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Phytomodulatory effects of *Dendrocalamus hamiltonii* (Nees & Arn. Ex Munro) shoots on the hepatic status of healthy male Balb/c mice

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Abstract
Objectives: Bamboo shoot being rich in nutrients and bioactive compounds is emerging as a potential ingredient for modern functional foods and nutraceuticals. The young shoots however need to be processed for safe human consumption due to the presence of antinutrients. Processing can alter the value of nutrients and bioactive components with either desirable or adverse effects on the therapeutic quality of shoots. The present study was designed to evaluate the biochemical and histological alterations occurring in the liver of Balb/c mice by fermented, brine preserved and boiled shoots of *Dendrocalamus hamiltonii*.
Methods: The levels of serum bilirubin, albumin, globulin, alkaline phosphatase (ALP), serum glutamate oxaloacetate transaminases (SGOT), serum glutamic pyruvic transaminase (SGPT) and lactate dehydrogenase (LDH) were analyzed by using commercially available standard kits.
Results: Results revealed significant structural alterations in the liver on administration of aqueous extract of 5% brine preserved shoots which was also associated with enhanced activity of SGOT and SGPT. Fermented shoots decreased the level of SGOT, SGPT and LDH significantly but boiled shoots did not cause any major change in the liver function parameters studied.
Conclusion: Fermentation is not only beneficial for extending shelf-life, but can also enhance therapeutic properties of bamboo shoots in safe and effective manner.

Keywords: Bamboo shoot, fermentation, hepatic tissue, serum glutamic pyruvic transaminase, alkaline phosphatase

1. Introduction
Bamboo shoot is a fascinating food utilized in many Asian countries and also gaining demand in the other countries as well [1]. It is known for its several health benefits, including anti-inflammatory, anti-oxidants, anti-hyperlipidemic, anti-ulcer, and anti-cancer [2-5]. In recent times, it is emerging as a potential ingredient for modern functional foods and nutraceuticals. Shoots are commonly consumed as pickles, vegetables, salads, and in various other forms in different countries [6, 7] but in market they are also available as fresh, fermented and canned version [8-10]. India is one of the leading countries of the world, covering an area of bamboo forest around 10.03 m ha, which accounts 12.8% of the total forest cover in the country, but shoots are consumed only in North Eastern parts and the Western Ghats of Southern India [11]. It is estimated by the Planning Commission that the Indian bamboo shoot industry has the potential to grow at the rate of 255 per annum and capture a market worth rupees 300 crores. Bamboo shoot moreover has the potential to solve world's biggest problems such as food insecurity, hunger and malnutrition [12]. The current research on bamboo is leading to emergence of new avenues to utilize different parts of bamboo especially bamboo shoots for the production of novel food products and nutraceuticals [13-16].

Although, shoots are rich in nutrients and bioactive compounds but freshly harvested shoots need to be processed prior to exploiting them as a food additive and for safe human consumption [17] due to the presence of antinutrients (cyanogenic glycosides, thiocyanate, glucosinolate) [18], and perishable nature of fresh bamboo shoots. However, consumption of improperly prepared or processed bamboo shoots is associated with food poisoning and may produce symptoms like rapid respiration, drop in blood pressure, dizziness, stomach pains, headache, vomiting convulsion, and coma [19]. The major processing techniques which are commonly used for enhancing shelf life of shoots and removal of anti-nutrients are soaking, boiling, salting and fermentation. Processing improves palatability, increases shelf-life and, detoxifies shoots by removing anti-nutrients [20]. But along with enhancement of shelf life and palatability, processing often brings about changes in many attributes of shoots including its nutritional and therapeutic value [21-22]. Hence, appropriate processing methods need to be developed to ensure that the shoots are free from toxicity and at the same time causes minimum loss of nutrients and health enhancing bioactive compounds.

The liver is one of the vital organs of the body, responsible for hundreds of chemical actions that the body needs to survive, and also a prime target for damage because it processes everything that enters the mouth and is swallowed. Any change in food during processing can affect liver's ability to function, and over time, can lead to permanent damage or disease. Keeping this into consideration, a study has been carried out to find the impact of boiling, brine preservation and fermentation on hepatic status in Balb/c mice with a view to providing preliminary information toward effective utilization of processed shoots in developing safe functional foods and neutraceuticals.

2. Material and Methods

2.1 Collection of plant material

The juvenile shoots of *Dendrocalamus hamiltonii* were collected from Shillong, Meghalaya. The shoots were harvested two weeks after emergence above the ground and packed properly to ensure safe transportation. Thereafter, the shoots were transported from Shillong to Botany Department, Panjab University, Chandigarh, India by air.

