

Original Research Article

Effects of a Medicinal Alkaline Food Additive of Ethnic Community of North East India on Microscopic Structural Organization of Small Intestine and Hepatic Tissue of Albino Mice

Dhan Hang Limbu and Moushumi Das*

Department of Zoology, University of Science and Technology, Meghalaya, India

*Corresponding Author: dasmoushumi092000@yahoo.co.in

Received: September 26, 2018; revised: November 20, 2018; accepted: November 25, 2018

Abstract: The ethnic communities of north east India are rich with their traditional delicacies. They make an alkaline preparation from parts of banana plants especially *Musa bulbisiana* species. Aquous extract of either dry peels of ripen banana or stem of the plant is used as common alkaline food additive among many of the ethnic communities. The preparation is commonly known as “*Kalakhar*” among the Assamese people. In the present study, it has been hypothesized that excess intake of this food additive may cause tissue injury. Thus, in vivo administration of this alkaline preparation was carried out in albino mice at a dose of 15ml/kg body weight per day. Administration through oral route was done for short duration (20 days) and long duration (40 days) in two separate groups of animals. The experimental mice were sacrificed at the end of the period and collected liver and intestine for histological studies. The results showed cellular degeneration in different rate in liver tissue. Infiltration of neutrophils in hepatic tissue indicates cellular necrosis. Effects on the intestinal epithelial cells are recorded following long duration (40 days) treatment. It causes collapse of surface cell layer at certain areas of intestine. It is to be noted that the effects of the extract could be dose and duration dependent. A health friendly effects of this food additive prevailed in the society for centuries can not be ignored. The extract intake in specific dose could be helpful in correction of eating abnormaliries and thus, protect the system against metabolic disorder.

Key words: *Ethnic foods, kalakhar, histoarchitecture, toxicity*

Introduction

Assam as well as the whole north eastern part of India is famous for its ethnic foods and unique food habits. Assam being the hub of north east India carries an unmatched blend of demographic pattern and so it is reflected in its food and food habit. One can taste the flavor of mongoloid recipe and at the same time the aroma of East Bengal and other North Indian cuisine are equally confluent in contemporary Assamese food habits. Assam is also famous for its various indigenous food additive with enormous traditional medicinal uses. Tradition prevails among many of the ethnic communities of states of north east India of using alkaline preparation of banana plants as a food additive, especially for medicinal

purpose. The alkaline preparation commonly known as “*Kalakhar*” throughout Assam is a popular traditional food additive in traditional Assamese delicacy. The alkaline preparation is aquous extract of parts of Banana plant, especially the *Musa bulbisiana* species (Fig. 1. A & B). The practice of use of this alkaline preparation is centuries old; however, a detailed scientific investigation on its effects on biological system is yet to be carried out. The alkaline preparation is added to various vegetarian and non vegetarian dishes among various communities (Rabha *et al.*, 2014). In many parts of Assam *kalakhar* is also use as a salt substitute (Deka *et al.*, 2007). Use of salt substitute is an alternative way

to taste and take food without using common table salt. Such substitute of common salt limits sodium intake that help in control of physiological anomalies associated with blood pressure (Neog and Deka, 2013). The pH level of this alkaline preparation ranges from 11-13.

Kalakhar contains potassium, sodium, carbonate and chloride as the major constituent, along with non alkali metals of insignificant quantity (Deka and Talukdar, 2006). In addition of its use as food additive, “*Kalakhar*” is also used antimicrobial, cleansing agent, to prevent bacterial attack on cut injuries, to cure worm infection, to kill leeches and prevent their attack in agricultural fields and for cure and prevention of certain cattle diseases (Deka and Talukdar, 2006; Kalita and Deb, 2004). Bacterial Pathogens like *Pseudomonas aeruginosa* are reported to be more sensitive towards *kalakhar*. Such bioactivity of *kalakhar* certainly attributes to the high alkaline nature of the preparation (Mudiar *et al.*, 2014). Among many ethnic groups *kalakhar* is a common additive of their daily meal. In such situation, there is an apprehension of its toxic effects on human health. It has been reported that frequent use of *kalakhar* as dietary constituent may cause oesophageal cancer (Phukan *et al.*, 2001). In the present research it has been hypothesized that the frequent and excess dietary uptake of *kalakhar* as dietary additive may link to the certain level of tissue damage and which may even subsequently lead to cancer. Adverse cellular effects may be reflected on the microscopic structure of small intestine and hepatic tissue.

