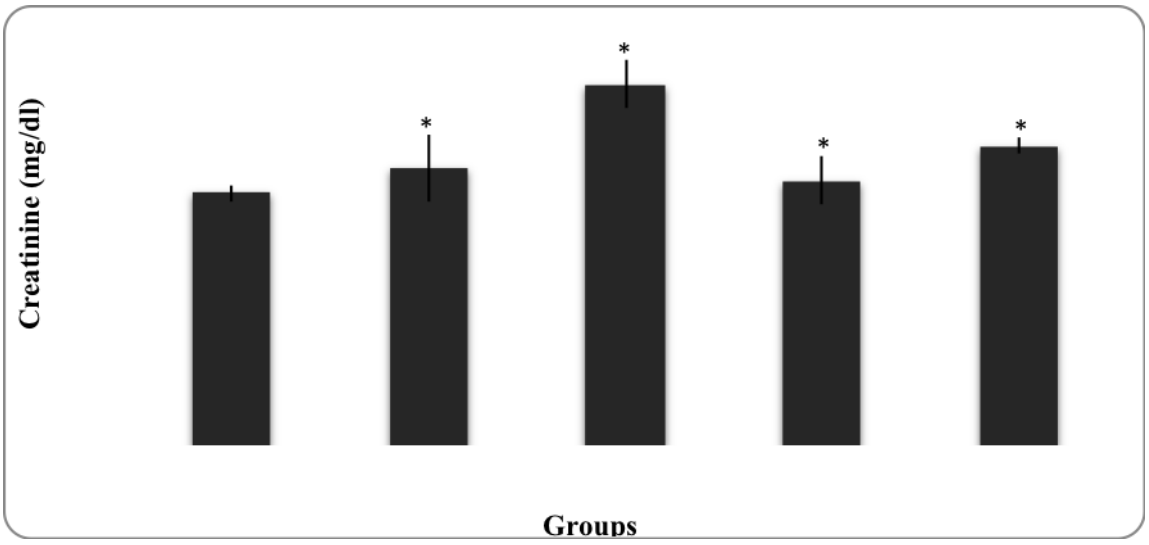

Figure 2. Effect of fresh and processed shoots extract on creatinine level, Group I: Control, Group II: Fresh shoots; Group III: Fermented shoots; Group IV: Brine treated shoots; Group V: Boiled shoots; Data is analyzed by one-way ANOVA followed by post-hoc test; (*P < 0.05) significant as compared to control group

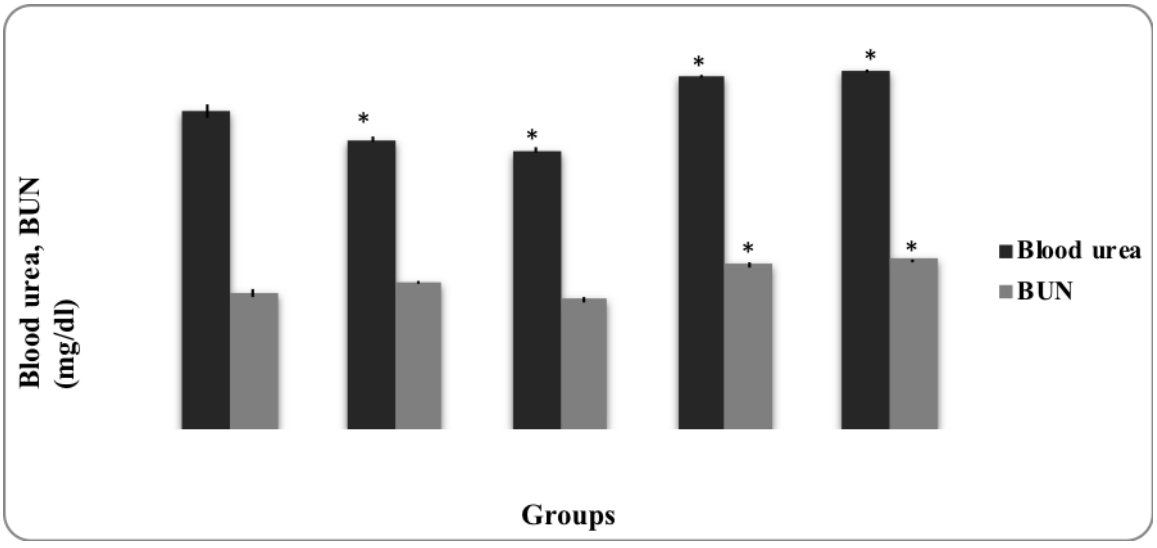

Figure 3. Effect of fresh and processed shoots extract on the level of blood urea and BUN, Group I: Control, Group II: Fresh shoots; Group III: Fermented shoots; Group IV: Brine treated shoots; Group V: Boiled shoots; Data is analyzed by one-way ANOVA followed by post-hoc test; (*P < 0.05) significant as compared to control group

