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Hepatoprotective Activity of Aqueous and Ethanol Extract of *Lippia adoensis* Leaf Against Carbon Tetrachloride-Induced Hepatotoxicity in Mice

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Abstract: Liver disease is one of the fatal diseases. Medicinal plants may serve as one ofthe best sources of remedies for treatment of liver disease. Identification of a potential therapeutic agent for protection of liver from hepatotoxins provides a useful way for the prevention of liver related illnesses. The aim of the present study was, therefore, to investigate the hepatoprotective activity of 80% ethanolic and aqueous extract of leaves of *Lippia adoensis* againstcarbontetrachloride induced liver injury in mice. Mice were pretreated with extract of *Lippia adoensis* (200 & 400 mg/kg bw. po) for 7 days and then challenged with CCl_4 (0.2 ml/100 gm p. o) on the 7th day. Serum biomarkers (AST, ALT, ALP and total bilirubin, total protein and albumin) were estimated in all the study groups. Histopathological examination was also carried out for all groups of mice. CCl_4 significantly ($P < 0.001$) increased the AST, ALT, ALP and total bilirubin levels and decreased the level of total protein and albumin indicating hepatic damage. Pretreatment with theextract of *Lippia adoensis* (200 & 400 mg/kg) produced significant ($P < 0.001$) hepatoprotective effects as evidenced by decreased serum enzymes (AST, ALT, ALP) and serum bilirubin and increase the level of total protein and albumin as well as by histopathological findings of the liver. Histopathological examination of the liver tissues of CCl_4 group represented the presence of marked foci of mononuclear infiltration in the hepatic parenchyma tissue, sinusoid and around central vein, as well as disorganization of hepatic plates, whilepretreatment with extract of *Lippia adoensis* overcome most of these changes to normal histological architecture of the liver. Body weights of *Lippia adoensis* extract treated groups increased significantly ($P < 0.001$) as compared to carbontetrachloride treated group. The absolute and relative liver weights of extracts treated mice decreased significantly ($P < 0.001$) as compared to those treated with CCl_4. The results obtained from both ethanolic and aqueous extract of *Lippia adoensis* were comparable with those of the standard drug, Silymarin. It can be concluded from the present study that bothethanolic and aqueous extracts of *Lippia adoensis* have hepatoprotective effect.

Keywords: *Lippia adoensis*, Aqueous Extract, Ethanol Extract, Hepatoprotective, CCl_4, Silymarin, AST, ALT, ALP, Bilirubin, Protein, Albumin and Mice

1. Introduction

Liver is the largest organ in the body and plays a significant role in protecting various biological function and help in detoxification and excretion of various endogenous and exogenous compounds (Mohamed*et al.*, 2010). The

liver is often the target organ for chemically induced injuries. Many oxidative reactions produce reactive metabolites that can induce lesions within the liver. The types of injury to the liver depend on the type of toxic agent, the severity of intoxication, and the type of exposure, whether acute or chronic (Comfort *et al*., 2013; Singh *et al*., 2011).

*Lippia adoensis*is included under Verbenaceae that is a large family with about 70 to 80 genera and over 3, 000 species; distributed throughout the world mainly in the tropics and temperate regions. *Lippia* is a genus with 200 species in tropical Africa and America (Toshihiro *et al*., 1992). In Ethiopia, the family is represented by 9 genera and 30 species. *Lippia adoensis* (locally known as "Kesse") is a shrub having a height of 1 to 3 meters. Two varieties of *Lippia adoensis* are recognized in Ethiopia, the wild variety (var. *adoensis*) and the cultivated variety (var. *koseret*). The fragrant leaves are used by the Gurage and Oromo tribes as one of the condiments in the preparation of spiced butter. The special taste and flavor of the "Gurage Kitfo" is attributed to the oils imparted by the leaves **(**Toshihiro *et al*., 1992). In Ethiopia, the wild variety (var. *adoensis*) is used for washing kitchen utensils to impart fresh and spicy fragrance (Abegaz *et al*., 1993). The chemical compositions of *Lippia adoensis*, investigated so far are essential oils. The oils were predominantly monoterpenoids with minor sesquiterpenoid fraction. In one study, out of more than 10 compounds isolated, linalool comprised 81.30% and 94.56% of the leaf and flower oils, respectively (Asres and Bucar, 2002). It is well documented that *Lippia adoensis* is one of the rich sources of flavonoids, Phenolics, tannins, saponins and alkaloids *(*Riot *et al.,* 2005). The leaves of *L. adoensis* are used in Ethiopian traditional medicine for the treatment of various skin diseases including eczema and superficial fungal infections (Asres *et al*., 1986). Review of literature revealed that this rare medicinal plant remained unexplored for many of its claimed pharmacological activities. The present study was conducted to establish the use of *Lippia adoensis* as hepatoprotective against CCl_4 induced hepatotoxicity in mice.

2. Methodology

2.1. Collection of Plant Materials

Fresh leaves of *L. adoensis* were collected in Addis Ababa, Ethiopia, on January 18, 2014. Plant identification and authentication was done with the help of local floras. The specimen has been preserved in AAU herbarium with the voucher number of 084908.

2.2. Preparation of Plant Extracts

One kilogram of dried powder plant material was macerated in ethanol (80%) in a ratio of 1:1. It was then filtered and the filtrated extract was then concentrated by evaporation using Rota vapor at temperature not exceeding 40°C. Then the resulting aqueous residue was placed in an oven for about 48 hours at a temperature of 40°C. One kilogram of dried powder plant material was macerated in water in a ratio of 1:1. The mixture was then filtered and the filtrated extract was lyophilized for 24 h. The resulting powder was stored in to amber colored glass bottle in desiccators over silica gel for use in subsequent experiments (Bimlesh *et al.,* 2011).

