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The modulatory effect of methanol extract of *Piper guineense* in CCl_4-induced hepatotoxicity in male rats

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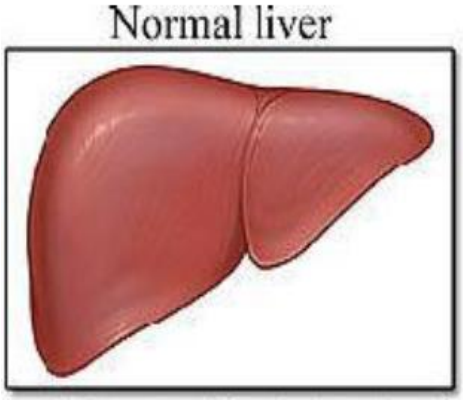
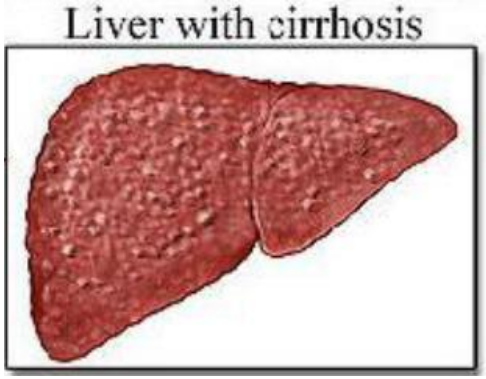
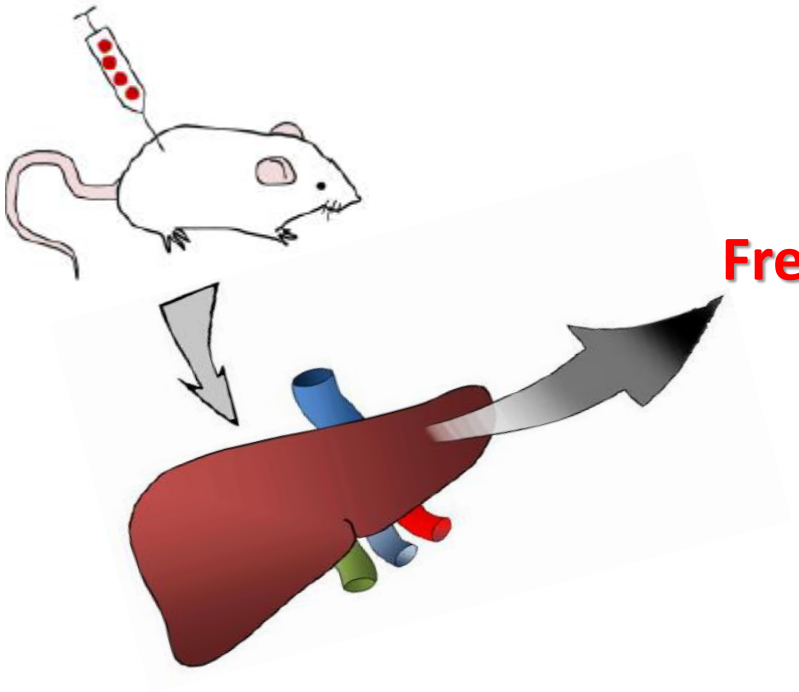


Introduction

The current increase in the incidence and prevalence of liver diseases remain a major globally health burden (Williams, 2006; Suryaprasad *et al*., 2014).

Liver plays an essential role in metabolism and excretion of xenobiotics from the body (Huang *et al*., 2012).





