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Hepatoprotective activity of Tridecan-1-ol isolatedfrom *Flaveria trinervia* (Speng). C. Mohr

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Abstract

Background- *Flaveria trinervia* (Asteraceae), a medicinal herb has been used in Indian traditionl medicine to cure jaundice. Reports on phytochemical and pharmacological investigations are very scarce. A phytoconstiuent Tridecan-1-ol and screened for hepatoprotective activity. **-**To evaluate hepatoprotective effects of Tridecan-1-ol against CCl_4 induced hepatic damage on Wister albino rats.

Methods- Tridecan-1-ol was isolated from petroleum extract of *Flaveria trinervia* using silica gel column chromatography and the structure was confirmed by IR, MASS,and ^1H NMR spectroscopic studies. The parameters employed to confirm the hepatoprotectivity were the estimation of liver function serum markers such as total bilirubin, total protein, alanine transaminase, aspartate transaminase, alkaline phosphatase activities and the histological profile of the liver tissue.

Results-The LD_{50} of petroleum ether extract and the constituent Tridecan -1-ol was found to be 500 and 150 mg/kg body weight respectively. Tridecan -1-ol showed significant hepatoprotective activity than the crude extract and it was compared with the standard hepatoprotective drug silymarin. The histological profile of the liver tissue ofTridecan-1-ol administered rats showed the presence of normal hepatic cords, absence of necrosis and fatty infiltration as similar to the controls.

Conclusion-Tridecan-1-ol afforded significant hepatoprotectivity against CCl_4 induced liver damage and supported the traditional claim of *Flaveria trinervia* as a hepatoprotective herb.

Keywords: *Flaveria trinervia*; Hepatoprotective; Tridecan -1-ol; CCl_4

1. Introduction

Liver is the largest organ in the body serves as a site of serum enzyme synthesis, cleaning station for toxins and erythrocyte ghosts. It is well known that CCl_4 induced a substantial increase in steatosis and fibrosis and leads to lethal cirrhosis of the liver[1]. In indigenous system of medicine several plants and phytoconstituents were known to act as potent hepatoprotective drugs for healing jaundice and their therapeutic property was evaluated by many investigators using animal models such as *Wedelia chinensis* [2][3][4][5].

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Flaveria trinervia(Spreng).C.Mohr.(Asteraceae) is as a potent hepatoprotective plant used to cure liver disorders by the traditional practitioners of Karnataka, India and its curative property is more appreciable against liver cirrhosis [6] studied the antiprotozoal activity of the crude extracts to treat gastrointestinal infections and were screened against *Entamoeba histolytica and Giardia lamblia axenic trophozoites.* So far, no reports are available on the pharmacological screening of the phytoconstituents of this species. This paper deals with the potential hepatoprotective effect of the petroleum ether extract and its constituent Tridecan-1-ol against CCl_4-induced hepatic damage in rats.

2. Material and methods

2.1. Plant material

The plant *Flaveria trinervia* was collected from the agricultural fields of Davanagere district of Karnataka, India. Plant was authenticated by comparing with the voucher specimen deposited in Kuvempu University, Shankaraghatta, Karnataka, India [7].

2.2. Isolation of Phytoconstituent

The plant material was shade dried, powdered mechanically and was subjected for soxhlet extraction using petroleum ether(40-60°Cb.p) for about 48 cycles. The extract was filtered and concentrated in vacuum under reduced pressure using rotary flash evaporator (Buchi, Flawil, Switzerland) and allowed it for complete evaporation of the solvent. Crude petroleum ether extract was subjected to qualitative studies for the detection of phytochemical groups. TLC studies showed the separation of a single compound with the solvent system Hexane: Chloroform in the ratio of 9:1. Using the same solvent proportion the active constituent was separated by column chromatography (180g silica gel of 60-120 mesh, 80x2cm), fractions were collected at the intervals of 5min and were monitored by TLC to check purity of constituent. The compound was re-crystallized in Hexane and the yield was about 74mg/5gms of petroleum ether crude extract. The characterization of the isolated compound was carried bysubjecting it to IR, MASS and ^{1}HNMR spectral studies.