2.3. Experimental Animals

A total of 42 healthy male mice weighing 25-35 gram were obtained from animal house of department of pharmacology, School of medicine, AAU and Ethiopian Public Health Institute *(*EPHI*)* were used throughout the study. They were kept under standard environmental conditions at 22±3°C with 12:12 hour light–dark cycle in ventilated plastic cages. The animals were housed in steel mesh cages, (6 per cage) and had a free access to feed with a standard pellet diet and water *ad libitum*. The animals were acclimatized to laboratory conditions for one week prior to experimentation.

3. Experimental Design

3.1. Hepatoprotective Activity

The body weights of all mice were recorded at the beginning of the experiment. Experimental mice were divided in seven groups (n-7) of six mice/ group. The animals were fasted overnight before the initiation of the study. Group Iserved as normal control which received vehicle(5% gum acacia; 1 ml/kg; p. o) orally daily for seven days. Group II received CCl_4 (0.2 ml/100 gm p. o) on seventh day. Group III served as standard and received silymarin (100 mg/kg p. o) daily for seven days and thirty minutes post dose of silymarin mice received CCl_4 (0.2 ml/100 gm p. o.) on the seventh day. Group IV and Group V were treated with the aqueous extract of *L. adoensis*at the doses of 200 mg/kg and 400 mg/kg, respectively in 5% aqueous gum acacia orally daily for seven days. Group VIand Group VII were treated with the ethanolic extract of *L. adoensis* at the doses of 200 mg/kg and 400 mg/kg, respectively in 5% aqueous gum acacia orally daily for seven days. Thirty minutes post dose of extract administration, animals received CCl_4 at the dose of 0.2 ml/100 gm orally(Sunita *et al.,*2014; Sabreena *et al*., 2014; Showket *et al*., 2014).

3.2. Blood Collection

The blood sample from each mouse was collected separately in sterilized dry centrifuge tubes by cardiac puncture and allowed to coagulate for 10 minutes at 37°C. The clear serum was separated at 2500 rpm for 10 minutes. The serum collected was used for biochemical analyses. All biochemical analyses were carried out within 24 hours of serum separation. (Maliha and AL-Marzooq, 2014; Iyanda and Adeniyi, 2014).

3.3. Histopathological Examination

After collection of blood sample, the mice were scarified and then the liver was excised from the animals and washed with normal saline. The absolute weight of liver tissue was measured. The tissues of liver were fixed in 10% formalin and processed separately for histopathological observation. The microscopic slides were examined under a microscopeby a pathologist who was blind to the protocol of the study (Alaezi et al., 2014; Bhaskara and Mukalel, 2014).

3.4. Statistical Analysis

The results were expressed as means ± standard errors of mean (SEM). The datawere analyzed by one way analysis of variance (ANOVA). P < O.05 was considered statistically significant. The statistical analysis was carried out using the SPSS program (version 21.0).

4. Results

4.1. Yields of the Aqueous and 80% Ethanol Extracts

Leaves of *Lippia adoensis* were extracted with aqueous and 80% ethanol using maceration technique and these extracts were used for investigation of hepatoprotective activity. The percentage yields of these extracts are given in Table 1. The yields of aqueous and ethanol extracts were found to be 16.3% and 12.7%, respectively.

Table 1. *Percentage yields of the aqueous and 80% ethanol extracts of the dried and powdered leaves of Lippia adoensis.*

Plant species	Extracts	Parts used	Percentage yield
Lippia adoensis	Aqueous Extracts	Leaves	16.3
Lippia adoensis	80% Ethanol Extracts	Leaves	12.7

4.2. Limit Dose Test

Limit test was performed following OECD (Organization of Economic Co-Operation Development) guidelines–423. The purpose of this study was to allow selection of the appropriate starting dose for the main study. The animals did not show any signs of toxicity and behavioral changes after 24 hrs and 72 hrs. The oral LD_{50} was estimated to be above 3000mg/kg.

4.3. Effects of Lippia adoensis Extract on Body Weight, Liver Weightand Relative Liver Weights Ratioin CCl_4 Intoxicated Mice

Both aqueous and ethanol leaves extracts prevented reduction significantly (p<0.01) in the bodyweights of mice treated with carbon tetrachloride at both doses comparable to that of the standard as shown in table 2. The same table shows a significant decrease in both absolute and relative liver weight with both doses of both extracts comparable to those of the standard.

Table 2. *Effects of Lippia adoensis leaves extracts on body weight, and absolute and relative liver weights.*

Groups Treatment	Body weight (gm)		Body weightChange	Absolute Liver weight(gm)	Relative liver Weight (%)
	Initial	Final			
Control	29.00 ± 0.93	32.33 ± 0.88	2.83 ± 0.60	1.47±0.05	4.53±0.084
$CCl_4$2ml/kg	32.17±0.74*	28.33 ± 0.76**	-3.50 ±0.61#	2.32±0.06#	7.97±0.43#
Sylimarin100mg/kg	29.83 ±0.48***	32.50±0.43**	2.67±0.33**	1.50±0.03**	4.52±=0.06**
Aqueous extract200mg/kg	28.83 ± 0.79*	30.83±0.65***	1.83±0.30**	1.76±0.06**	5.53±0.23**
Aqueous extract400mg/kg	28.33 ± 0.67*	30.67±0.56***	2.33± 0.49**	1.69±0.07**	5.50±0.28**
Ethanolic extract200mg/kg	27.83 ± 0.83**	30.67±0.88***	2.83±0.40**	1.71±0.10**	5.62±0.42**
Ethanolic extract400mg/kg	29.17±0.7031*	31.83±0.83*	2.83±0.48**	1.65±0.16**	5.20±0.57**

Values are expressed as means ± standard error of means (SEM) of six mice treated for **7** days.
* P <0.01, ** P < 0.001, #P < 0.001, *** P <0.05 were statistically significant. Symbols represent statistical significance;# significantly different from control ** significantly different from CCl_4 treated group.