Histopathological studies of kidney

Figure 4 (A-E) shows the histopathological structure of the renal tissues from Balb/c mice administered with aqueous extract of fresh shoots (B), fermented shoots (C), brine preserved shoots (D) and boiled shoots (E) at the dose 800 mg/kg bw for six consecutive weeks. Neither fresh nor processed shoots extract caused any pathological alteration in the kidney of the mice. The glomeruli and tubules of the kidney of all the groups were normal when compared with the control group (A).

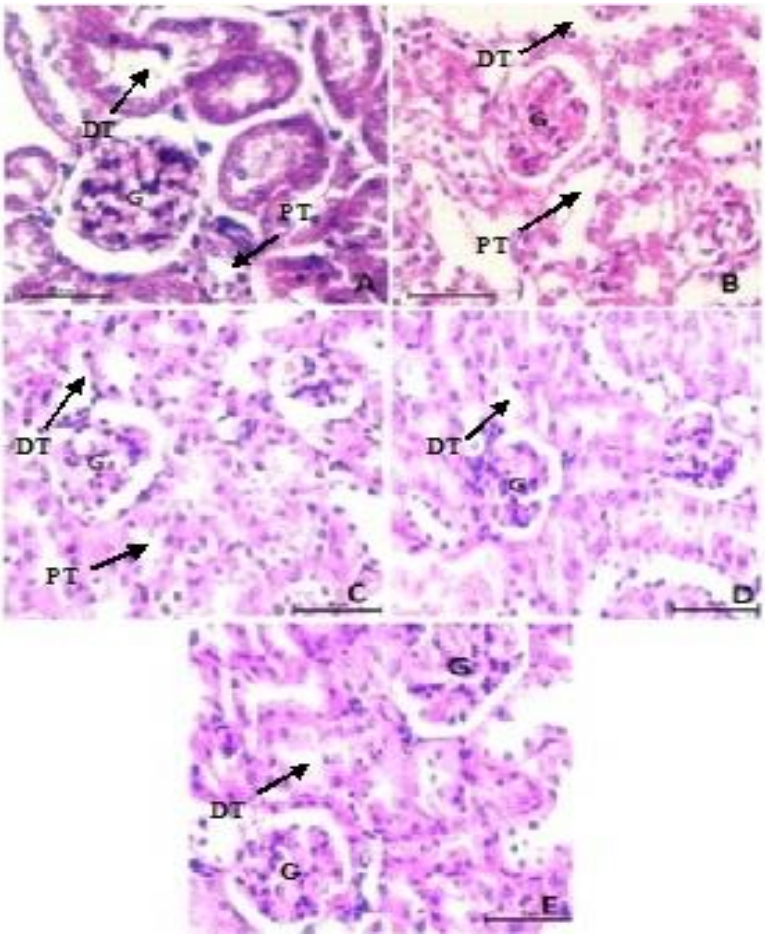

Figure 4. Histomicrographs of renal tissue from control group (A) and the groups treated with aqueous extract of fresh shoots (B), fermented shoots (C), brine preserved shoots (D) and boiled shoots (E), section of kidney showing normal glomerulus and tubules (400×), (G=glomerulus, DT= distal convoluted tubule, PT= proximal convoluted tubule)

Discussion

Dendrocalamus hamiltonii is the most preferred bamboo species in the north-eastern parts of India. In Meghalaya, Mizoram and Sikkim tender edible shoots of *D. hamiltonii* (Nees & Arn.) are harvested and consumed most followed by *Melocanna baccifera*, *Bambusa balcooa* and *Chimonobambusa hookeriana* (Bhatt *et al*. 2003). Young shoots of *D. hamiltonii* are not only delicious but are also rich in nutrients and bioactive compounds which are beneficial for health. Recent studies have indicated the antioxidant, anticancer, antimicrobial and cholesterol lowering properties of the juvenile bamboo shoots (Park and Jhon 2009, 2010; Fujimura *et al*. 2005; Hiromichi 2007; Woo *et al*. 2012; Tanaka *et al*. 2011, 2013). Juvenile shoots of some bamboo species after harvest need to be stored in various processed forms until use by consumers due to short shelf life and presence of anti-nutrient cyanogenic glycoside. Traditional processing and preservation methods such as boiling, soaking, canning, drying, fermentation etc. need to be standardized for eliminating toxic content while keeping the nutritional properties intact. The effects of aqueous extract of fresh, fermented, brine preserved and boiled shoots of *Dendrocalamus hamiltonii* on blood glucose level and kidney function in Balb/c mice were investigated.