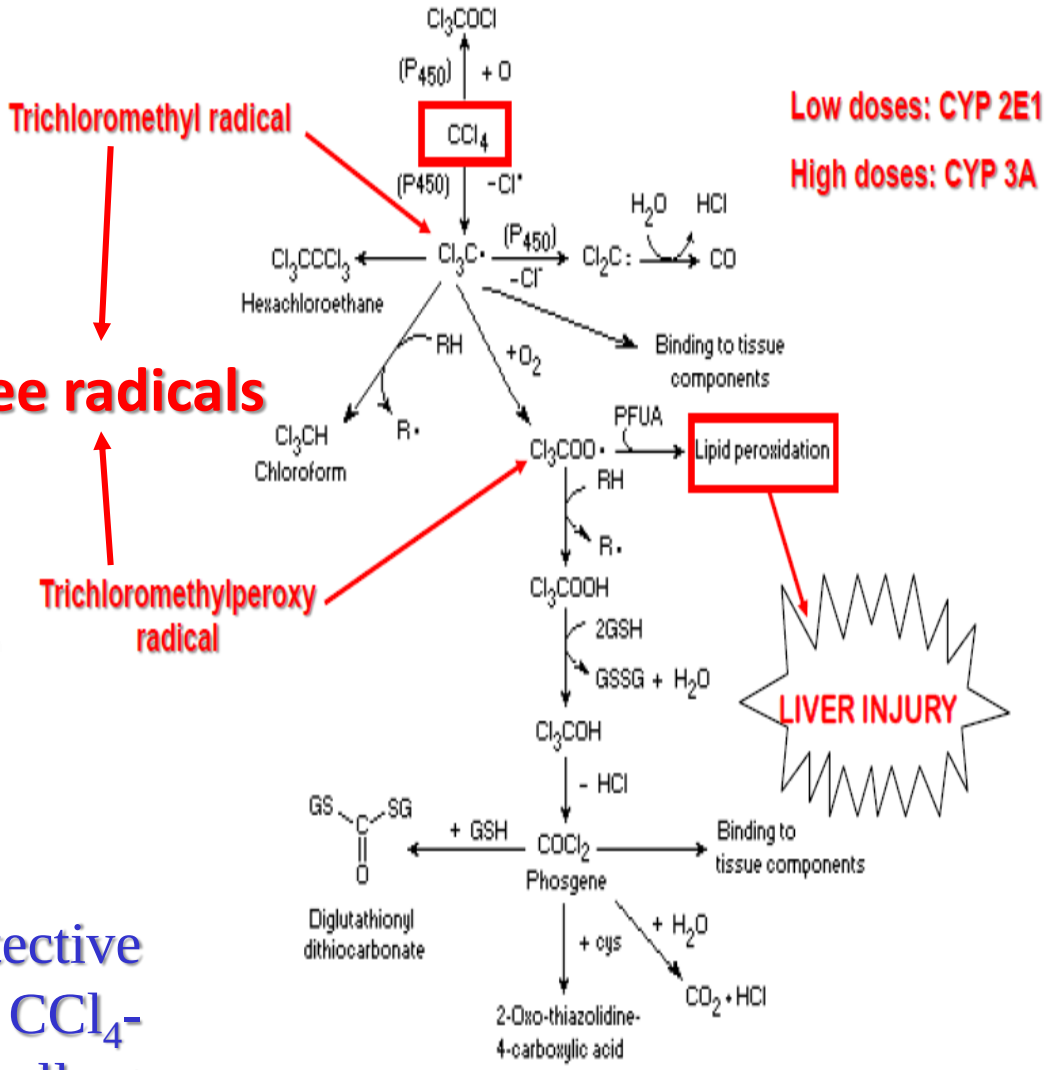
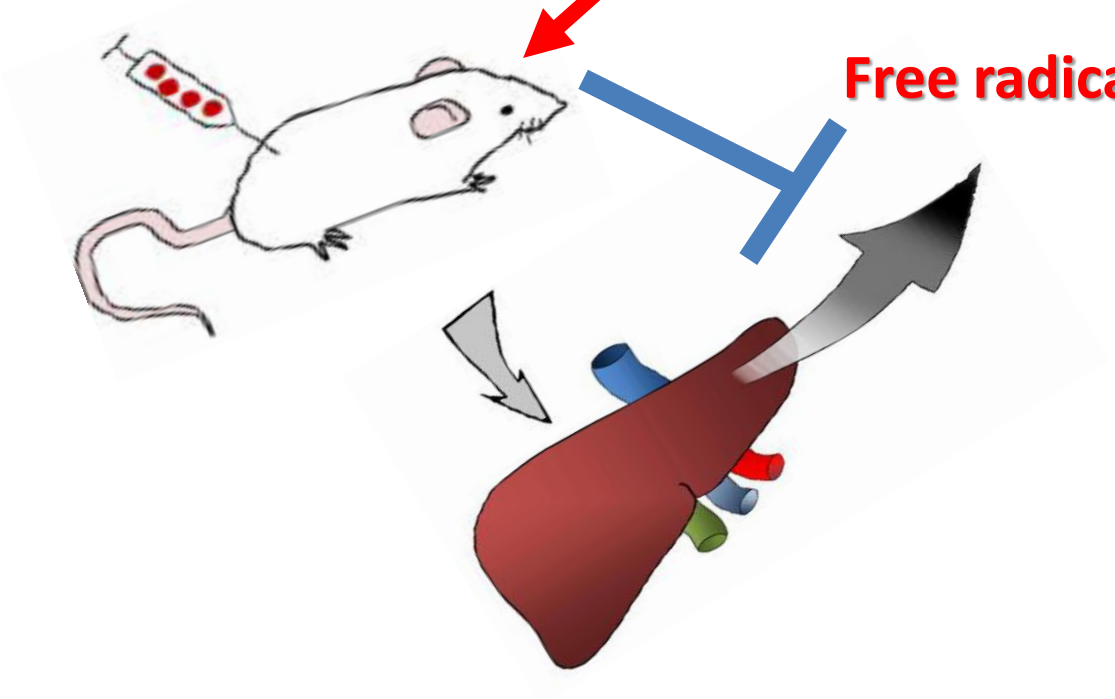


