Hepatoprotective Activity of Methanolic Extract of Young Bamboo Shoot *Melocanna Baccifera* and *Chakhwi* – Two Traditional Tribal Food Ingredients of Tripura

[1]**Namasudra, Uma , [2]Bhaumik Debabrata, [3]De, Biplab and [4]Ghosh, Ranjib**
[1]Post Graduate Teacher, [2]Rtd. Reader, [3]Associate Professor, [4]Associate Professor
[1]Department of Human Physiology.
[2]Department of Human Physiology, MBB College, Agartala, Tripura.
[3]Regional Institute of Pharmaceutical Science And Technology (RIPSAT), Abhoynagar, Agartala, Tripura.
[4]Department of Pharmacology, Tripura Medical College and Dr. B. R. A.T. Hospital, Hapania, Tripura.
[1]Ramkrishna Krishna Mahavidyalaya, Kailashahar, Unakoti Tripura

ABSTRACT

The present study aimed to determine the hepatoprotective activity of a methanolic extract of bamboo shoot (*Muia*) and *chakhwi* - a prepared food ingredient.The hepatoprotective potential was evaluated in CCl_4-induced hepatotoxic albino swiss male mice. Animals were divided into five groups, while group I- normal control, group II- toxic control(CCl_4) and the remaining three groups (III, IV and V) were treated with Standard drug Liv. 52, bamboo shoot (*Muia*) and *chakhwi* - a prepared food ingredient respectively. Except normal group, animals of all other groups received CCl_4 (1 ml/kgbw) by subcutaneous injection for 7days alternately. Normal group received water. On eighth day, all the animals from all groups were sacrificed, blood was collected from each animal for serum analysis. SGPT, SGOT, Total Bilirubin and Direct Bilirubin were assayed.The present study showed that both the tribal food ingredients have significant hepatoprotective activity.

KEY WORDS: - *Muia*, *Chakhwi* , hepatoprotective activity, CCl_4 and *Melocanna baccifera.*

INTRODUCTION

The liver, one of the largest organs in the human body, serves a vital role in the metabolism of carbohydrates, lipids and proteins, the regulation of immune responses, and the clearance of toxins and pathogens. The liver is frequently exposed to various chemicals, which may cause cell swelling, degeneration, necrosis, apoptosis, hepatic fibrosis and dysfunction. As the fifth most common cause of mortality following heart disease, stroke, lung disease and cancer, the rates of liver disease, unlike other major causes of mortality, are increasing. However, the available synthetic drugs, including interferon and corticosteroids, are expensive and may present the risk of adverse effects(https://www.). The liver is necessary for survival, a human can only last up to 24 hours without liver function. Liver damage occurs by either due to direct damage to the liver cells or due to a secondary damage resulting from obstruction of the bile flow. Rarely, it can be due to obstruction to the blood flow either in the portal vein or in the hepatic vein(Hemavathy et al., 2016). Therefore, treating liver disease with alternative medicine seems attractive, as a number of medicinal plants, which have been traditionally used for centuries, are accessible and appear to exhibit decreased toxicity. Liver injury is a common pathological state in various types of liver disease; severe or persistent liver damage is the basis of hepatic failure(Hines et al., 2004). CCl_4 is a commonly-applied chemical substance which may induce acute and chronic liver injury in animals.

The present study aimed to investigate the effect of two tribal food ingredients *Muia* and *Chakhwi* on carbon tetrachloride (CCl_4)-induced acute liver injury in mice and to provide the theoretical foundation of the clinical application of in acute liver injury.

Nutrition has great deal for survival of society & for its people and exploration of the nutritional status is still unfolded in case of Tribal people of Tripura. That is why concentration is given on searching the hepatoprotective effect of *Muia* (young bamboo shoot) and *Chakhwi* –two food ingredients of Tribal people of Tripura.

METHODS & MATERIALS

The experiment was conducted in vivo in adult albino swiss male mice which was carried out in TMC (Tripura Medical College & Dr. B. R. Ambedkar Teaching Hospital), Hapania, Agartala, Tripura. The Ethical No. of TMC is 1006/GO/ac/06/CPCSEA. The experiment lasted for 8 days.

Methods of preparation of different tribal dishes along with food ingredients was come to know by conducting a survey, through an eventually prepared printed format, extensive interviews among the ten numbers aged personalities and housewives.