4.4. Effects of Lippia adoensis Leaves Aqueous and Ethanol Extract on AST, ALT, ALP, Total Bilirubin, Albumin and Total Protein Serum Levels

The serum levels of AST, ALT, and ALP significantly (P< 0.001) increased in carbon tetrachloride treated mice; as compared to those in the control mice (table 3). Pretreatment with 200mg/kg and 400mg/kg bothethanolic and aqueous extracts of *Lippia adoensis* leavessignificantly reduced (P<0.001) the levels of AST, ALT and ALP after carbon tetrachloride administration (table 3). The standard drug, silymarin also significantly (P < 0.001) reduced the serum levels of AST and ALP as shown in table 3.

The level of total bilirubin in serum significantly(P<0.001) increased in carbon tetrachloride treated mice compared to that of the normal control mice as shown in table 3. The same table shows that both doses of *Lippia adoensis* ethanolic and aqueous extracts (200mg/kg) reduced the levels of total bilirubin significantly (P < 0.001) The level of serum totalprotein significantly (P<0.001) reduced in carbon tetrachloride treated mice, while Administration of both doses of both extracts significantly (P<0.001) increased the serum total protein level comparable to that of the standard (table 3).

The same table shows the level of albumin significantly (P<0.001) reduced in carbon tetrachloride treated mice; while both doses of both extracts increased thelevel significantly (P<0.001) comparable to that of the standard

Table 3. *Effects of extracts of Lippia adoensis on CCl_4 induced liver damage in mice.*

Sr. no	Group	Dose (po)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Total Protein (gm/dl)	Total Bilirubin mg/dl)	Albumin mg/dl
1	Control	Vehicle(5%Acacia)	104.17± 4.41	126.67±1.61	201.17±4.52	6.75± 0.21	1.15±0.13	2.18 ±0.15
2	CCl_4 control	2ml/kg	251.83±3.46*	286.83±2.52*	446.50±3.67*	4.63±0.15*	4.48 ±0.12*	1.67±0.20*
3	Silymarin+ CCl_4	100mg/kg	112.50±3.24**	129.83±2.02**	220.67±2.95**	6.67±0.12**	1.17±0.07**	2.47±0.17**
4	Aqueous extract + CCl_4	200mg/kg	135.50±4.27**	143.67±2.44**	257.83±2.75**	6.17±0.11**	2.05±0.14**	2.62±0.09**
5	Aqueous extract + CCl_4	400mg/kg	127.0±1.79**	138.33±3.29**	225.5±2.44**	6.23±0.09**	1.983±0.25**	2.63±0.07**
6	Ethanolic extract + CCl_4	200mg/kg	128.83±2.89**	141.83±2.76**	233.67±3.45**	6.15±0.11**	2.22±0.11**	2.65±0.085**
7	Ethanolic extract + CCl_4	400mg/kg	126.17±4.38**	140.50±2.01**	227.67±3.05**	6.22±0.08**	2.10 ±0.13**	2.67±0.11**

Values are mean ± S. E. M, (n=6); $P < 0.001$

Symbols represent statistical significance * considered significantly different at $P < 0.001$ when compared with normal control group; ** considered significantly different at $P < 0.001$ when compared with CCl_4 alone control group.

4.5. Effect of Lippia adoensis Extracts on Histopathology

Histopathological analysis of liver sectionsrevealed that carbon tetrachloride (0.2 ml/100 gm p. o) intoxication caused marked foci of mononuclear infiltration in the hepatic parenchyma tissue, sinusoid and around central vein, as well as disorganization of hepatic plates in contrast to thecentral veins and thin sinusoids observed in normal mice (Figure 1). In c Moreover, in carbon tetrachloride treated mice, there was massive necrosis throughout the liver, in some areas whole lobules being degenerated, in others the more central hepatic cells showing the greater disintegration (Figure 2). Pretreatments with *Lippia adoensis* extractsat both doses resulted in normal hepatic cells, central vein and sinusoids with no necrosis and foci of mononuclear infiltration comparable to that observed with the standard as shown in figure 3.

In the liver section from 200 mg/kg *Lippia adoensis* aqueous extract pretreated mice hepatic cells, central vein and sinusoids were normal, except the presence of a minimal focus of mononuclear infiltration at the periphery (Figure 4); whereas, in the one from 400 mg/kg *Lippia adoensis* aqueous extract pretreated mice there was a marked protection with normal hepatic cells, central vein and sinusoids (Figure 5). Similarly, in the liver section from 200 mg/kg *Lippia adoensis* ethanolic extract p pretreated mice there was a marked protection with normal hepatic cells, central vein and sinusoids (Figure 6).

There was also no histopathological change in the sections of the liver from 400 mg/kg *Lippia adoensis* ethanolic extract pretreated mice with normal hepatic cells, central vein and sinusoids (Figure 7). The histological appearance of the *Lippia adoensis* extractpretreated groups was quite similar to that of the control group, and tissue damage and necrosis were of less extent in *Lippia adoensis* extracttreated group than the CCl_4 group.

