Chapter-6

DISCUSSION
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Plant based medicines gained priority for their highest level of safety and efficacy compared to safety and efficacy offered by synthetic drugs. Now-a-days plant products are tremendously used as medicine for curing liver diseases. In spite of the availability of a number of commercial hepatoprotective medicines, the demand of a suitable liver protective drug is yet to be fulfilled (Bhandarkar and Khan, 2004; Ozbek et al., 2004; Kumar et al., 2012). Therefore, it is necessary to find suitable liver protective agent from natural plant products. Phytomedicines are the secondary metabolites which have curative properties against diseases. Presence of secondary metabolites or organic chemical constituents in plants can be known by the study of qualitative phytochemical screening. Qualitative phytochemical screening of Pajanelia longifolia (Willd.) K. Schuman suggested that tannin was present in the ethyl acetate extract and alkaloids, tannin, reducing sugar, flavonoids and steroids were present in acetone and methanol extract. The information regarding the polarity of different chemical constituents were provided by the Thin Layer Chromatography (TLC) technique. Compound showing high Rf value in the less polar solvent system have low polarity and with less Rf value have high polarity. The suitable solvent system has been chosen for performing Column Chromatography for further separation of pure compound(s).

Hepatic diseases are now-a-days a major health problem but the suitable drug for curative purposes are not available; therefore it is always demandable to search for alternative drugs. Pajanelia longifolia (Willd.) K. Schuman has been chosen for the assessment of hepatoprotective and antioxidant effect as because in Southern Assam the plant is
traditionally known to be a very effective folkloric medicine for curing jaundice. But there is little documented scientific evidence regarding the hepatoprotective and antioxidant activity of this plant. Before starting the investigation about the hepatoprotective and antioxidant activity, it was important to check the toxicity level of this plant. Therefore, the toxicity study was assessed with graded dose levels (200, 400, 600, 800, 1000, 1200, 1600 and 2000 mg/kg b.w.p.o. respectively) of acetone and methanol crude bark extracts for 21 days. The toxicity study of acetone and methanol bark extracts did not exhibited mortality or any visible behavioral changes at any of the dose level upto 21 days of treatment. After dissection of mice, cyst like structure was found in higher dose (1600 mg/kg b.w.p.o. and 2000 mg/kg b.w.p.o.) treated mice liver but in lower dose (200, 400 and 600 mg/kg b.w.p.o.) treated mice liver no any cyst like structure could be found. From histopathological observations, hepatic necrosis and tissue degradation were observed at higher dose levels (1000, 1200, 1600 and 2000 mg/kg b.w.p.o.) but in lower dose levels (200, 400 and 600 mg/kg b.w.p.o.) slight fatty changes were noticed, however these changes were negligible. The findings of acute toxicity results were further cleared by measuring the serum biochemical parameters where it was observed that the levels of SGOT, SGPT and bilirubin were significantly (P<0.001) increased from lower dose (200, 400, 600 mg/kg dose) to higher dose (1000, 1200, 1600 and 2000 mg/kg) levels, which was the indication of increasing the toxic effect from lower dose to higher dose levels. From the result of toxicity study, it can be said that the bark extracts of *Pajanelia longifolia* (Willd.) K. Schuman at higher dose amount has toxic effect but the toxic effect which was observed in lower dose levels were negligible.
On the basis of acute toxicity results the dose level of *Pajanelia longifolia* (Willd.) K. Schuman crude extracts was selected for bioactivity studies. For *in vivo* assessment of bioactivity, Carbon tetrachloride (CCl₄) was chosen for the production of toxic effects in Swiss albino mice liver. CCl₄ induced hepatic injury is similar to that of acute viral hepatitis. The mechanism by which CCl₄ causes damage involves CCl₄ bioactivation through CYP2E1 activity and the results of this activation is the release of a trichloromethyl free radical CCl₃. Free radical CCl₃ then convert into CCl₃O₂, which is a more reactive trichloromethylperoxyl radical. This peroxyl radical causes lipid peroxidation by increasing the plasma membrane permeability, leading to hepatic damage (Recknagel *et al.* 1989). The hepatoprotective activity was monitored by estimating the levels of serum transaminases, serum alkaline phosphatase and serum bilirubin. By the study of the serum biochemical parameters a good idea can be taken about the functional status of the liver (Rao and Mishra, 1997). The hepatic cells consist of higher concentration of GOT and GPT in Cytoplasm and GOT particularly exists in mitochondria. Due to the necrosis or membrane damage the hepatospecific enzymes are released in blood circulation and therefore it can be measured by measuring the serum enzyme levels (Drotman and Lawhorn, 1978). High levels of SGOT indicate liver damage as that of viral hepatitis. SGPT is more specific to the liver as it catalyses the conversion of alanine to pyruvate and glutamate and is released into the blood circulation, therefore SGPT is a better parameter for detecting liver injury. The highest concentration of bilirubin in the serum is an indication of increased erythrocyte degeneration rate (Rao, 1973; Singh *et al.*, 1998). On the other hand ALP level in serum is related to the function of hepatic cells. Increased by ALP level in serum is due to increase synthesis in the presence of increasing biliary pressure (Akindele *et al.* 2010). Acetone and methanol crude bark extracts of
Pajanelia longifolia (Willd.) K. Schuman at different dose concentration manner exhibited hepatoprotective activity. Acetone crude bark extract at a dose of 200 mg/kg b.w. p.o. exhibited best level (P<0.001 compared to toxic group and P<0.001 compared to control group) of protective efficacy, whereas, the methanol crude bark extract at a dose of 300 mg/kg b.w.p.o. exhibited least level (P<0.001 compared to toxic group and P<0.01 compared to control group and standard group respectively) of protective efficacy in serum enzymes and bilirubin levels compared to other doses of crude bark extracts. In toxicity study 200 mg/kg dose of both acetone and methanol crude bark extracts exhibited slight fatty changes in histopathological observations. But the same dose when treated in hepatotoxic mice (CCl₄ treated), then offered hepatoprotection.