Materials and methods

Preparation of the extract

In the present investigation the alkaline preparation “*Kalakhar*” was made from banana peels of species *Musa balbisiana* belonging to the family Musaceae. The Banana peels of ripen fruit were collected and dried under sun light for several weeks. The dry material was burnt into ashes and subjected to extraction with water. Weighed quantity of ash was suspended in distilled water in a ratio of 1:10 (W/V) in a glass beaker and stirred. Ashes are allowed to soak in water for 4 to 5

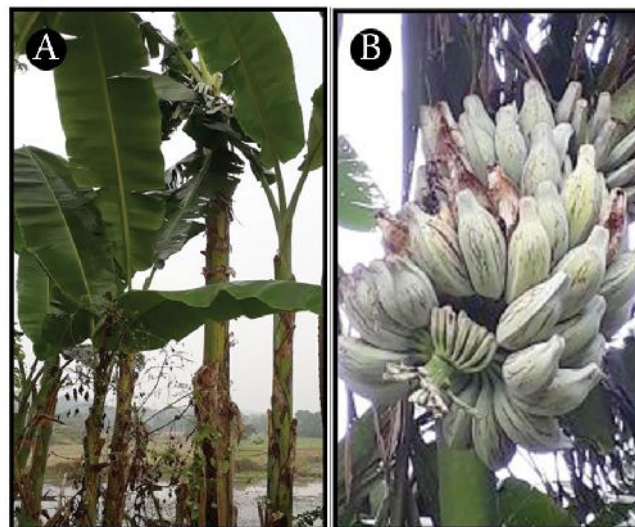


Fig. 1. (A&B). The plant *Musa balbisiana* with banana fruit.

hours followed by its filtration using Whatman filter paper (Grade 4). The filtrate extracts is known as ‘*Kalakhar*’ which has been used in the present in vivo study.

Experimental design and sample collection

Adult albino mice of both gender weighing 35 ± 5 g (60-90 days old) were used for the present in vivo studies. All animals were kept in the institute’s animal maintenance facility for research. Animals were maintained under natural light and temperature, feed with routine diet like Bengal gram; corn and water *add libitum*.

The animals were divided into three groups (n=4). Group I served as a control, treated with vehicle (distilled water). Group II and III were administered with the alkaline extract (*kalakhar*). The alkaline extract (pH-12) was administered to the animals at a dose of 15ml/kg body weight per day in two separate groups for two different durations (group II for 20 days and group III for 40 days respectively). Duration of the treatment was considered arbitrarily keeping in mind the daily use of the additive to routine meal among the people of the community as well as the preliminary study on the food additive. Administration was done through oral route in between 8.00-9.00 hours every day after morning food is added to the cage. Treated animals were sacrificed on 21st day (group-II) and 41st day (group-III) by cervical dislocation for sample collection. Along with the treated

animals, control mice were sacrificed in similar manner for collection of liver and parts of small intestine for further histological studies.

Histological study

The hepatic lobes and intestine were collected, washed in buffered saline and fixed in Bouins fixative for 48-72 hours. The intestinal part prior to place in fixative was washed thoroughly, clean the lumen passing buffered saline throughout for removal of food materials inside. Fixed tissues were processed for histological study using routine eosin-hematoxyline (EH) stain following the method of Culling (1974). Tissues were dehydrated in graded alcohol and subsequently embedded in paraffin wax. Serial sections (6mm) of paraffin embedded tissues were cut in a rotary microtome and stretched on Poly-L lysine coated glass slide for further staining. Sections were EH stained and observed in bright field microscope (Mugnus, Olympus). Appropriate areas were photomicrographed to collate the most significant results in the histoarchitecture of the intestine and liver.

Results

The histological study was carried out to study the effects of the traditional alkaline preparation “*kalakhar*” on the selected tissues of digestive system, i.e the liver and small intestine. Objective of the study was to determine the toxicity (if any) of the traditional food additive “*kalakhar*” on hepatocytes and intestinal cells in its two different duration of treatment.

Histological study of liver

The histological structures of hepatic tissue of both control and treated mice is presented in Fig. 2. The liver of the control mice showed distinct central vein and the radially arranged hepatocytes (Fig. 2. A & B). The hepatocytes of the control mouse liver tissues appeared with distinct nucleus with clear cytoplasm indicating functional liver tissues. Administration of alkaline extract induces changes in hepatocytes' appearance with presence of irregular cell structure and morphology. The animal group having 20 days alkaline extract exposure showed the tendency of cellular death with evidence of vacuole formation in the cytoplasm (Fig. 2. C & D). Infiltration of