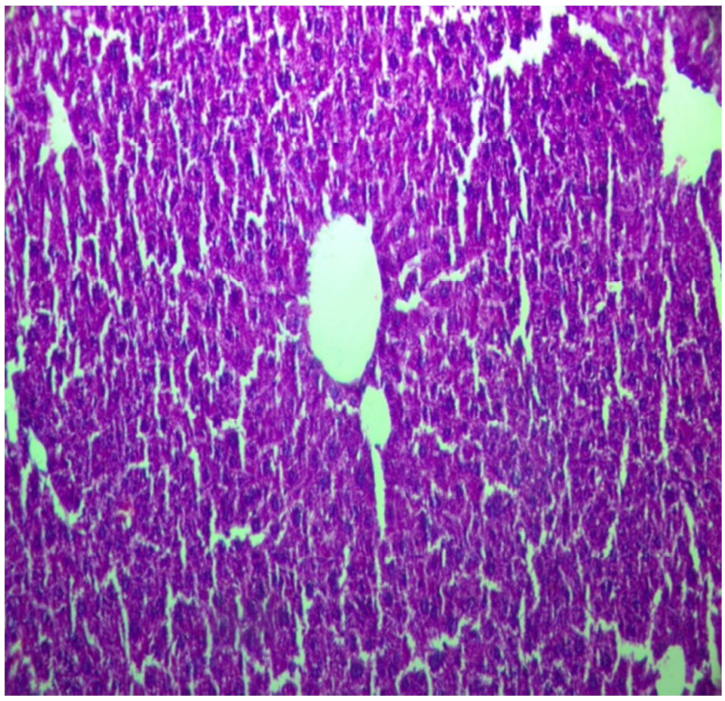

Figure 1. *Section of liver from the control group showing normal architecture of the hepatic lobule with no histopathological change H & E staining (x100).*

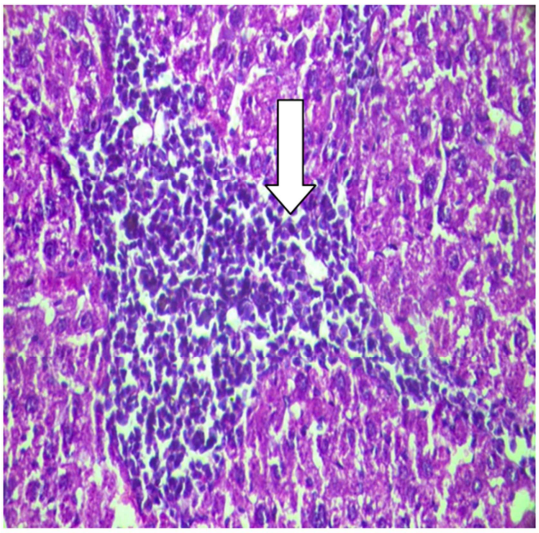

Figure 2. *Liver section from mice treated with CCl_4 at a dose of 0.2ml/100 gm p. o; bw showing a focal mononuclear infiltration (arrow), H & E staining (x100).*

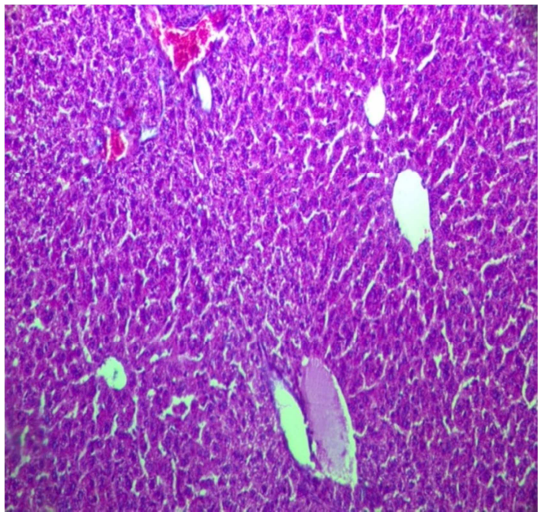

Figure 3. *Liver section from mice treated with silymarin and CCl_4 showing normal architecture of the hepatic lobule with no histopathological change H & E staining (x100).*

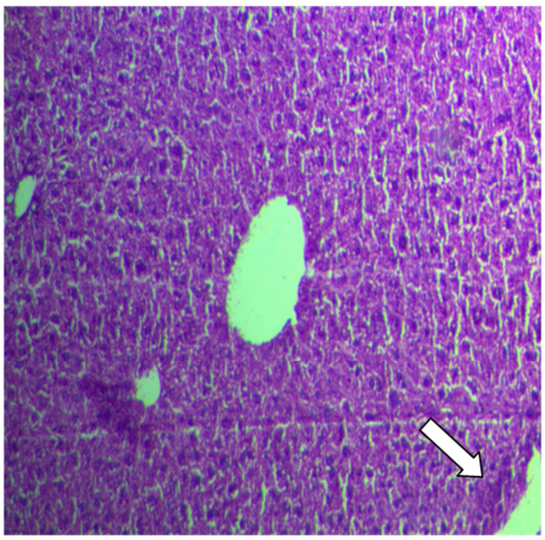

Figure 4. *Liver section from mice treated with 200mg/kg Lippia adoensis aqueous extractandCCl_4 showing normal architecture of the hepatic lobule with minimal focus of mononuclear infiltration at the periphery (arrow) H & E staining (x100).*