The food ingredient *Chakhwi* prepared as per standard procedure(Das, P.), as published by Tripura Tribal Cultural Research Institute & Museum (Govt. of Tripura) was subjected for Hepatoprotective activity. The raw ingredients i.e. tender shoot of bamboo (*Melocanna baccifera, family: Poaceae*) (known as *Muia* in *Kokborok*) was also subjected for methanolic extraction and then was studied for Hepatoprotective activity.

PREPARATION OF EXTRACT OF *MUIA*-SAMPLE-1

Very young stem of bamboo *(Melocanna baccifera)* i.e. *muia* was collected. Removing the outer shell and internodes, 2 kg of *muia* was pieced (1.5mm in length). Certain compounds may get denatured in sunlight, so it was dried under shade to avoid decomposition and followed by grinding into fine powder by electric grinder(BAJAJ, REX 500, India). After shed dry, it was 113.4 gm and soaked into 400 ml methanol for 7 days. The extract was filtered through cotton plug followed by vaccum suction. The filtrate was obtained. Further the filtrate was allowed to dry to get powder like substance.

Though *Muia* is taken by Tribal people either raw or cooked preferably, then also the methanolic extract preferred to have ingredients as extracts in raw form could not be preserved due to fungal/bacterial decomposition & cooked extract contains some other ingredients from other vegetables/spices also.

Chakhwi was prepared in present study from ash of bamboo only. Therefore the results will reflect the comparative assessment in two different form of bamboo and bamboo shoots.

PREPARATION OF *CHAKHWI*-SAMPLE -2

To prepare *chakhwi*, dry stem and shoots of bamboo (M. baccifera) was allowed to burn. The burnt ash was taken in a specially prepared basket called *cheyakhok* in *Kokborok* (The popular Tribal Language/dialect). The basket is hanged from a suitable support & a container is kept below it to collect the extract of ashes. The water is poured slowly on ash to bath the whole ash. This extract is collected in the container which is known as *chakhwi. Chakhwi* was also allowed to evaporate under very low flame. When the water portion was evaporated entirely, the whitish substance like powder was obtained from the bottom of the container and allowed to dry which is treated as sample 2.

Animal Experiment Protocol

A CCl4-induced acute liver injury model of mice was established as described by Li et al., 2015. Adult albino swiss male mice weighing between 18-30 gm was selected for the study(De et al., 2008). The animals were divided into five groups. In each group there were 5 animals. The animals were housed under standard environmental condition (25 ± 2^0 C) and relative humidity (50±5%) and fed with standard diet and water adlibitum. The animals were acclimatized to laboratory environment for a period of 14 days before performing the experiments.

Normal saline was used as vehicle.

Group – I has served as normal control which received only vehicle (water) 1 ml/kg bw(Aykae et al., 1985 and Manokaran et al., 2008).

Group - II has received only carbon tetrachloride(CCl_4) in 1 ml/kgbw on alternate days for one week with vehicle by subcutaneous injection(Ghosh M.N.).

Group - III received Liv. 52 (standard drug), dissolved in normal saline/water in 50 mg/kg bw, four times orally 30 min after the administration of vehicle along with the administration of CCl_4 on alternate days for one week.

Group –IV and V have received the test drugs (*muia* & *chakhwi*-150 mg/kgbw) 30 min after the administration of vehicle orally along with the administration of CCl_4 on alternate days for one week.

All the animals were subjected to anaesthesized on eighth day by mild ether. Blood was collected by direct cardiac puncture. The serum was separated by centrifugation which was done at 250 rpm for 10 mins at 37°C. SGOT, SGPT, Total Bilirubin and Direct Bilirubin were assayed by using commercial kits and AU480 Chemistry analyser (BECKMAN COULTER).

Biochemical parameters were assayed by using assay commercial kits (Erba Mannhein, India) and AU480 Chemistry analyzer(Beckman Coulter).

Biochemical Markers

SGPT & SGOT

Both the serum transaminase enzymes SGPT and SGOT were estimated by IFCC method(Bradely et al., 1972) using working reagent as per protocol supplied by manufacturer's Kits.

Total & Direct Bilirubin

Serum Bilirubin both Total bilirubin and Direct Bilirubin were assayed by Diazo method as per protocol supplied by manufacturer's Kits(Henry et al., 1974).