Fig. 1. Biotransformation of carbon tetrachloride (From Harris & Anders, 1981; Anders & Jakobson, 1985; McGregor & Lang, 1996)

To ascertain the hepatoprotective effect of drugs during screening, CCl_4-induced hepatic injury is an excellent model commonly used (Huang *et al*., 2012).


Piper guineense


CCl4


Free radicals

Aim of the study

This study was designed to assess the possible protective mechanism of *Piper guineense* (PG) against CCl_4-induced hepatotoxicity and its potential role in inhibition of oxidative stress in animal-model.

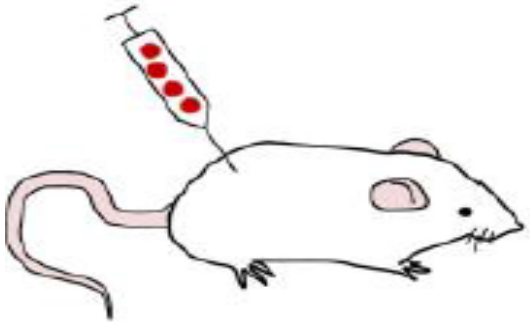
MATERIALS AND METHODS

Chemicals

All the chemicals and reagents were purchased from Sigma Chemical (St Louis, MO, USA) and Merck (Germany). Randox assay kits were used.

Plant material and extract preparation

One kilogram of seeds was coarsely powdered using an electric blender and macerated with 80 % methanol (200 g/ 1000 ml) with intermittent stirring for 72 hours.



Animals and experimental design

Thirty Male Wistar albino rats (weighing 180 - 200g) were divided into five groups and classified into; control, CCl_4, pre-treatment (T_1), post-treatment (T_2) and standard drug (T_3).

Preparation of blood and tissue homogenates for biochemical analyses and histological examination

- Blood samples were collected and allowed to coagulate at room temperature

- Liver samples were quickly excised and washed in ice-cold 1.15 % KCl solution

- homogenized in 4 volumes of 56 mM Tris/HCl buffer (pH 7.4)

- Small pieces of liver sections were fixed in 10 % formal saline for histopathological examinations.

Biochemical assays

- Ferric reducing antioxidant potential (FRAP) as discribed by Benzie and Strain, 1996.

- Oxygen radical absorption capacity (ORAC) as discribed by Ou *et al*., 2001 .

- AST, ALT and ALP as discribed by Reitman and Frankel, 1957.

- Serum total proteins concentration was determined according to the method of Henry (1964), total cholesterol and triglyceride were evaluated by routine enzymatic methods using Randox commercial kits.

- Lipid peroxidation was determined by measuring the thiobarbituric acid reactive substances (TBARS) following the method of Varshney and Kale (1990).

- GSH levels were estimated using the method described by Beutler et al (1963).

- GST activity was determined according to Habig et al., (1974).

- The level of SOD activity was determined by the method of Misra and Fridovich (1972).

- Catalase (CAT) activity was determined by adopting the method described by Sinha, 1972.

Statistical analysis

The values were presented as means ± SD of different groups. Differences between the mean values were estimated using one-way analysis of variance (ANOVA). The results were considered statistically significant when $p < 0.05$.