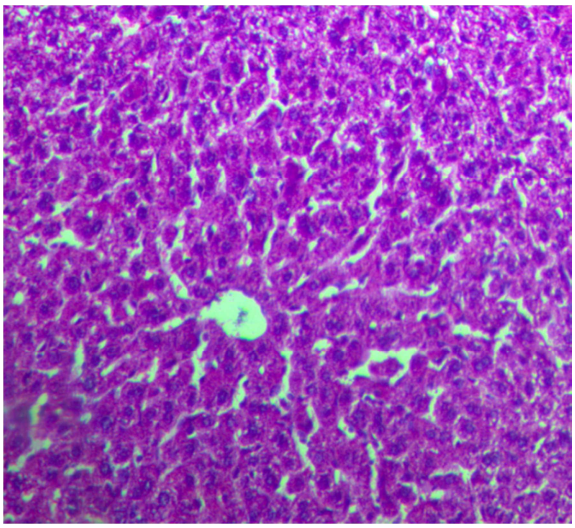

Figure 5. *Liver section from mice treated with 400 mg/kg Lippia adoensis aqueous extract and CCl_4 showing normal architecture of the hepatic lobule with no histopathological change H & E staining (x100).*

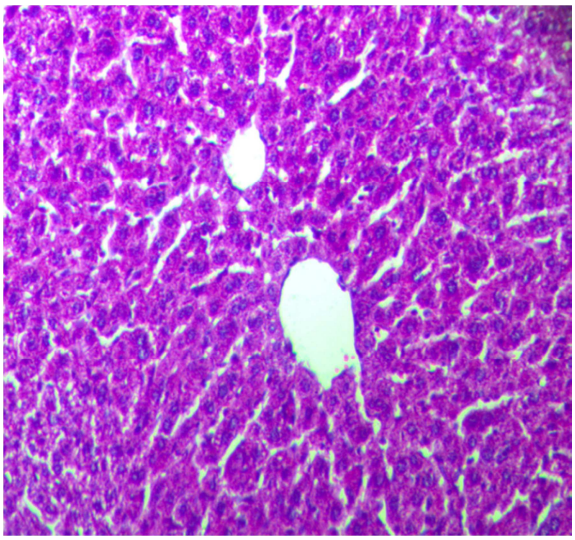

Figure 6. *Liver section from mice treated with 200 mg/kg Lippia adoensis ethanolic extract and CCl_4 showing normal architecture of the hepatic lobule with no histopathological change H & E staining (x100).*

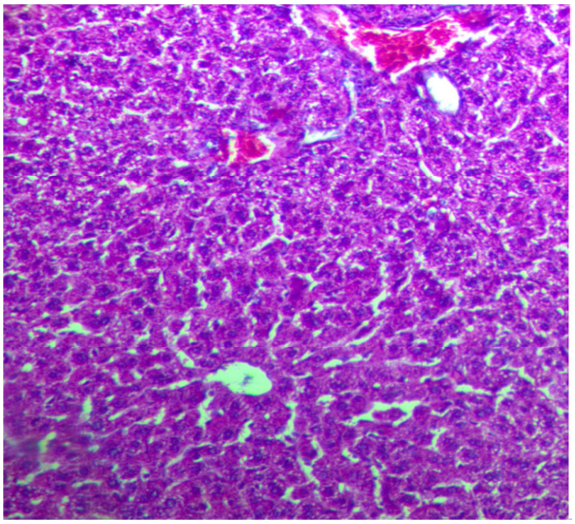

Figure 7. *Liver section from mice treated with 400 mg/kg Lippia adoensis ethanolic extract and CCl_4showingnormal architecture of the hepatic lobule with no histopathological change H & E staining(x100).*

5. Discussion

Medicinal plants are getting great attention as important sources of bioactive substances, with health beneficial effects. However, a great limitation to the use of medicinal plants is the issue of safety and toxicity. Liver damage is a widely used indicator of toxicity of medicinal plants *in vivo* (Kadejo *et al.,* 2012; Manuj *et al.,* 2014).

The significant increase in body weight and decrease in absolute and relative liver weights in extract pretreated mice indicates that the plant extract protects liver from hepatotoxic agents. The results from the present study moreover reveal a relationship between body weight/absolute/relative liver weights and liver function.

Muhammadet al. and Douhri *et al.* reported similar results those of the present study. Bhaargavi*et al.* demonstrated that in hepatotoxicity, liver weight increases generally as a consequence of necrosis and fibrosis or hypertrophy of the liver and body weight decreases due to infiltration of hepatic parenchyma, disorganization of hepatic plates and fatty change. The alterations in the body weight and the liver weight in mice after CCl_4 administration were considered to result from direct toxicity of the liver and indirect toxicity related to liver damage (Andréia *et al.,* 2013). Change in relative liver weight is a valuable index of the extent of acute hepatic damage (Douhri *et al.,* 2014).

The aminotransferases (ALT, AST), ALP and bilirubin are among serum biomarkers of hepatic function, with their increase in the serum indicating hepatic damage (David *et al*., 2014), where as decreased level of total protein and albumin in the serum indicating hepatic damage (Kanwal *et al*., 2012).

Pretreatment of normal mice with *Lippia adoensis*did not result in any toxicity or adverse effects as indicated by the insignificant change in serum levels of ALT, AST, and ALP, total bilirubin, total protein and albumin in liver damage. Results from the present study confirmed that CCl_4 induced hepatotoxic effects manifested by a significant increase in activity of liver function marker enzymes ALT, AST, ALP and bilirubin in the serum of CCl_4 treated mice, whereas total protein and albumin in the serum of CCl_4 treated mice decreased significantly. These observations are in accordance with those in similar studies (Senthilkumar *et al.,* 2014; Isha *et al.,* 2014; Mary *et al.,* 2014). In the present study, the levels of bilirubin and total protein and albumin were restored to the normal values after administration of the plant extract indicating its hepatoprotective action. This effect was found to be comparable to that of standard drug (silymarin). The results generally suggest that the imbalanced antioxidant system in liver treated with carbontetrachloride is normalized by the protective effect of *L. adoensis* extract.