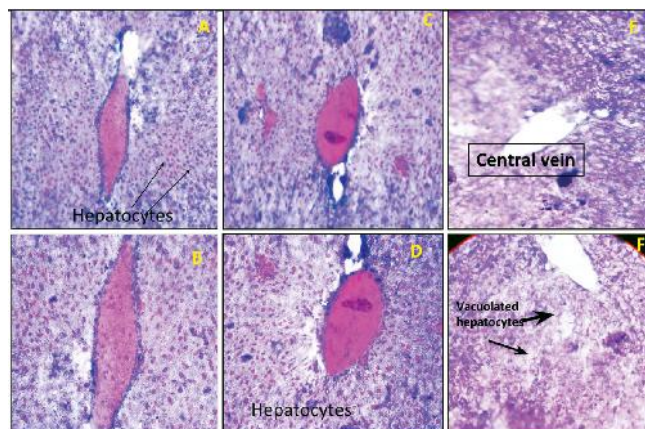


Fig. 2. (A-B) photomicrograph of control mice liver. (C-F) Photomicrograph of kalakhar treated mice liver for 20 and 40 days (E & F) respectively. Original magnification: A & C10X & B,D,E,F40X)

leucocyte has been observed in the hepatic tissue in the treated mice liver. Administration of the alkaline extract for consecutive 40 days showed enhanced cellular effects with increased vacuoles formation around the nucleus. With the vacuolated hepatocytes the disintegration of normal cellular arrangements has also been observed. Infiltration of neutrophils also observed in the liver tissues (Fig. 2. E & F).

Histological study of intestine

The intestinal villi of control showed normal pattern of cellular structure with distinct nucleus (Fig. 3. A & B). The histological structure of intestine treated for 20 days in did not show any toxic effects on intestinal tissues.

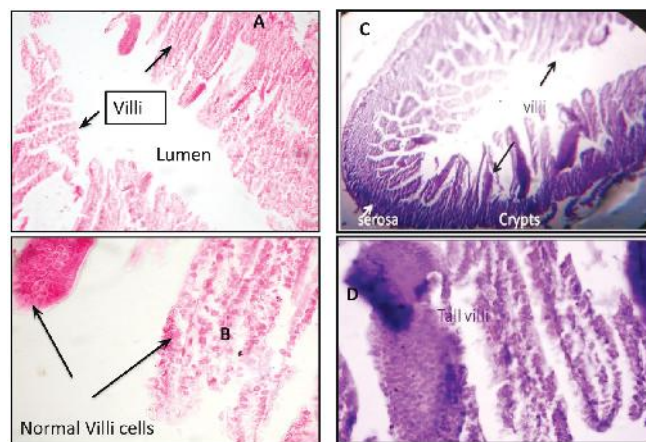


Fig. 3. (A-B) Photomicrograph of control mice small intestine shows intestinal villi and mucosa region. (C-D) Photomicrograph of 40 days *kalakhar* treated small intestine showing the villi of intestine. Original magnification (A & C10X, B & D 40X)

The oral dose of *kalakhar* at a dose of 15ml/kg/day for consecutive 40 days showed little changes than the normal histoarchitecture of small intestine. The treatment of *kalakhar* showed normal villous collapse and sloughing of epithelium into lumen. Slight necrosis of villi tissues also observed (Fig. 3. C & D).

Discussion

The traditional use of *kalakhar* as a food additive is as old as the Assamese culture. Therefore, it is generally not at all considered as a risk factor of health. However, some firsthand information reveals that *kalakhar* added food is not given to young children. The research carried out by the Indian council of medical research center Dibrugarh has drawn a link between the risk of esophageal cancer and *kalakhar* (Phukan *et al.*, 2001). *Kalakhar* has identified as a significant risk factor in a hospital-based case-control study which has been conducted with 502 cases and 1,004 controls. Other researchers showed that the *kalakhar* contain high amount of potassium carbonate which is highly alkaline. Generally potassium carbonate does not have serious health hazardous effects when its concentration is low. However, being a highly alkaline food it may have some deleterious effects on health. Still there are no known significant toxic properties of the banana. Bananas contain high levels of biogenic amines such as dopamine and serotonin. High level intake of banana has previously been implicated in the occurrence of endomyocardial fibrosis (EMF) (Foy & Parratt, 1960). Another study determined that serotonin is rapidly removed from circulating plasma and does not contribute to elevated levels of biogenic amines in healthy individuals (Ojo, 1969). Subsequent studies by Shaper (1967) also determined that there is no evidence for implicating the banana/plantain as several lectins have been isolated from banana fruit, including BanLec, which belongs to the mannose-specific jacalin-related lectins (Peumans *et al.*, 2000). This lectin is an important murine T-cell mitogen and can induce human T-cell proliferation (Koshte *et al.*, 1990).