2.2 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 was also noted. The shoots were then cut into thin slices, divided into four equal portions and subjected to processing (boiling (15 min), brine treatment (5%), fermentation) and dried at 40ºC. Thereafter, the shoots were pulverized to fine powder using mortar and pestle.

2.3 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 hr. Extract was then filtered and dried using a hot air oven at low temperature. The dried crude extract was weighed and stored in a refrigerator at 4ºC in air tight bottles and used for further experiment.

2.4 Experimental design

The male Balb/c mice weighing 25-30 g each, procured from Central Animal House, Panjab University, Chandigarh, 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 hr light/dark cycle at 25 ± 2ºC. 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. Mice were randomly assigned into five groups (N=6). Group-1 served as control group; 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 preserved 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 weight of all the animals was 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 and then mice were sacrificed under mild ether anesthesia for histopathological investigation. Blood (500 µl) was withdrawn in micro centrifuge tubes and incubated in an upright position at 37ºC for 3 hr to allow clotting. After incubation, the samples were centrifuged at 3000 rpm for 10 min and the serum was carefully aspirated for further study.

2.5 Assessment of liver function

Liver function in all the experimental groups were monitored by analyzing the levels of serum bilirubin, total proteins, albumin, globulin, alkaline phosphatase (ALP), serum glutamate oxaloacetate transaminases (SGOT), serum glutamic pyruvic transaminase (SGPT) and lactate dehydrogenase (LDH) by using commercially available standard kits (ENZOPAK) obtained from Reckon Diagnostics P. Ltd. and total proteins content was determined by the method of Lowry *et al*. [23]

2.6 Histopathological studies

For histopathological studies, formalin fixed hepatic 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 haematoxylin and eosin to investigate histopathological alterations.

2.7 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 $F < 0.05$.

3. Results and Discussion

Bamboo is a multipurpose plant and due to its unique biological characteristic, it has attracted worldwide prominence in recent years. A large number of studies have been conducted to examine the use of different parts of bamboo in terms the biological activity [24, 25]. Many nutritious and active components such as proteins, amino acids, vitamins, phenols, polysaccharides, minerals, dietary fiber and phytosterols can be extracted from bamboo shoots, and all these have anti-cancer, anti-oxidant, anti-hyperlipidemic, anti-aging, anti-viral and anti-bacterial functions [4, 24]. But the anti-nutritional factor and toxic influence of unprocessed shoots used as human food certainly calls for concern. Therefore, efficient and reliable ways and means of eliminating or reducing their levels to the barest minimum should be used because antinutrients reduce nutrients utilization, thereby contributing to impaired gastrointestinal and metabolic performance. Soaking, boiling, salting and fermentation are the major processing techniques which are commonly used for enhancing shelf life of bamboo shoots and removal of anti-nutrients [26]. But along with enhancement of shelf life and palatability, processing often brings about changes in many attributes of shoots including loss of vitamins, reduced amount of fiber and other bioactive compounds which can be disadvantageous for the consumers. A poor diet will impact overall health of a person and contribute to dysfunction of vital organs of the body including liver. The liver is one of the most important organs of our body relies on to the balance of biochemical and enzymatic reactions [27]. A balanced nutrition can help to support the liver to function efficiently and plays a crucial role in health [28]. Commonly used tests to check liver function are the serum bilirubin, total proteins, albumin, globulin, ALP, SGOT, SGPT and LDH. The results of the present study showed increase in the bilirubin content in mice treated with bamboo shoots when compared with the control group (0.273 mg/dl). Increase was significant (*P*< 0.05) in the mice administered with fermented and boiled shoots while, non-significant in the case of fresh and brine preserved shoots treated mice (Fig. 1). This might be due to the caloric deprivation because bamboo shoots are low in fat and calories. But the fundamental mechanism responsible for the increased total bilirubin which occurs during caloric deprivation is not yet clear [29].