2.3. Animals

Male Wistar albino rats weighing 180-200g and albino mice weighing 20-25g were procured from Central Animal House, NGSM Institute of Pharmaceutical Sciences, and were maintained at standard housing condition. The animals were fed with commercial diet (PranavAgro IndustriesLtd., Sangli) and waterad-Libitum during the experiment. The Institutional Animal Ethical Committee (Reg.No.NGSMIPS/IAEC/DEC-2020/217) permitted the study.

2.4. Acute Toxicity studies

Acute toxicity study was conducted according to"staircase"method (Ghosh1984).

2.5. Drug Formulation

LD_{50}ofthepetroleumetherextractandtheisolatedconstituent Tridecan-1-ol for oral suspensions were found to be 500mg/kg and 150mg/kg body weight respectively. One tenth of these doses (50 and 15mg/kg, body weight respectively) was selected as the therapeutic dose for the evaluation of hepatoprotective activity. Drug was prepared in gum tragacanth (1%w/v) in distilled water [8].

2.6. Induction of hepatotoxicity

Rats were divided into five groups(*n*=6). Group I (control) animals were administered a dose of water (1ml/kg body weight, p.o.) and received liquid paraffin (1mL/kg,s.c.) daily for 14days. Group II received water(1 ml/kg body weight, p.o.) and received CCl_4 : liquid paraffin (1:1,1ml/kg body weight, S.C.) once daily for 10days. Group III received standard drug silymarin (50mg/kg,p.o.) once daily for 14days. Test group animals Groups IV were administered orally a dose of 50mg/kg b.wt. , of petroleum ether extracts and Group V animals were administered orally a dose of 15mg/kgb.wt., of isolated phytoconstituent Tridecan-1-ol respectively, in the form of aqueous suspension once daily. The Groups III–V animals were administered concomitantly CCl_4 : liquid paraffin (1:1,1ml/kg body weight, s.c.) after 30min of administration of the Silymarin, crude extract and Tridecan-1-ol respectively. The drugs were administered concomitantly for 14 days. Animals were sacrificed after 3hrs of the last treatment under light ether anesthesia. The blood samples of each animal were collected separately by carotid bleeding into sterilized dry centrifuge tubes and allowed to coagulate for 30min at 37 C. The clear serum was separated by centrifugation at 2500 rpm for 15min and subjected to biochemical investigations. Liver was dissected and was used for histopathological studies.

2.7. Biochemical determinations

The biochemical tests conducted were consists of liver function parameters like determination of total bilirubin [9]. Total protein, alanine and aspartate amino transferase [10], serum alkalinephosphatase[11].

2.8. Histopathological studies

The liver tissue was dissected out from the animals of each group after draining the blood, washed with the normal saline and fixed in 10% formalin. The tissue was dehydrated in ethanol-xylene series and embedded in paraffin. Sections of 5 m thickness were prepared, processed, stained with alum-haematoxylin and eosin (H–E) dye and observed under microscope. The histological changes including cell necrosis, fatty change, hyaline regeneration, ballooning degenerations were evaluated.

2.9. Statistical analysis

The results of biochemical estimations were expressed as mean ± S.E.M of six animals in each group. The statistical analysis was carried out using one way ANOVA. The difference in values at P 0.05 was considered as statistically significant.

3. Results

3.1. Isolation of phytoconstituent Tridecan-1-ol.

The yield of petroleum ether extract was 26.0gms/kg of the crude. The qualitative phytochemical evaluation showed the presence of glycosides, favonoids, steroids, triterpenes and fatty oils. By column chromatographic technique a fatty acid alcohol was isolated using the solvent system hexane : chloroform in the ratio 9:1. Physically the compound is a white amorphous and exhibited pleasant straw berry odour. Its R_f value is 0.42 and melting point is 67-70°C. The molecular formula and chemical structure of the compound was established with the help of following spectroscopic data. The IR spectrum of the isolated compound showed the presence of hydroxyl group by exhibiting a broad peak at 3445.18cm^{-1}. The peak in the region 1651.22cm^{-1} is for a methyl group and 1005.00cm^{-1} for secondary alcoholic group (Fig1). The molecular weight of the compound was confirmed from its APCI-MASS positive mode spectrum and it showed a pseudo molecular ion at m/z 169(Fig2). The ^{1}HNMR spectra showed the presence of peak at 4.72 is due to presence of CH_2OH group (Fig3). Based on FAB-Mass spectral studies the molecular formula was deduced as $C_{13}H_{28}0$ and the compound is characterized as Tridecan-1-ol with the following structure (Fig.4). Most of the essential oils were found to be rich in tridecan derivatives and its esters are characterized by the presence of pleasant straw berry odour. The constituent Tridecan-1-ol isolated and characterized from *Flaveria trinervia* for the first time, however, its presence was reported in many plants which were belongs to different families viz. *Pilocarpus pennatifolius* [12], *Passiflora incarnate*[13], *Acmella radicans*[14] *Aloe ferox*[15], *Allium tuberosum* [16] and *Cichorium intybus*[17].