*Lippia adoensis*pretreatment of CCl_4 treated mice significantly reduced the elevated levels of ALT, AST, ALP and bilirubin. The diminished levels of these serum biomarkers may be attributed to the stabilizing effect of the *Lippia adoensis* phytochemical constituent(s) and various active ingredients on the plasma membrane of the hepatocytes, probably brought about by the stimulation of hepatocellular protein synthesis and ability to induce microsomal enzymes either by accelerating the excretion of CCl_4 or by inhibition of oxidative stress induced by CCl_4(Farhan *et al*., 2012; Sagar *et al*., 2012).

Food rich in plant bioactive compounds and polyphenols and flavonoids in particular may exert beneficial effects towards human health (Francesco *et al.,* 2011). Health benefits of phytochemicals, especially antioxidant properties of phenolic compounds, which is known to exert preventive activity against degenerative diseases, inflammation and allergies via antioxidant, proteins andenzymes neutralizationor modulation mechanisms (Tulay *et al*., 2014).

Among plants containing natural antioxidants, *Lippia adoensis* has attracted particular interest due to a high content of biologically active compounds (Riot*et al., 2005*). It has been considered to play an important dietary antioxidant role in the prevention of oxidative damage in living system (Tadewos *et al., 2014*).

CCl_4 induced hepatic damage observed was confirmed by histopathological examination of the CCl_4 treated mice which revealed severe hepatic degeneration and marked foci of mononuclear infiltration of hepatic parenchyma tissue and around central vein, disorganization of hepatic plates and tissue necrosis (Snehal *et al*., 2014).

Carbontetrachloride is a widely used experimental hepatotoxicant biotransformed by cytochrome P-450 system to produce trichloromethyl free radical, which in turn covalently binds to cell membranes and organelles to elicit lipid peroxidation, disturb Ca^{2+} haemostasis and finally result in cell death (Muhammad *et al.,* 2014). Liver damage induced by carbontetrachloride is a classical model for screening the hepatoprotective activity (Showket *et al.,* 2014).

Histopathological findings of liver samples were in agreement with the results obtained in serum biochemical studies, indicating that *L. adoensis* extract is able to protect carbontetrachloride induced hepatotoxicity. Phenolics and flavonoids display a wide range of biological and pharmacological properties, and they normally scavenge the free radicals and play an essential role in preventing oxidative stress (Kutaiba *et al.,* 2014).

The outcome of the present investigation indicates that treatment with *L. adoensis* was effective in inhibiting the hepatotoxic effect of carbontetrachloride in vivo models, most likely because of high content of flavonoids, alkaloids, phenolics, tannins and saponins and active principle (Amita *et al.,* 2014). However, the precise molecular mechanism by which *L. adoensis* mediates its hepatoprotective action is still not clear. In the present study, Carbontetrachloride treatment produced centrizonal necrosis (zone 3), marked foci of mononuclear infiltration to hepatic parenchyma tissue, sinusoid and around central vein, disorganization of hepatic plates (Gayathiri *et al.,* 2012).

Pretreatment with *Lippia adoensis* extract protected hepatic architecture and liver tissue frommarked foci of mononuclear infiltration of hepatic parenchyma tissue, sinusoid and around central vein, as well as disorganization of hepatic plates and tissue necrosis, by preventing the toxic chemical reaction, oxidative stress, molecular changes in the liver tissues ultimately leading to necrosis. Similar reports were observed from some other plants including*veronica ciliatafisch* (Li *et al.,* 2014*), Mung Bean* (Norlaily *et al.,* 2013), *Deinococcus radiodurans*(Cheng *et al.,* 2014) and*Lumnitzera racemosa* (Sundaram and Murugesan, 2011).

Histopathological changes indicating liver damage after CCl_4 administration has been reported in the previous findings that CCl_4 causes necrosis (Sampath *et al*., 2013), fibrosis (Jamilah *et al.,* 2014), mononuclear cell infiltration (Jamilah *et al*., 2014), steatosis and degeneration of hepatocytes, in the liver and also CCl_4 causes apoptosis in liver (Sampath *etal.,* 2013). Therefore, histopathological findings in the liver due to CCl_4 administration are in agreement with previous studies.

Hepatoprotective drugs may play a role in the process of regeneration, prevention of fibrosis, or formation of nodules which may be expressed in the long termuse of the drug (Elsohafy *et al.,* 2013). This study, however, showedmarked foci of mononuclear infiltration to hepatic parenchyma tissue, sinusoid and around central vein, disorganization of hepatic plates and necrosis by hepatotoxic (CCl_4) and prevention of such changes and restoration to normal by *Lippia adoensis* extracts. In conclusion, the present results show that *Lippia adoensis* leaves ethanolic and aqueous extracts at the doses of 200mg/kg and 400mg/kg have potential hepatoprotective effect.

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References

[1] Abegaz, B., Asfaw, N. and Lwande, W. Constituents of essential oil from wild and cultivated *Lippia adoensis, J. Essent. Oil Res*.1993; 5: 487-491.

[2] Alaezi CM, Adisa JO, Okorochi EC and Okechi OO. A progressive Nuclear stain substitute for haematoxylin And eosin staining technique in the Demonstration of invasive ductal Carcinoma. *GJSR Journal*.2014; 2 (2): 51-55.

[3] Amita Jain, Rashmi Ranade, Prem Pritam, Neelu Joshi, Sirisha Lakshmi Vavilala, Ankita Jain. A comparative study of antioxidant activity, total phenolic and flavonoid contents in different parts of *Helicteres isora*. *L. American journal of life sciences*.2014; 2 (5): 292-302.