Mammalian cells contain antioxidant enzymes, including catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx). For the maintenance of the bodies redox balance the body cells tightly maintained the levels of these enzymes and also non-enzymes like Lipid peroxidation (LPO) and reduced glutathione (GSH) (Saeed et al., 2005). Free radicals are detoxified by antioxidant enzymes and are converted to more stable molecules. The activity of catalase enzyme results in the conversion of hydrogen peroxide to water and oxygen. Melondialdehyde is a breakdown product of lipid peroxidation therefore; it is a useful index of lipid peroxidation. In another case water and oxidized glutathione were produced by the combined activity of reduced glutathione and hydrogen peroxide, where glutathione peroxidase helps to this conjugation (Akindele et al., 2010). A significant (P<0.001 compared to control) decrease in the values of SOD, CAT, GPₓ and GSH and a significant increase (P<0.001 compared to control) in the value of LPO were recorded after 5th day of Carbon tetrachloride (CCl₄) intoxication, indicating a considerable hepatocellular

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injury. Pretreatment with *Pajanelia longifolia* (Willd.) K. Schuman crude extracts at different dose dependent manner subsequently recovered the changes occurred in SOD, CAT, GPx, GSH and LPO levels of CCl₄ intoxicated mice. *Pajanelia longifolia* (Willd.) K. Schuman acetone bark extract at a dose of 200 mg/kg b.w.p.o. exhibited best level (P<0.001 compared to toxic group and control group respectively and P<0.05 compared to standard group) of protective efficacy and 300 mg/kg dose of methanol bark extracts exhibited least level (P<0.001 compared to toxic group, control group and standard group respectively) of protective efficacy in GSH, CAT, SOD, GPx and LPO levels compared to other doses of crude extracts. Histopathological study was also supported this findings. In histopathological examinations control group animal exhibited well formed hepatocytes whereas, toxic group (CCl₄ treated) exhibited necrotic lesions with cellular degradation. Treatment with acetone and methanol bark extracts exhibited healing of necrosis. Maximum level of healing of necrosis was offered by the treatment with 200 mg/kg b.w.p.o. dose of acetone crude bark extract. Silymarin treated group (standard) exhibited normal hepatocytes.

In this work one compound was isolated from acetone crude bark extract and the purity level of this compound was analysed by analytical HPLC technique. The analytical HPLC chromatogram of the isolated compound indicated the material to be fairly pured (impurity present in minor quantity). From spectroscopic data analysis the compound was identified as 2,3,6-trimethyloct-6-enal. From literature survey there was no any reported document could be found about this compound. Therefore, this is the first report of
isolation of this compound from this plant. The compound possesses skeletal similarity with citronellal and bioassays were performed thereafter with this isolated compound.

2,3,6-trimethyloct-6-enal at a dose level of 100 mg/kg exhibited a maximum level (significant at P<0.001 compared to toxic group) of hepatoprotective efficacy on serum enzymes and bilirubin levels compared to other two doses (200 mg/kg and 300 mg/kg b.w.p.o.). The antioxidant efficacy of 100 mg/kg b.w.p.o. dose (P<0.001 compared to toxic) of 2,3,6-trimethyloct-6-enal was similar to that of standard drug Silymarin. 2,3,6-trimethyloct-6-enal at different dose concentration manner also exhibited antioxidant efficacy by lowering the CCl₄ induced elevated LPO level and by increasing the CCl₄ induced decreased levels of SOD, CAT, GPₓ and GSH. The antioxidant efficacy exhibited by 100 mg/kg b.w.p.o. dose of isolated compound was similar to that of standard drug Silymarin (50 mg/kg b.w.p.o.). Histopathological study was also supported the bioactivity results of 2,3,6-trimethyloct-6-enal.

From overall data it was proved that Pajanelia longifolia (Willd.) K. Schuman being able to restored the damage of hepatocytes caused due to the administration of single dose of CCl₄. From bioactivity study of Pajanelia longifolia (Willd.) K. Schuman, it can be demonstrated that the possible hepatoprotective and antioxidant efficacy of Pajanelia longifolia (Willd.) K. Schuman against CCl₄ induced liver damage in Swiss albino mice might be due to the following effects: (1) Inhibiting the cytochrome p450-dependent oxygenase activity; (2) Preventing lipid peroxidation; or (3) Stabilizing the hepatocyte membrane.