Statistical Analysis

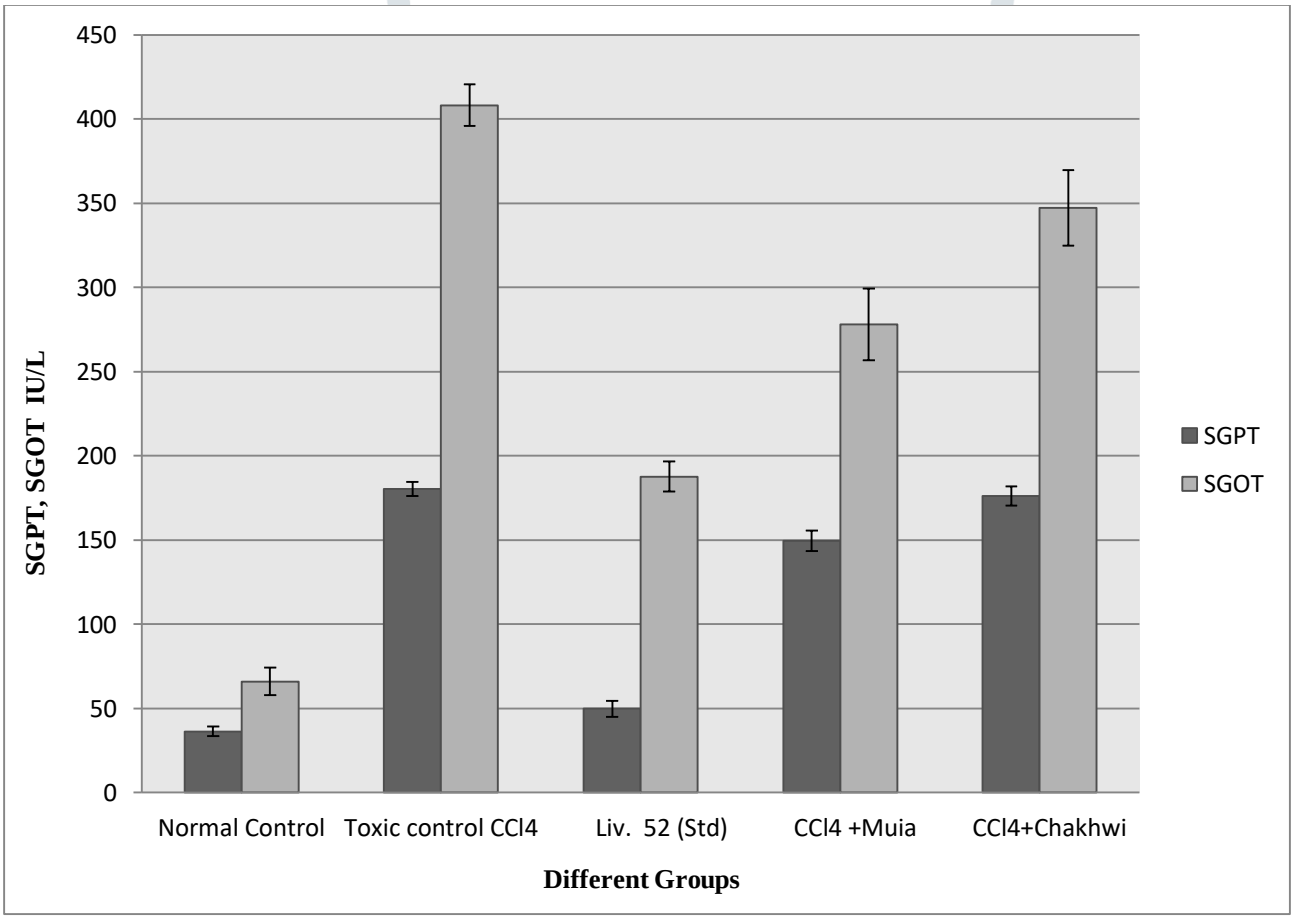
The Values were expressed as Mean±SEM where n=5. Copmarisons between different groups was carried out by unpaired Student's t test. Differences were considered statistically significant at ***$p<0.001$, or **$p<0.01$ or *$p<0.05$ when compared with normal control vs toxic control, normal control vs Test groups, normal control vs standard and standard vs Test groups, toxic control vs test groups, toxic control vs standard and test vs test groups.