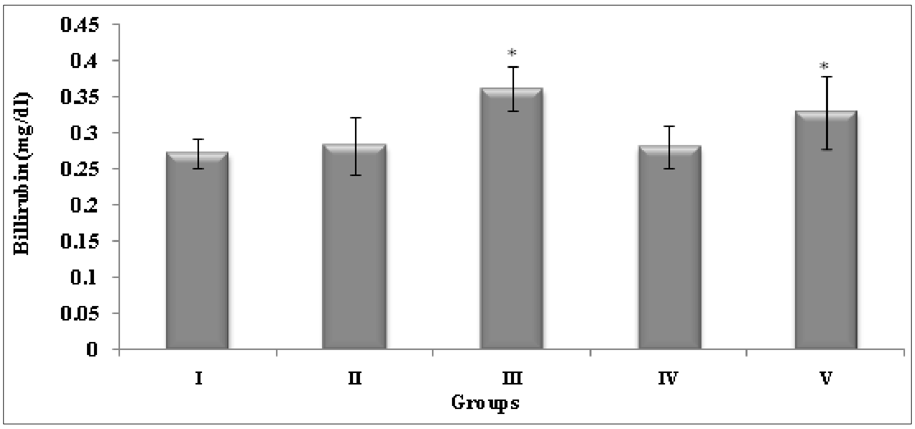


Fig 1: Modulatory effects of fresh and processed shoots on the level of billirubin, Group I: Control, Group II: Fresh shoots; Group III: Fermented shoots; Group IV: Brine preserved shoots and Group V: Boiled shoots treated mice; (*P < 0.05) significant as compared to control group

Serum total protein and globulin level decreased (*P*< 0.05) in all the groups but decrease was non-significant in the case of boiled shoots treated mice. However, albumin level remained unchanged in all the groups (Fig. 2). The change in the level of serum total protein and globulin is also probably related to the diet. Serum albumin is a major component of serum proteins, which sustains osmotic pressure or transports many kinds of substance or hormones to organs. Its level can be changed by decrease in synthesis, leakage outside the body or malnutrition. Fermented shoots did not cause any significant change in the level of serum albumin and alkaline phosphatase. However, ALP (U/L) level increased significantly (*P*< 0.05) in mice treated with boiled and brine preserved shoots (Fig. 3).

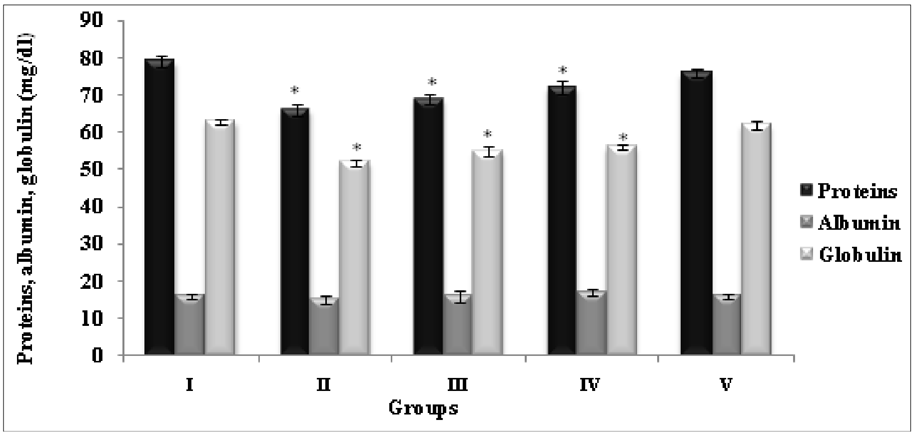


Fig 2: Modulatory effects of fresh and processed shoots on the level of proteins, albumin and globulin, Group I: Control, Group II: Fresh shoots; Group III: Fermented shoots; Group IV: Brine preserved shoots and Group V: Boiled shoots treated mice; (*P < 0.05) significant as compared to control group

Similarly, SGOT and SGPT level got reduced (*P*< 0.05) in mice administered with fresh, fermented and boiled shoots but increased significantly (*P*< 0.05) in the animals that received brine preserved shoots when compared with the control counterparts. When compared among the groups, the group received fermented shoots had the lowest level of SGOT and SGPT while, brine preserved shoots treated group had the highest level (Fig. 4). As can be seen from Fig. 3, LDH level decreased (*P*< 0.05) in all the groups, with highest decrease (35%) in the group treated with fermented shoots indicated that, incorporating fermented shoots into diet is a natural and healthy way to keep the liver functioning at its best. On the other hand, high salt (5%) intake may lead to high risk of liver damage.