Results and Discussion

Table 1. Antioxidant capacity of *Piper guineense* methanol extract

Parameter	Activity in *Piper guineense*
FRAP (μmol AAE/mL)	693.01 ± 0.28
ORAC (μmol TE/mL)	207.41 ± 0.16

Ferric reducing antioxidant potential (FRAP) and oxygen radical absorption capacity (ORAC)

Table 2. The effect of *Piper guineense* on Indices of hepatotoxicity

Groups	AST activity (U/L)	ALT activity (U/L)	ALP activity (U/L)
Control	34.84 ± 1.12^{b}	21.35 ± 0.18^{b}	$109.87 \pm 0.43^{a,}$
CCl_4	56.12 ± 1.08^{a}	29.09 ± 0.12^{a}	145.88 ± 0.38^{b}
T_1	$42.55 \pm 1.51^{a,b}$	21.87 ± 0.13	117.72 ± 0.08
T_2	51.96 ± 1.36^{a}	23.90 ± 0.71	138.09 ± 0.18^{a}
T_3	$44.70 \pm 1.34^{a,b}$	23.34 ± 0.16	119.09 ± 0.51

Values shown are mean ± S.D. (n = 6). Mean differences are significant ($P < 0.05$) when compared with: [a] control group, [b] CCl_4 only

Table 3. Effect of *Piper guineense* on serum total protein, total cholesterol and triglyceride levels

Groups	Total protein (g/dl)	Total cholesterol (mg/dl)	Triglycerides (mg/dl)
Control	29.26 ± 0.23^{b}	142.44 ± 0.26^{b}	46.64 ± 0.14^{a}
CCl_4	23.49 ± 0.36^{a}	261.22 ± 0.20^{a}	98.42 ± 0.82^{b}
T_1	$34.36 \pm 0.36^{a,b}$	$189.66 \pm 0.02^{a,b}$	49.19 ± 0.11
T_2	27.51 ± 0.14	$223.03 \pm 0.16^{a,b}$	63.98 ± 0.28^{a}
T_3	32.77 ± 0.27	$201.86 \pm 0.23^{a,b}$	$51.21 \pm 0.24^{a,b}$

Values shown are mean ± S.D. (n = 6). Mean differences are significant ($P < 0.05$) when compared with: [a] control group, [b] CCl_4 only

This result is an indication that there was inhibition in protein metabolism in the liver.

In like manner, since the liver is also responsible for the metabolism of hormones and lipid (cholesterol, triglycerides, and high-density lipoproteins), it is expected that might be disturbance in total cholesterol (TC) and triglycerides (TG) metabolism.

Therefore, the increase witness in serum concentration of TC may be attributed to inhibition of cholesterol catabolism or mobilizations of fatty acids from adipose tissues by lipolysis while the increase in serum TG level may be due to increased biosynthesis or dismissed clearance of TG from the blood

Table 4. Effect of *Piper guineense* on assessment of oxidative stress

Groups	LPO	GSH	GST	SOD	CAT
Control	13.35 ± 1.45^{b}	41.21 ± 0.41^{b}	19.12 ± 1.89^{b}	7.09 ± 0.11^{b}	24.21 ± 0.71^{b}
CCl_4	28.40 ± 1.82^{a}	28.26 ± 0.26^{a}	12.61 ± 1.08^{a}	3.41 ± 0.26^{a}	16.79 ± 0.86^{a}
T_1	19.14 ± 0.89^{a}	37.56 ± 0.43^{b}	18.86 ± 1.13^{b}	7.23 ± 0.16^{b}	23.61 ± 0.53^{b}
T_2	23.92 ± 1.14^{a}	35.05 ± 0.33^{b}	15.08 ± 1.79	5.91 ± 0.13	19.20 ± 0.29
T_3	17.69 ± 1.86^{a}	37.34 ± 0.21^{b}	19.59 ± 1.46^{b}	7.01 ± 0.08^{b}	21.44 ± 0.12^{b}

Values shown are mean ± S.D. (n = 6). Mean differences are significant ($P < 0.05$) when compared with: [a] control group, [b] CCl_4 only

The elevation observed in the hepatic levels of TBARS is an indication of induction of lipid peroxidation.

It is generally accepted that antioxidant enzymes are the first line of defence in response to oxidative challenges in order to protect cellular integrity and the pathogenesis of various diseases.

During oxidative stress, depending on the extent of disturbances in the normal redox state within the cells, there is an overwhelming effect on the status of the enzymatic antioxidant enzymes notably SOD, CAT, GST and GPx as well as the non-enzymatic antioxidant GSH, due to overproduction of free radicals.