Results

Significant increase in the level SGPT and SGOT were observed in the animals of CCl_4 induced toxic group(180.35 ± 4.136 and 408.134 ± 12.38 respectively). Treatment with Standard drug (Liv. 52), *Muia* and *Chakhwi* on CCl_4-induced hepatotoxic animals for 7 days on alternate day showed significant protective effect (Table-1) when compared to toxicant animals. In compare to *Chakhwi*, *Muia* is more strong in decreasing the level of SGPT, SGOT, Direct Bilirubin than *chakhwi* activity. Significance levels of Mean difference between Liv.52 & *Muia, and* Liv.52 & *Chakhwi* were not considered significant. It has been shown from the result that in decreasing the level of Total Bilirubin, *Chakhwi* is more potent than *Muia.*

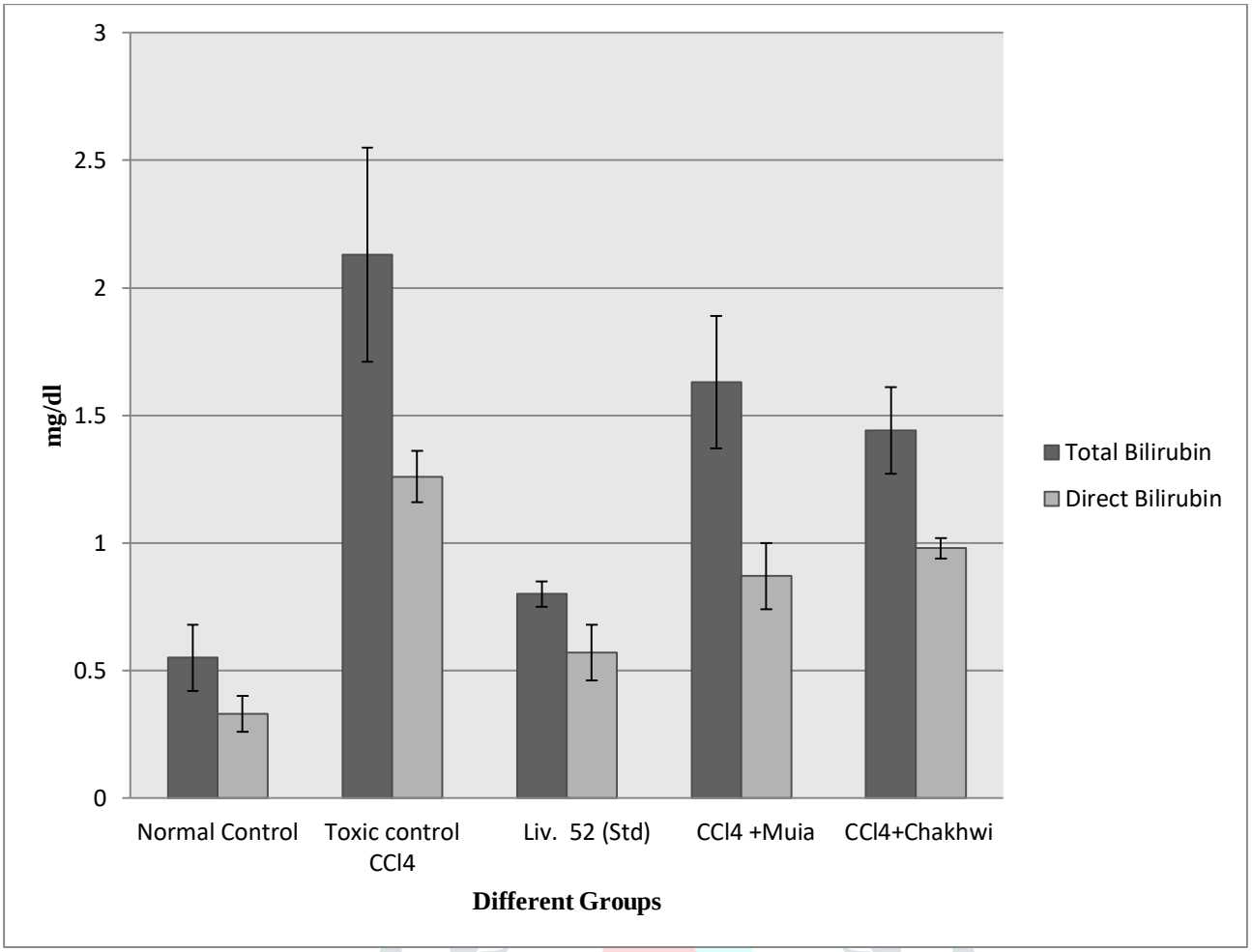
Table1: Level of SGPT, SGOT, Direct Bilirubin and Total Bilirubin

