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HEPATO-PROTECTIVE EFFECT OF AQUEOUS EXTRACT OF SEED, LEAF AND FRUIT OF JACKFRUIT (*ARTOCARPUS HETEROPHYLLUS* LAM.) AGAINST CCL_4 INDUCED HEPATOTOXICITY ON SWISS ALBINO MICE.

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ABSTRACT

Jackfruit (*Artocarpus heterophyllus* Lam.) is one of the important and commonly found tree in home gardens of India. It consists of therapeutically important secondary metabolites and is widely used in traditional medicine. The aqueous extract of seed, leaf and fruit of this plant were evaluated for its effects on CCl_4 induced hepatotoxicity in Swiss albino mice. Animals of either sex were treated with CCl_4 for one day followed by oral administration of a constant dose of aqueous extract of Jackfruit seed, leaf and fruit for 30 days. The hepataprotective activity of the extracts was determined by assessing the biochemical parameters in serum. Serum ALP, AST, ALT, Bilirubin and total protein levels were significantly changed (either increase or decrease) in CCl_4 treated animals. Post administration of aqueous extract of Jackfruit seed, leaf and fruit these levels recovered close to normal levels excepting total bilirubin level. The present study reveals that the aqueous extract of Jackfruit seed, leaf and fruit have beneficial effects on liver function. It is assumed that this hepataprotective effect may be due to the presence of secondary metabolites that respond to stimuli in natural environment and the influence of abundant calcium, potassium, magnesium content.

KEYWORDS: *Artocarpus heterophyllus*, Jackfruit, Hepatotoxicity, Hepataprotective, Carbon tetrachloride, biochemical parameter.

INTRODUCTION

Plant based herbal drugs are used in the treatment of various ailments all over the world including India (Shafi et al., 2015). One of the common serious health problem is liver diseases (Gupta et al., 2013). Hepatitis, a high incidence ailment around the world is induced by viruses, alcohol, lipid peroxidative products, various drugs and autoimmune diseases (Gupta et al., 2013; Das et al., 2012). According to world health statistics, the strategies for the treatment and prevention of liver disease still have many limitations despite of tremendous advances in modern medicine (Afroz et al., 2014). It is because the pathogenesis of liver diseases as well as the causative role of oxidative stress and inflammation in liver diseases is well established, inhibiting oxidation and inflammatory processes could be one of the most important therapeutic strategies for the treatment and prevention of liver damage. Hepatic disease (Liver disease) is a term that affects the cells, tissues, structures or functions of the liver (Kumar et al., 2012). In spite of using modern medicine, the treatment by using many formulations containing herbal extracts are considered to be more effective and safe medicament for hepatotoxicity. Therefore, search for newer drugs (with minimum side effects) obtained from traditional medicine continues (Gupta et al., 2013).

The genus Artocarpus (Family: Moraceae - mulberry family) received a great level of scientific interest as it consists of therapeutically active secondary metabolites and is widely used in traditional medicine (Periyanayagam et al., 2013). *Artocarpus heterophyllus* popularly known as jackfruit is one of the important and commonly found tree in home gardens of India and Bangladesh and also considered as 'poor man's food' (Periyanayagam et al., 2013; Vazhacharickal et al., 2015). In India, it is widely distributed in the states of Assam, West Bengal, Uttar Pradesh, Kerala, Maharashtra and Karnataka (Vazhacharickal et al., 2015). It's vernacular name kathal in Assamease, sohphan in khasi. Extracts of its plant parts have been applied traditionally for the treatment of diarrhea, snakebite and glandular swellings (Gogte et al., 2000), diabetes, malarial fever, tapeworm infection and as wound healing, antisyphilic and also to treat anemia, asthma and dermatitis (Periyanayagam et al., 2013; jagtap et al., 2010; Fernando et al., 1991; Sato et al., 1996). The Artocarpus species contain a diversity of compounds including phenolic compounds, flavonoids, stilbenoids, carotenoids, volatile acids sterols and tannins (Jagtap et al., 2010). The jack fruit seed contain β-carotene, α-carotene and also jacalin is the major protein representing over 50% in seed and capable of binding to human IgA and T-Antigen (Vazhacharickal et al., 2015). The reported pharmacological activities include antimicrobial, anticancer, antihypertensive, antiulcer,

antioxidant, Immuno-modulatory, Anti-inflammatory and anti-ageing (Swami et al., 2012; Haq et al., 2006; Devi et al., 2014; Prakash et al., 2009; Ko et al., 1998; Kabir et al., 1998; Rahman et al., 1999; Dutton et al., 1985; Gupta et al., 2011; Gupta et al., 2004).