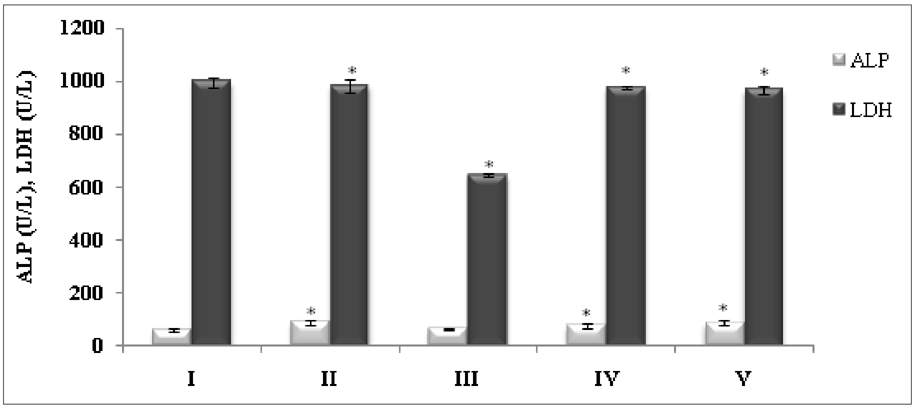


Fig 3: Modulatory effects of fresh and processed shoots extract on the level of ALP and LDH, Group I: Control, Group II: Fresh shoots; Group III: Fermented shoots; Group IV: Brine preserved shoots and Group V: Boiled shoots treated mice; (**P* < 0.05) significant as compared to control group

Fig. 5A to 5E shows the histopathological structure of the liver. Here, there were no significant changes observed between control group (Fig. 5E) and groups administered with fresh (Fig. 5A), fermented (Fig. 5B) and boiled shoots extract (Fig. 5D). The lobular structure of the liver was preserved. Portal tracts were of normal size, without increase of collagen fibers and without inflammatory infiltrates. Proper bile ducts were present in all portal tracts. The structure of the cytoplasm was also preserved in all hepatocytes. But, the mice administered with brine preserved shoots extract (Fig. 5C) depicted inflammation in the liver both around the portal tract and scattered in the lobule.

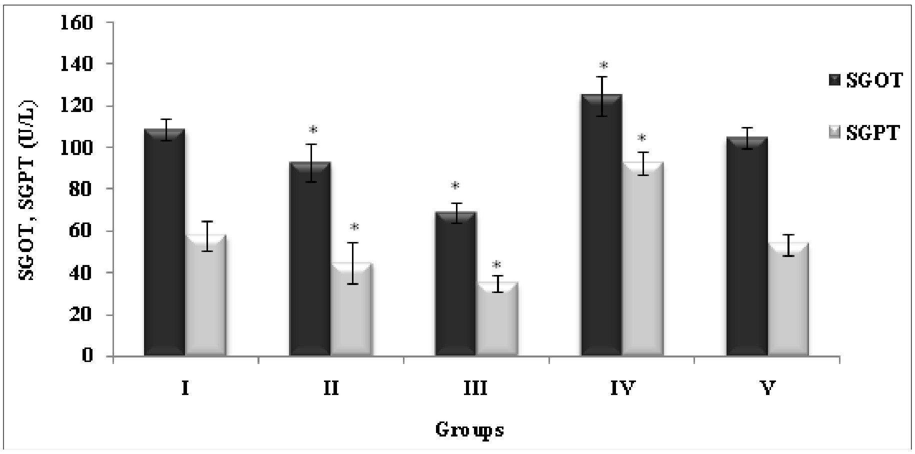
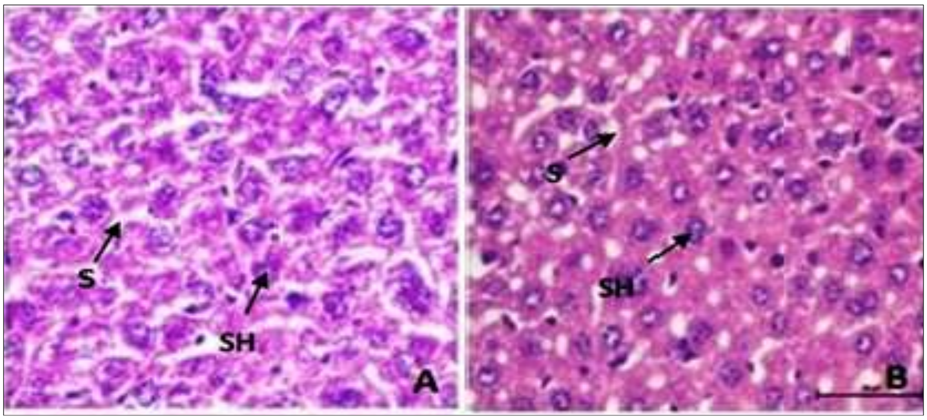