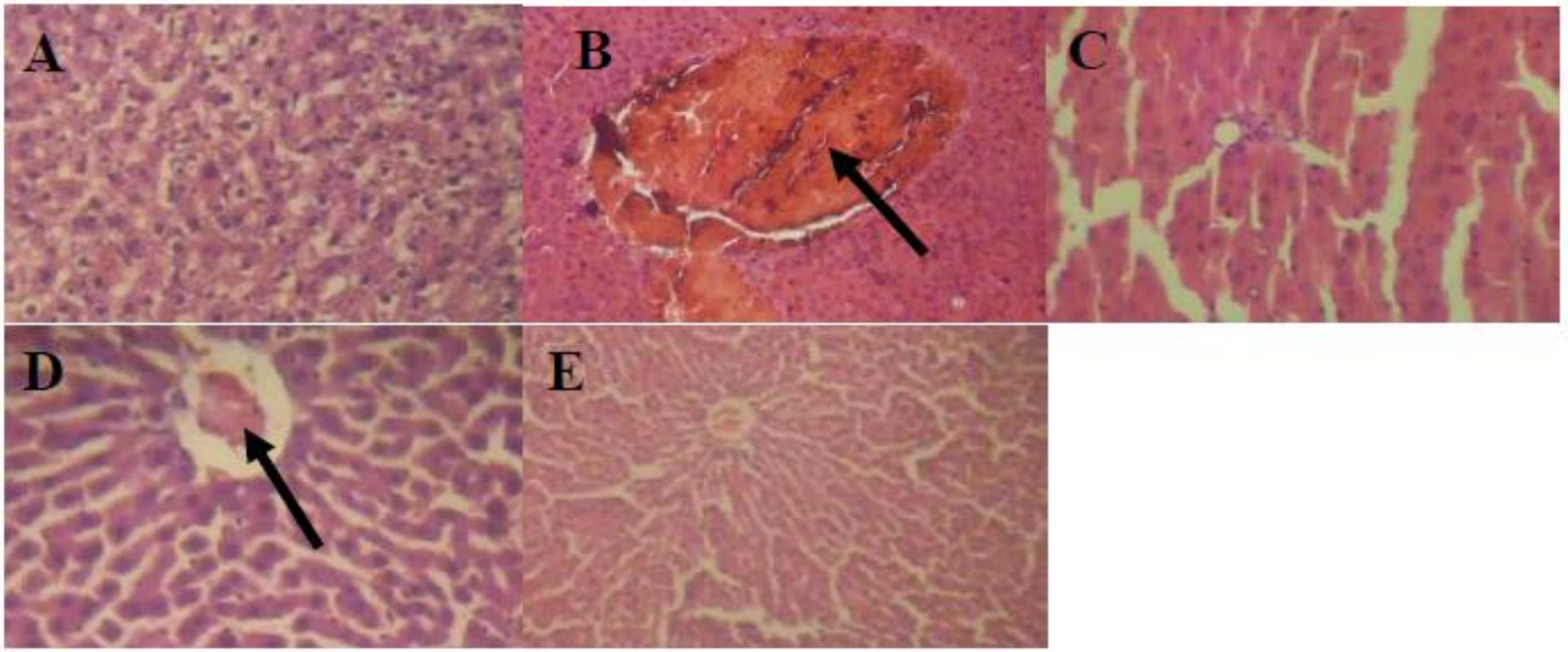


Figure 1. Histological examination of rat livers stained with hematoxylin and eosin (H&E).

(A) Control: showing normal hepatic architecture, with no lesions or abnormalities;

(B) CCl_4: showing congestion in central vein associated with infiltration of inflammatory cells;

(C) T_1: showing mild hepatocytes necrosis and mononuclear cellular infiltration;

(D) T_2: showing mild portal tract and lobular chronic inflammation with focal hepatocyte destruction

(E) T_3: showing improved hepatic architecture, inflammatory cell infiltration and hepatocytes necrosis were hardly detected. (×400)

Conclusion

Obtained results from this study demonstrates that extracts from *P. guineense* possess antioxidant and hepatoprotective properties comparable to that of Livolin forte with better efficacy when pre-treated with 400 mg/kg bw 14 days prior to CCl_4-exposure in animal model.

We propose that the possible mechanism, by which PG brought about the observed changes in the present study, may be due to synergistic interactions of its bioactive components.


THANKS
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