[4] Andréia Assunção Soares, Anacharis Babeto de Sá-Nakanishi, Adelar Bracht, Sandra Maria Gomes da Costa, Eloá Angélica Koehnlein, Cristina Giatti Marques de Souza and Rosane Marina Peralta. Hepatoprotective effects of *mushrooms*. Molecules.2013; 18: 7609-7630.

[5] Asres K and Bucar F. Lippia adoensisvar. Adoensis: studies on the essential oil composition and antioxidant activity, *Ethiop. Pharm. J.*2002; 20:32-38.

[6] Asres, K, Bibbons, W, David, P. and Polo, M. Alkaloids of Ethiopian *Calpurniaaurea*, subsp. aurea, *Phytochemistry.* 1986; 25 (6): 1443-1447.

[7] Bhaargavi, Vuyyuri, G. S. L. Jyotsna, and Reshma Tripurana. "A review on hepatoprotective activity." *International Journal of Pharmaceutical Sciences & Research*. 2014; 5 (3): 690-702.

[8] Bhaskara KL and Mukalel SL. Curative efficacy of methanolic extract of Monochoria vaginalis against Carbontetra chloride (CCl4) induced liver fibrosis. *Journal of Pharmacy and Biological Sciences (IOSR-JPBS).* 2014; 9 (1): 71-76.

[9] Bimlesh Kumar, Harleen Kaur Sandhar, Sunil Prasher, Prashant Tiwari, Manoj Salhan, Pardeep Sharma. Phytochemistry and Pharmacology. Internationale Pharmaceutica Sciencia.2011; 1 (1): 25-41.

[10] Cheng, Jianhui, Zuofa Zhang, Zhiguo Zheng, Guoying Lv, Liangyan Wang, Bing Tian, and Yuejin Hua. "Antioxidative and Hepatoprotective Activities of Deinoxanthin-Rich Extract from *Deinococcus radiodurans* R1 against Carbon Tetrachloride-Induced Liver Injury in Mice. " *Tropical Journal of Pharmaceutical Research* 2014; 13 (4): 581-586.

[11] Comfort JA, Augusta AE, Gbadebo EA, Margaret OOand Emmanuel NM. N-Nitrosation of dimethylamine hydrochloride and its toxicology in the wistar rats fed different levels of dietary protein. *European Journal of Experimental Biology.* 2013; 3 (2): 94-103.

[12] David EK, Naga PC, William ML. Hepatic histological findings in suspecteddrug-induced liver injury. Hepatology.2014; 59 (2): 661–670. doi:10.1002/hep.26709.

[13] Douhri B., Idaomar M, Skalisenhaj N, Ennabili A, Abrini J. Hepatoprotective effect of *Origanum elongatum*. *European journal of medicinal plants 2014;* 4 (1): 14-28.

[14] El Sohafy, Samah M., Saleh I. Alqasoumi, Aly M. Metwally, Abdallah A. Omar, Masouda M. Amer, Mohammed I. Abou Shoer, S. A. El Toumy, and Maged S. Abdel-Kader. "Evaluation of the hepatoprotective activity of some plants belonging to the tribe Cynareae growing in Egypt. " *Journal of Medicinal Plants Research.* 2013; 7 (7): 324-328.

[15] Farhan H, Rammal H, Hijazi A, Hamad H, Daher A, Reda M, Badran B. In vitro antioxidant activity of ethanolic and aqueous extracts from crude *malva parviflora*. Grown in lebanon. *Asian J Pharm Clin Res*.2012; 5 (3): 234-238.

[16] Francesco Visioli, Catalina Alarcón, Cristina Andres, Michael Aviram, Conceição Calhau, Alfredo Cassano, Massimo D'Archivio, Ana Faria, Gaëlle Favé, Vincenzo Fogliano, Rafael Llorach. Polyphenols and human health. *Critical Reviews in Food Science and Nutrition*.2011; 51: 524–546.

[17] Gayathiri S, Vetriselvan S, Shankar J, Ishwin S, Hemah D, Shereenjeet K, Yaashini A. Hepatoprotective activity of aqueous extract of *hippophae rhamnoides.* International *journal of biological & pharmaceutical research.* 2012; 3 (4): 531-537.

[18] Isha I. Sait, Jyoti Harindran, A. Abdul Vahab, Jeena J. L, Nasli S. Jolly John. Potential hepatoprotective effect and antioxidant role of methanol extract of morinda tinctoria in carbon tetrachloride induced hepatotoxicity in albino rats. *Int J Pharm* 2014; 4 (1): 363-368.

[19] Iyanda A A and Adeniyi F A. Buffer conditions for measuring alkaline phosphatase activity in Indices of Hepatorenal Function. *J Phys Pharm Adv*. 2014; 4 (1): 310-317.

[20] Jamilah M Hashemi. Hibiscus Sabdariffa Calyx Extract alleviate hepato toxicity induced by carbon tetrachloride on male albino rats. *NatSci.* 2014; 12 (6): 111-120.

[21] Kadejo OA, James DB, Sallau BA, Ozigi A A. In vivo antioxidant effect of aqueous root bark, stem bark and leaves extracts of Vitex doniana in CCl_4 induced liver damage rats. *Asian Pac J Trop Biomed*. 2013; 3 (5): 395-400.

[22] Kanwal Z, Muhammad IS, Muhammad AM. Effect of *Momordica charantia* (bitter melon) on serum glucose level and various protein parameters in acetaminophen intoxicated rabbits. *J Intercult Ethnopharmacol.*2012; 1 (1): 7-12.

[23] Kutaiba Ibrahim Alzand, Mohamed Ali Abdullah, Mukul Tailang. Structural elucidation and biological activity of the flavonoid from the roots of cudrania tricuspidata. *World Journal of Pharmaceutical Research*. 2014; 3 (10): 981-997.