Blood glucose level is the amount of sugar present in the blood of a human or animal. The body naturally regulates blood glucose levels as a part of metabolic homeostasis. A healthy diet is a key to blood sugar management and preventing or treating diabetes. Bamboo shoots being a rich source of nutrients and bioactive compounds can help stabilize blood sugar level. In the present study, glucose level in the normal control mice was 68 ± 2.92 (mg/dl). Treatment with aqueous extract of fresh and processed shoots at concentration 800 mg/kg bw for consecutive six weeks resulted in increase in serum glucose level as compared to control mice. The highest increase (57%) was seen in the animals treated with fermented shoots while, lowest (12%) was observed in mice treated with fresh shoots. However, the glucose level in all the bamboo shoot treated groups was not more than 110 (mg/dl). The results of the present study indicated a less decrease in fasting glucose level in bamboo shoots treated mice as compared to the control group. It has been reported that, the regular consumption of high-fiber diet helps in maintaining the serum-insulin level, hence cause less or even no fall in blood sugar level. The removal of fiber from food, and also its physical disruption, can result in faster and easier ingestion, decreased satiety, and disturbed glucose homoeostasis which is probably due to inappropriate insulin release. These effects favor over nutrition and, if often repeated, might lead to diabetes mellitus (Haber *et al*. 1977). Dietary fiber content increased to a significant level after fermentation (Bajwa *et al*., 2016a) which might be responsible for well maintained fasting serum glucose level in the animals administered with fermented bamboo shoots as compared to other processed forms. This indicates that, the regular

consumption of high-fiber diet like bamboo shoots could help in maintaining the serum-insulin level and also energy level of the body even in the conditions of fasting.

Kidney function is an indication of the state of the kidney and its role in renal physiology. The results of these tests are used to assess the excretory function of the kidneys. Kidney function in all the experimental groups were monitored by analyzing the levels of serum creatinine, blood urea and blood urea nitrogen (BUN) which are commonly measured to determine kidney health. Creatinine is a breakdown product of creatine phosphate in muscles, and is usually produced at a fairly constant rate by the body. It passes into the bloodstream, and is usually passed out in urine. Urea is also a waste product formed from the breakdown of proteins and passed out in the urine. BUN tests measure the amount of nitrogen in the blood. Generally, a high blood level of creatinine, urea and BUN indicate that the kidneys may not be working properly. In the present study, a significant increase in the serum creatinine level was observed in all the groups treated with fresh and processed shoots with highest increase (43%) in the animals that received fermented shoot extract. Blood urea and blood urea nitrogen level was found to increase in brine preserved and boiled shoots treated animals while a slight decrease was seen in the group administered with fermented shoots. Besides the increase in serum glucose and creatinine level, no alteration was found in the structure of kidneys. The histopathological studies of the renal tissues from bamboo shoots treated mice did not depict any pathological change in the glomeruli and tubules of the kidney. The increase in serum glucose or creatinine level may be contributed by the primary metabolites that are present in the shoots of *D. hamiltonii*, or more specifically by the carbohydrates and proteins in this context. It has been reported that, high-protein diet is associated with increased glomerular filtration rate (GFR), serum creatinine, urea, urinary calcium excretion, and serum concentrations of uric acid (Jolliffe *et al*. 1932; Yeap *et al*., 2012).

Conclusion

When Balb/c mice were treated with aqueous extract of fresh and processed shoots of *Dendrocalamus hamiltonii*, less fall in fasting glucose level was recorded. Similarly, serum creatinine level was also increased in all the groups when compared to the control group. This might be due to the high dietary fiber and high protein content present in bamboo shoots. Dietary fiber content increased to a significant level after fermentation which might be responsible for well maintained fasting serum glucose level in the animals administered with fermented bamboo shoot extract as compared to the other processed bamboo shoots. The normal histoarchitecture of kidney confirmed the safety of fresh as well as processed bamboo shoot extract. Thus, it can be stated that, bamboo shoots could be a safe and effective source of nutrients,

bioactive compounds and antioxidants for maintaining the energy level of the body, prevention of heart diseases and to restore health.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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