The present study focused on the effect of aqueous extracts of seed, leaf and fruit of Jackfruit against CCl_4 induced hepatic damage on Swiss albino mice on which a few work has been done.

(A) (B) (C) (D)

Fig. 1. (A) Flower, (B) Fruit, (C) Seed and (D) Leaf of jackfruit plant (*Artocarpus heterophyllus* Lam.).

MATERIALS AND METHODS

Plant Material

Seeds, leaves and fruits of Jackfruit were collected from the village area of Lakhimpur district, Assam, India. The seeds and leaves were dried in room temperature, whereas fruits were cut into small pieces and only the fleshy parts were dried in a room having temperature more than 30^0C. Then all these were powdered individually in a mechanical grinder and stored in well closed container.

Preparation of extract

The seed, leaf and fruit powder was separately suspended in double distilled water in a 1:3 ratio w/v and kept in magnetic stirrer for 24 hours respectively. After 24 hours the suspension was filtered by using sterile muslin cloth and the filtrate was centrifuged at 2000 rpm for 30 mins. The supernatants were collected and stored at -20^0C until experimentation.

Animals and exposure conditions

The animals used were Swiss albino mice weighing about 19-24.4g obtained from Pasteur Institute, Shillong. The animals were housed in polypropylene cages, given water ad libitum and fed standard pellets diet for one week for adaptation. Mice were exposed to a 12:12 light/ dark cycle, at a room temperature of 18-22°C.

Treatment

The animals of either sex (19-24.4 g body weight) were divided into five groups (group A1, group A2, group A3, group A4 and group A5) comprising 5 mice in each. The group A1 was taken as normal control. The remaining groups were commonly treated with 0.025ml of CCl_4 in liquid paraffin (1:1) as per 1.25 ml/kg body weight intraperitoneally (IP). From the next day groups A3, A4 and A5 were given orally aqueous extract of seed, leaf and fruit of Jackfruit in a ratio of 1.5:5 (v/v)/kg/day for one month where the group A2 was taken as negative control.

Sample Collection

At the end of one month, mice were fasted overnight and blood sample was collected from these animals by Retro orbital Venus plexus. Later on the blood sample was kept to clot for one hours and serum was separated by cooling centrifuge and stored at -80^0C until analysis.

Biochemical studies

Biochemical parameters including AST, ALT, ALP, Total Proteins, Serum Bilirubin (Total, direct and indirect) were estimated by reported methods (Shafi et al., 2015; Varley et al., 1980; Kemal et al., 2014; Jendrassik et al., 1938).

Statistical Analysis

The results are presented as mean values ± standard deviation (SD) (Cumming et al., 2007). Data were analyzed using Microsoft Excel 2013. Statistical analyses of biochemical data were conducted by using student t-test. A *P* value of <0.05 was accepted as statistically significant.

RESULTS

The results show that the levels of all hepatic enzymes elevated with the CCl_4 treated groups but subsequently declined with respective extract of Jackfruit administration during the treatment period. The results indicate that treatment with CCl_4 resulted in significant decrease

in serum protein level. After treatment with aqueous extract of Jackfruit seed, leaf, fruit resulted in a significant increase in serum protein level. Animals treated with CCl_4 as well as extract (seed, leaf or fruit) showed a significant increase in serum total bilirubin level as compared with the normal control group and the major portion was unconjugated or indirect bilirubin (>90%).

Table 1: The results of tested serum hepatic biomarkers.

Treatment	AST (U/l)	ALT (u/l)	ALP (U/l)	Total protein (g/dl)	Bilirubin (mg/dl)		
					Total	Direct	Indirect
Control	20.89±1.344	15.82±0.932	133.028±1.07	6.17±0.844	1.97±0.09	0.19±0.034	1.46±0.178
CCl_4 treated	39.56±2.64* a	42.78±1.147* a	367.98±0.938* a	3.17±0.827* a	1.85±0.373	0.13±0.026	1.7±0.224
CCl_4 + seed extract treated	14.87±0.941* b	37.14±1.097* b	253.28±1.44* b	4.77±0.841* b	2.81±0.381	0.023±0.002	2.78±.0.05
CCl_4 + leaf extract treated	17.94±1.8* b	25.17±1.135* b	268.78±1.273*b	3.82±1.02* b	2.11±0.378	0	2.10±0.135
CCL_4 + fruit extract treated	23.56±1.0377* b	5.15±0.826 *b	257.14±1.448* b	5.27±0.62 *b	1.96±0.374	0	1.91±0.042

Data are presented as mean ± SD of the five animals (duplication of each experiment i.e. n= 2).