Sl No	Treatment	SGPT (IU/L)	SGOT (IU/L)	Total Bilirubin (mg/dl)	Direct Bilirubin (mg/dl)
1	Group- I-Normal control (Mean ± SEM)	36.398 ± 2.836	66.1 ± 8.060	0.548 ± 0.125	0.332 ± 0.067
2	Group II-CCl_4 (T. Control) (Mean ± SEM)	180.35 ± 4.136	408.134 ± 12.38	2.128 ± 0.416	1.256 ± 0.102
3	Group –III-Liv.52(Std.) (Mean ± SEM)	49.86 ± 4.68	187.65 ± 8.97	0.8 ± 0.05	0.57 ± 0.107
4	Group –IV – *Muia* (Mean ± SEM)	149.538 ± 5.919	278.014 ± 21.37	1.632 ± 0.264	0.874 ± 0.133
5	Group- V- *Chakhwi* (Mean ± SEM)	176.308 ± 5.692	347.22 ± 22.534	1.442 ± 0.165	0.978 ± 0.035



Error bars in the graph represent the mean ± standard error

Fig-1: Serum SGPT & SGOT in Animals of different groups



Error bars in the graph represent the mean ± standard error

Fig-2: Level of Total & Direct Bilirubin in Animals of different groups.

DISCUSSION

The liver can be injured by several chemicals and drugs(Leo et al., 1982). In the present research, carbon tetrachloride (CCl_4) was selected as chemical to induce hepatotoxicity in experimental animals. CCl_4 is effective hepatotoxin which is used in preclinical laboratory to induce liver damage in animals(Hemavathy et al., 2016). It is closely linked to its metabolic activation to short lived reactive intermediates (Conner et al., 1986). Cytochrome P_{450} is an enzyme that is the terminal oxidase of the hepatic mixed function oxidase system(Nogushi et al., 1982). This cytochrome P_{450} acts on CCl_4 and converts it into active trichloromethyl radical(CCl_3). The later further reacts with O_2 to generate more free radicals(Hemavathy et al., 2016). The existence of free radicals during metabolism has been proven by spin trapping experiments (Conner et al., 1986). In radical form, CCl_4 bind to both cellular lipids and proteins. Apparently this is an essential step in blocking of lipoprotein secretion(Dianzani et al., 1984). CCl_4-induced liver damage progresses through a series of steps that contribute to various extents to the ultimate damage: reductive dehalogenation, covalent binding of resulting radicals, inhibition of protein synthesis (in particular apolipoprotein synthesis), assembly, packing and release of VLDL, HDL, fat accumulation, formation of CCl3-OO* radicals, lipid peroxidation, membrane damage, loss of Ca^{2+} sequestration, apoptosis and fibrosis(Clawson, 1989, Boll et al., 2001a, b). Serious damage occurs only in the presence of an induced cytochrome P_{450} system, the stronger the induction, the more damage results(Boll et al., 2001). The serum transaminase level is most widely used as a measure of hepatic injury, due to its ease of measurement and high degree of sensitivity. It is useful for the detection of early damage of hepatic tissue and requires less effort than that required for a histologic analysis(Ray et al., 2006). Biotransformation of CCl_4 into active trichloromethyl radical(CCl_3) elicits lipid peroxidation of membrane lipids in the presence of oxygen generated by metabolic leakage from mitochondria. All these events culminate in loss of integrity of cell membranes and damage of hepatic tissues(Mangathayaru et al., 2005). Damage to membrane integrity affects calcium homeostasis thought to be the cause of CCl_4- induced liver necrosis. Ca^{2+} was proposed as a toxic messenger(Nicotera et al., 1992).

Irreversible membrane damage , loss of Ca^{2+} sequestratation in hepatocytes, leakage of K+ and entry of Na+ may be the critical events in the initiation of cell death(Ozaki et al., 1993).

In the present research, administration of CCl_4 significantly altered the serum marker enzymes of liver. During liver damage serum marker transaminase like SGPT and SGOT were released into blood stream from hepatocytes. The elevated level of these enzymes along with Total Bilirubin and Direct Bilirubin level also increases in liver toxicity. These elevated levels are indicative of cellular leakage and loss of functional integrity of cell membranes in liver cells(Hemavathy et al., 2016). Biochemical parameters such as SGPT, SGOT, Total and Direct Bilirubin have been reverted significantly by the administration of *Muia* compared to Toxic Control group. Seven days treatment with test drugs (150 mg/kg) protected the animals but the effect of samples is not similar to that of standard drug Liv.52.

Conclusion: The present findings observed in this study revealed that, *Muia* and *Chakhwi* possess significant hepatoprotective activity against CCl_4-induced liver toxicity. *Muia* is more potent than *Chakhwi* to protect the injured liver. However further extensive research is required to find out the possible hepatoprotective molecules of the *Muia* and *Chakhwi*.

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