The oral administration of *kalakhar* for 20 consecutive days did not show any noticeable effects on the targeted

organs. This result indicates that *kalakhar* can be use as a food additive without any health risk when used at less frequently. However, the treatment with *kalakhar* for consecutive 40 days showed some effects on liver with vacuole formation and villous collapse. Being the major metabolizing organ liver is also responsible for metabolism of pharmaceutical drugs and other compounds and at the same time more susceptible to injuries from such compounds or drugs (Bale *et al.*, 2014). This may be one of the reasons that *kalakhar* induced noticeable changes in liver tissues as compared to intestinal tissues. Such results suggest that frequent and excess intake of such alkaline food may induce cellular damage to certain extent.

The possible outcome of the research investigation may throw a new insight in risk or beneficial factor associated with of traditional Assamese food habit. Dietary compounds with health risk factors shall be identified and other prevailing health beneficial effects of such compounds can be further processed for pharmaceutical formulations.

Acknowledgements

The authors are grateful to University of Science and Technology, Meghalaya and Veterinary college, Khanapara, Assam for providing laboratory facility and other technical supports. We are also thankful to M.V. Devi, S.R.K Ahmed and other members and students of Department of Zoology, USTM for their help and Support.

References

- Bale Shyam Sundar, Verneti Lawrence, Senutovitch Nina, et al. 2014.** In vitro plantforms for Evaluating Liver Toxicity. Exp. Biol. Med. 239(9): 1180-1191.
- Deka C Dibakar and Talukdar N Nripendra. 2007.** Chemical and spectroscopic investigation of *Kolakhar* and its commercial importance. Indian J. Tradit. Knowl. 6(1): 72-78
- Kalita Dilip and Deb Bikash. 2004.** Some folk medicines used by the Sonowal Kacharis tribe of the Bhrahmaputra valley. Nat. Prod. Radiance. 3:240-246

- Foy JM and Parratt JR. 1960. A note on the presence of noradrenaline and 5-hydroxytryptamine in plantain. J. Pharm. Pharmacol. 12: 360-364.
- Koshte VL, Van Dijk W, Van der Stelt ME and Aalberse RC. 1990. Isolation and characterization of BanLec-1, a mannoside-binding lectin from *Musa paradisiacal* (banana). Biochem. J. 272: 721-726.
- Mudiar Rinky Hemanta, Varsha Kelkar Mane and Bhagwat Ashok. 2014. Analysis of Traditional food additive Kalakhar for its Physico-chemical parameters and Antimicrobial Activity. Europ. Acad. Res. 2(8): 10531-10536.
- Neog Satya R and Deka Dibakar C. 2013. Salt substitute from banana plant (*Musa- balbisiana* Colla). J. Chem. Pharmaceut. Res. 5(6): 155-159.
- Ojo GO. 1969. Plantain meals and serum 5-hydroxytryptamine in healthy Nigerians. West African Med. J. 18(5): 174-5.
- Pathak Swarnali and Deka Dibakar Chandra. 2014. Comparison between the Catalytic activity of water soluble and water insoluble parts of the Catalyst derived from trunk of *Musa balbisianacolla* for Transesterification. Int. J. Pharm. & Life Sci. 5(1): 3217-3220.
- Pathak Swarnali and Deka Dibakar Chandra. 2014. Transesterification of dimethyl malonate with a *novel catalyst* derived from *Musa balbisianacolla*. Int. J. Sci. & Eng. Res. 5(1): 70-72.
- Peumans WJ, Zhang W, Barre A et al. 2000. Fruit-specific lectins from banana and plantain. Planta. 211: 546-554.
- Phukan RK, Chetia CK, Ali MS and Mahanta J. 2001. Role of dietary habits in the development of esophageal cancer in Assam, the north-eastern region of India. Nutrition and cancer. 39(2): 204-209.
- Rabha Hira Prabha, Mazumdar Minakshi and Baruah Uttam Kumar. 2014. Indigenous technical knowledge on ethnic dishes of snail in Goalpara district of India. Scholars Academic J. Biosci. 2(5): 307-317.
- Sharma D Jagannath, Kataki Amal C and Vijay CR. 2013. Population-based incidence and patterns of cancer in Kamrup Urban Cancer Registry, India. The Nat. Med. J. India. 26(3): 133-41.
- Shaper AG. 1967. Plantain diets serotonin and endomyocardial fibrosis. Am. Heart J. 73(3): 432-434.