AST-Aspartate aminotransferase, ALT-Alanine aminotransferase, ALP-Alkaline phosphatase. *P <0.05.

(a) Significantly different from control group. (b) Significantly different from CCl_4 treated group.

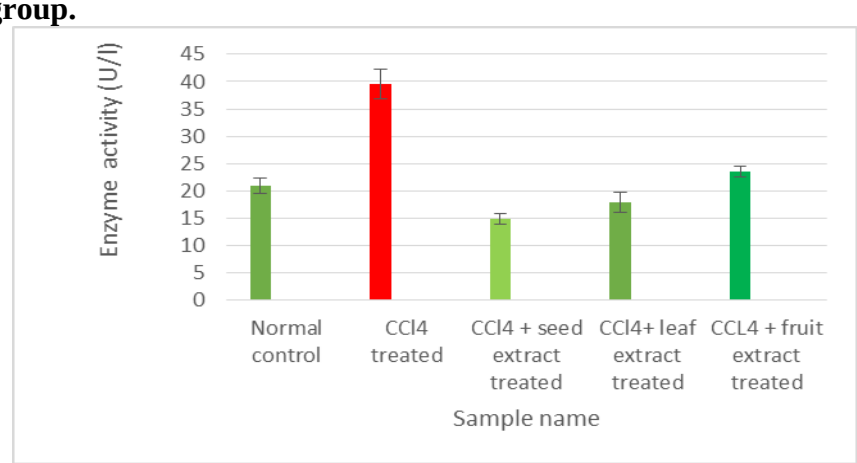

Fig. 2. Effect of aqueous extract of jackfruit seed, leaf and fruit on serum Aspartate aminotransferase (AST) activity (U/l) in the treated animals; the results indicate that treatment with CCl_4 resulted in significant increase in serum AST activity. After treatment with respective extract of jackfruit resulted significant decreased in AST activity. The seed and leaf extract showed significant decrease AST activity which were lower than the normal control.

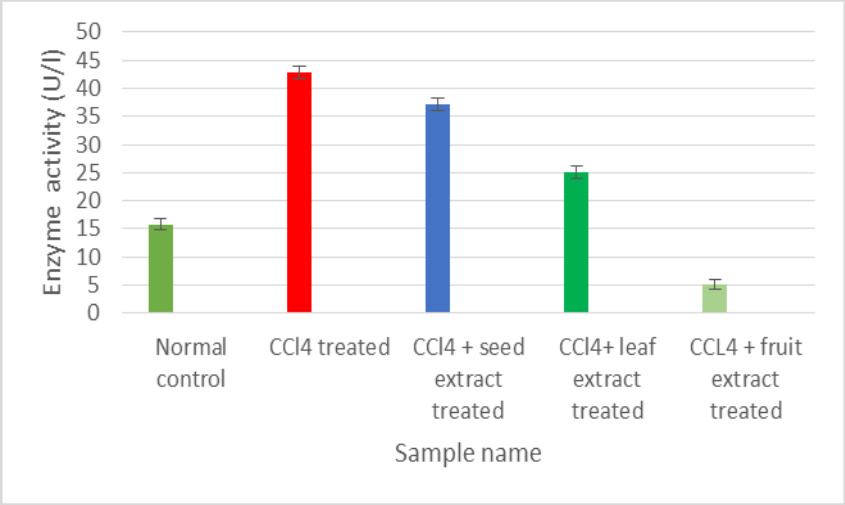

Fig. 3. Effect of aqueous extract of jackfruit seed, leaf and fruit on serum Alanine aminotransferase (ALT) activity (U/l) in the treated animals; the results indicate that treatment with CCl_4 resulted in significant increase in serum ALT activity. Post administration of aqueous extract of jackfruit seed, leaf, fruit resulted in a significant decreased in serum ALT activity. In this case fruit extract sowed significant diminished ALT activity which was lower than the control.