Fig 4: Modulatory effects of fresh and processed shoots extract on the level of SGOT and SGPT, Group I: Control, Group II: Fresh shoots; Group III: Fermented shoots; Group IV: Brine preserved shoots and Group V: Boiled shoots treated mice; (*P < 0.05) significant as compared to control group

High salt has also been known to cause hypertension and other side effects such as derangement of the hepatic cords and liver fibrosis [30, 31]. In addition, it cause swelling and fluid retention in the liver that makes it harder for the liver to filter waste and cause liver injury which can be detected from the raised level of hepatic enzymes in the bloodstream [32]. In contrast, fermented shoots which are rich in probiotic bacteria add beneficial bacteria and enzymes to overall intestinal flora, increasing the health of gut micro biome and digestive system and also enhancing the immune system [24]. Hence it is concluded that, fermented shoots being a rich source of dietary fibre, phenols and phytosterols can play an important role in liver health.



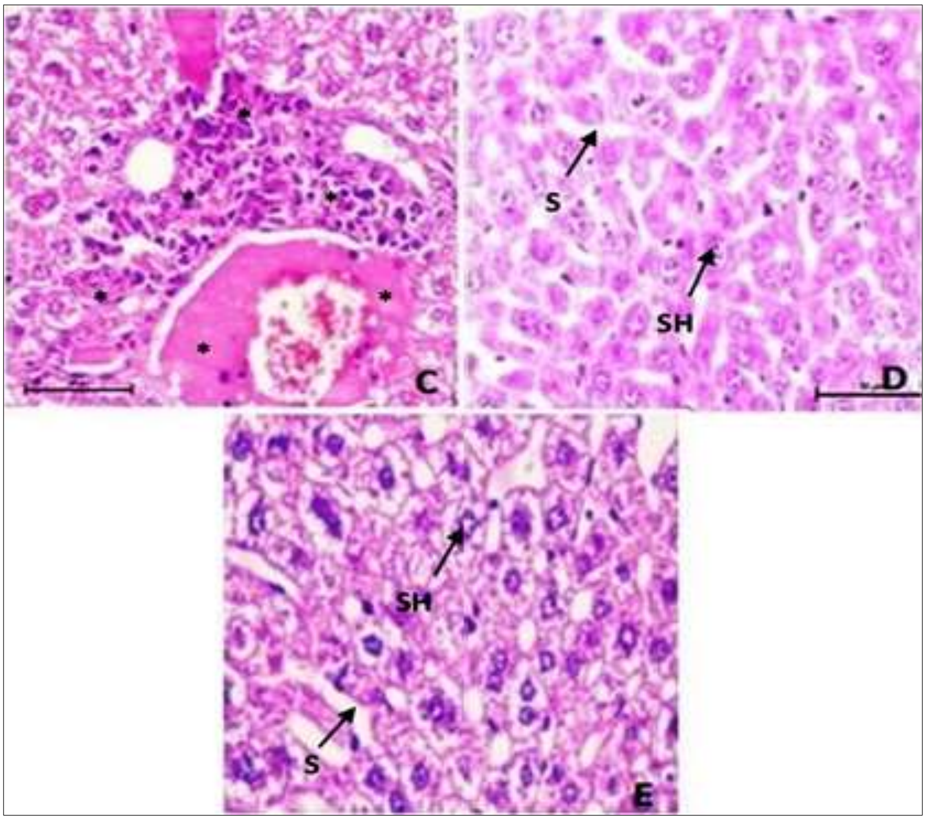


Fig 5: Histomicrographs of liver tissue from Balb/c mice administered with fresh shoots extract (A), fermented shoots extract (B), brine preserved shoots extract (C), boiled shoots extract (D) and control group (E) (400×); (*=inflammatory cells, S=sinusoids, SH=sheets of hepatocytes)

4. Conclusion

Bamboo shoots are gaining popularity among the people all over the world. But fresh juvenile shoots need to be processed before consumption to remove acridity. Various processing techniques (salting, boiling, fermentation) is expected to affect content, activity and bioavailability of nutrients and bioactive compounds. However, the health-promoting capacity of processed shoots strictly depends on their processing history. It is evident that administration of aqueous extract of 5% brine preserved shoots to mice caused histoarchitectural damage in liver which was associated with enhanced SGOT and SGPT activities. Fermented shoots caused significant reduction in the level of hepatic enzymes while, boiled shoots did not exhibit any drastic change when compared to the control counterparts. It may be inferred from the present study that, fermentation is the best processing technique to enhance the therapeutic qualities of bamboo shoots.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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