[24] Li Yin, Lei Wei, Rao Fu, Lisheng Ding, Yiran Guo, Lin Tang and Fang Chen. Antioxidant and hepatoprotective activity of *veronica ciliata fisch* Extracts against carbon tetrachloride-induced liver injury in mice. Molecules.2014; 19: 7223-7236.

[25] Maliha AAM. Phenoli. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transminases. *Journal of Food and Nutrition Sciences.*2014; 2 (5): 207-214.

[26] Manuj Kr. Bharali, Hage Konya, Luk Bahadur Chetry. Protective effect of *Oroxylum indicum* on acetaminophen induced liver injury in rat. *International Current Pharmaceutical Journal, January* 2014, 3 (2): 223-227.

[27] Mary Tolulope Olaleye, Ayodeji Emmannuel Amobonye, Kayode Komolafe, Afolabi Clement Akinmoladun. Protective effects of *Parinari curatellifolia* flavonoids against acetaminophen-induced hepatic necrosis in rats. *Saudi Journal of Biological Sciences.*2014; 21: 486–492.

[28] Mohamed STS, Madhusudhana C, Chetty S, Ramkanth V. S. T, Rajan KM. Hepatoprotective herbs. *Int. J. Res. Pharm* Sci. 2010; 1: 1-5.

[29] *Muhammad Imran, Muhammad Ali, Muhammad Ali*, Mohammad Saleem and Muhammad Hanif. Hepatoprotective effect of leaves of aqueous ethanol extract of*Cestrum nocturnum*. *Bangladesh J Pharmacol* 2014; 9: 167-170.

[30] NorlailyMohd Ali, HamidahMohd Yusof, Kamariah Long, Swee Keong Yeap, Wan Yong Ho. Antioxidant and Hepatoprotective Effect of Aqueous Extract of *Germinated* and *Fermented Mung* Bean on Ethanol-Mediated Liver Damage. *Bio Med Research International*. 2013; 9.

[31] Riot Sishu, Mariamawit Yonathan, Ameha Seyoum and Kaleab Asres. Radical scavenging activity of volatile oils of herbs traditionally used to spice cooking butter in Ethiopia. *Ethiopian Pharmaceutical Journal.*2005; 23: 7-14.

[32] Sabreena Rashid, Zahoor A Kaloo, Seema Singh & Iram Bashir. Medicinal importance of Genus Rheum onhepatoprotective, *International Journal of Advanced Research*. 2014; 2 (7): 261-267.

[33] Sagar D, Varsha Z, Dinesh D, Shital P. Hepatoprotective activity of Alcoholic leafextract of *Alianthus exelssa* root against carbon tetrachloride toxicity induced in albino rat. *Int. J. PharmTech Res.* 2012; 4 (2).

[34] Sampath Kumar GV, Sri Vidya VVL, Ch. Sandhya. Hepatoprotective activity of *borreria hispida l.* Roots against carbon tetrachloride induced hepatotoxicity in albino wistar rats. *International Journal of Biological & Pharmaceutical Research.* 2013; 4 (12): 1277-1282.

[35] Senthilkumar, Ravichandran, Rahul Chandran, and Thangaraj Parimelazhagan. "Hepatoprotective effect of *Rhodiola imbricata* rhizome against paracetamol-induced liver toxicity in rats. " *Saudi journal of biological sciences.*2014; 21 (5): 409-416.

[36] Showket Hussain Bhat, Rajni Shrivastava, Mohd Yousuf Malla, Mohd Iqbal, Tahira Anjum, Abrar Hussain Mir, Manik Sharma. Hepatoprotective activity of *argemone mexicanalinn* against toxic effects of carbon tetrachloride in rats. *World Journal of Pharmaceutical Research*.2014; 3 (3): 4037-4048.

[37] Singh, A, BhatTK, Sharma OP. Clinical biochemistry of hepatotoxicity. *J Clinic Toxicology.*2011; S4:001. doi:10.4172/2161-0495. S4-001.

[38] Snehal Gite, Supriya Yadav, Smita Nilegaonkar, Vaishali Agte. Evaluation of hepatoprotective potential of functional food formulations using in vitro and in vivo models of CCl_4 radical induced toxicity. *International Journal of Interdisciplinary and Multidisciplinary Studies (IJIMS),* 2014: 1 (10) 6-13.

[39] Sundaram R, Murugesan G. Hepatoprotective and antioxidant activity of a mangrove plant *Lumnitzera racemosa. Asian Pac J Trop Biomed. 2011; 1 (5): 348-352.*

[40] Sunita Shailajan, Mayuresh Joshi, Bhavesh Tiwari. Hepatoprotective activity of Parmelia perlata (Huds.) Ach. against CCl_4 induced liver toxicity. *Journal of Applied Pharmaceutical Science.* 2014; 4 (02): 070-074.

[41] Tadewos H, Abadi G, Henok K. Evaluation of physico-chemical, microbial and organoleptic properties of cow milk in smoked container with olive (*Olea africana*) and added leaves of *Lippia adoensis*. *American Journal of Food Science and Nutrition*.2014; 1 (2): 29-37.

[42] Toshihiro, A., Gebreyesus, T., Hiroshi, H. and Toshitake, T. Co-occurrence of C- 24 Epimeric 24-ethylsterols Possessing and Lacking a 25 bond in Kalanchoe petitiana, Phytochemistry.1992; 31: 163-166.

[43] Tulay Ozcan, Akpinar BA, Yilmaz EL, and Delikanli B. Phenolics in Human Health. *International Journal of Chemical Engineering and Applications*.2014; 5 (5): 393-396.