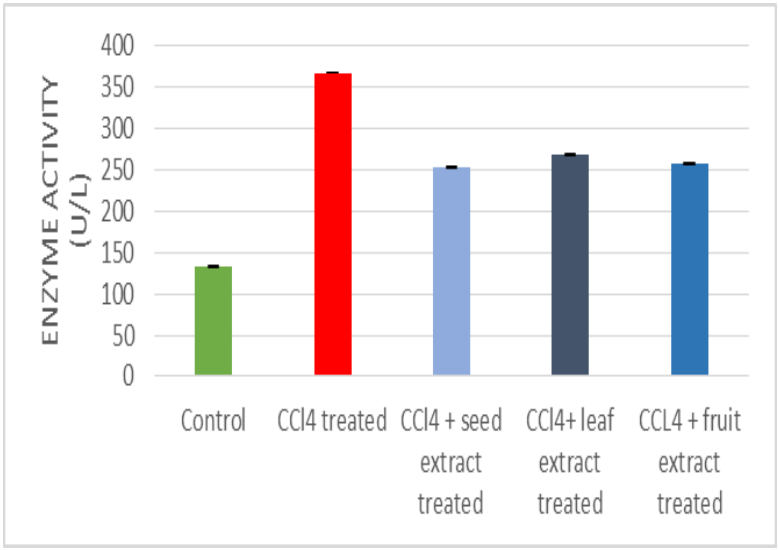

Fig.4. Effect of aqueous extract of jackfruit seed, leaf and fruit on serum Alkaline phosphatase (ALP) activity (U/l) in the treated animals; The results indicate that treatment with CCl_4 resulted in significant increase in serum ALP activity. After treatment with respective extract of jackfruit resulted significant decreased in ALP activity. In this case the seed and fruit extract showed significant decreased ALP activity which were higher than the normal control.

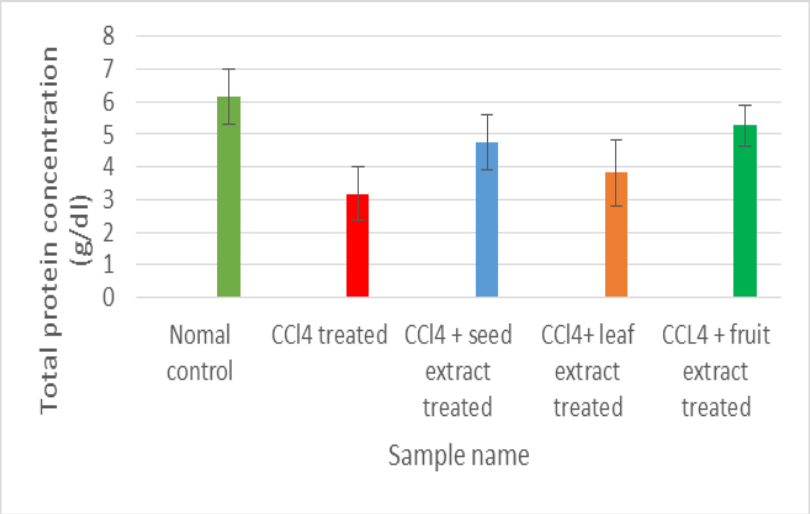

Fig.5. Effect of aqueous extract of jackfruit seed, leaf and fruit on serum total protein concentration (g/dl) in the treated animals; the results indicate that treatment with CCl_4 resulted in significant decrease in serum total protein level. Post administration of respective extract of jackfruit resulted in a significant increase in serum total protein level. In this case as compared to the other two extracts fruit extract showed higher efficiency in increasing in total protein level.

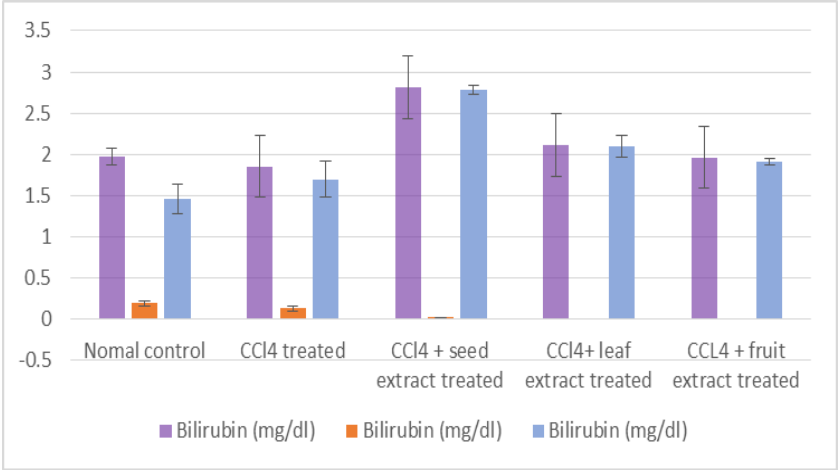

Fig. 6. Effect of aqueous extract of jackfruit seed, leaf and fruit on serum total, direct and indirect bilirubin (mg/dl) in the treated animals; Animals treated with CCl_4 as well as extract (seed, leaf or fruit) showed a significant increase in serum total bilirubin level as compared with the normal control group and the major portion was unconjugated or indirect bilirubin (>90%).

DISCUSSION

The liver is a vital organ that not only perform physiological functions but also protect against the hazards of harmful drugs, chemicals and xenobiotics. The liver is one of the

largest and highly complex organs with multifunction, including nutrient storage, maintenance of homeostasis, secretory and excretory function and synthesis of proteins (Al-Yahya et al., 2013). CCl_4 is also a well-established hepatotoxin; in present study CCl_4 administration to the animals caused a significant changes (increase or decrease) in biochemical parameters which indicates the extensive disruption of the function of the liver. Due to hepato injury, the transport function of hepatocytes gets disturbed resulting in the leakage of plasma membrane, thereby causing an increased enzyme level in the serum (Achliya et al., 2004).

In the present study CCl_4 administration to animals caused a significant increase in serum AST, ALT, ALP in the liver accompanied with a significant decrease in total protein, direct bilirubin in liver, which indicates the extensive disruption of the function of the liver. The tendency of these marker enzymes (SGPT, SGOT, ALP) at a near normal level in the groups of animals treated with aqueous extract of Jackfruit Seed, leaf and fruit is a clear manifestation of antihepato toxic effect of Jackfruit plant.

Out of the serum total protein, albumin is the abundant plasma protein produced only in the liver which maintains the oncotic pressure in the vascular system and carries transportation of endogenous and exogenous substances. Liver fails to synthesize albumin if its function is altered. It was reported that reduction in total protein (TP) content can be deemed as a useful index of severity of hepatocellular damage (Al-Yahya et al., 2013). According to the results, the lowered levels of total proteins in the serum of CCl_4 treated animals exhibited the severity of hepatopathy. Thus, this suggests that Jackfruit seed, leaf and fruit extract have the ability to promote protein synthesis leading to higher concentration of protein in the liver. It was reported that bilirubin shows the excretory function of the liver and its concentration elevated in the blood, either by increased production, decreased conjugation, decreased secretion by the liver, or blockage of the bile ducts. In cases of increased production or decreased conjugation, the unconjugated or indirect form of bilirubin will be elevated and called unconjugated hyperbilirubinemia (Shafi et al., 2015). The enzyme uridine diphosphoglucuronate glucuronyl transferase (UGT) in hepatocytes is necessary for the conjugation of bilirubin; either a lack of this enzyme, or the presence of drugs that interfere with UGT, impairs the liver's ability to conjugate bilirubin. It was also reported that unconjugated hyperbilirubinemia is due to decrease in UGT activity. Occasionally, cirrhosis can cause unconjugated hyperbilirubinemia, as hepatic fibrosis leads to capillarization of

sinosoids, causing decreased bilirubin uptake by hepatocytes. According to the results, it is assumed that this significant increase in unconjugated bilirubin in treated animals may be due to the above mentioned reasons. The activities of serum ALP is one of the most sensitive biomarker that can directly indicate the extent of hepatic damaged and toxicity (Afroz et al., 2014), where SGPT and SGOT give information about the inflammation and necrosis. Administration of CCl_4 to the animals caused massive elevation of serum ALP, SGPT, SGOT activity indicating hepatotoxicity. By treatment with aqueous extract of Jackfruit seed, leaf and fruit induced the opposite effect, markedly reducing the levels of these enzyme activity.

It is assumed that this hepatoprotective effect of aqueous extract of *A. heterophyllus* seed, leaf and fruit mainly due to the presence of secondary metabolites that respond to stimuli in natural environments, and that may not be expressed under culture conditions.

CONCLUSION

From the study, it can be concluded that the aqueous extract of *A. heterophyllus* seed, leaf and fruit have beneficial effects on liver function tests. Further pharmacological, histopathological and other biochemical investigations will clearly elucidate the mechanism of action and will be helpful in projecting this plant as a therapeutic target in medical research.

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Disclosure statement

The authors declare that there is no conflict of interest regarding the publication of the paper.

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