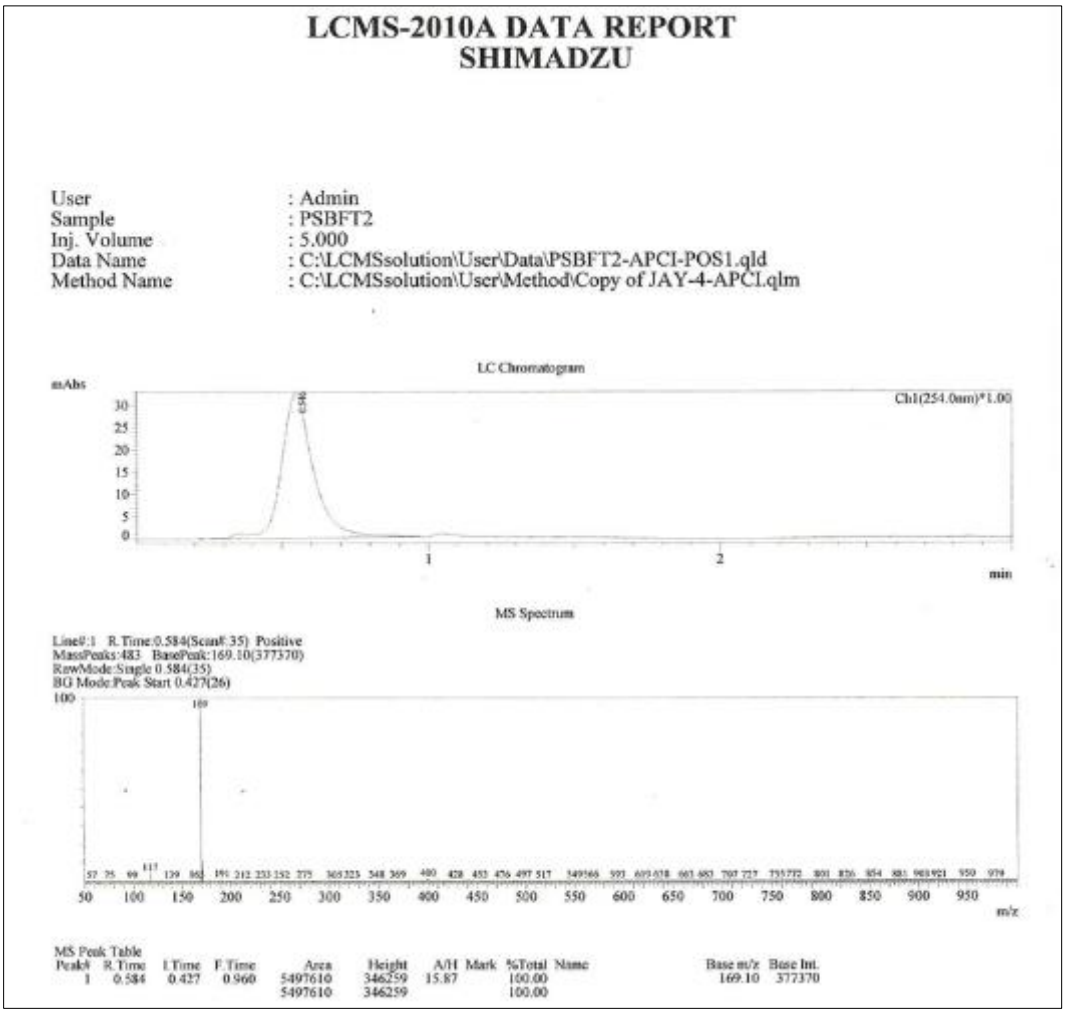

Figure 1 Mass spectrum of the phytoconstituentTridecan-1-ol

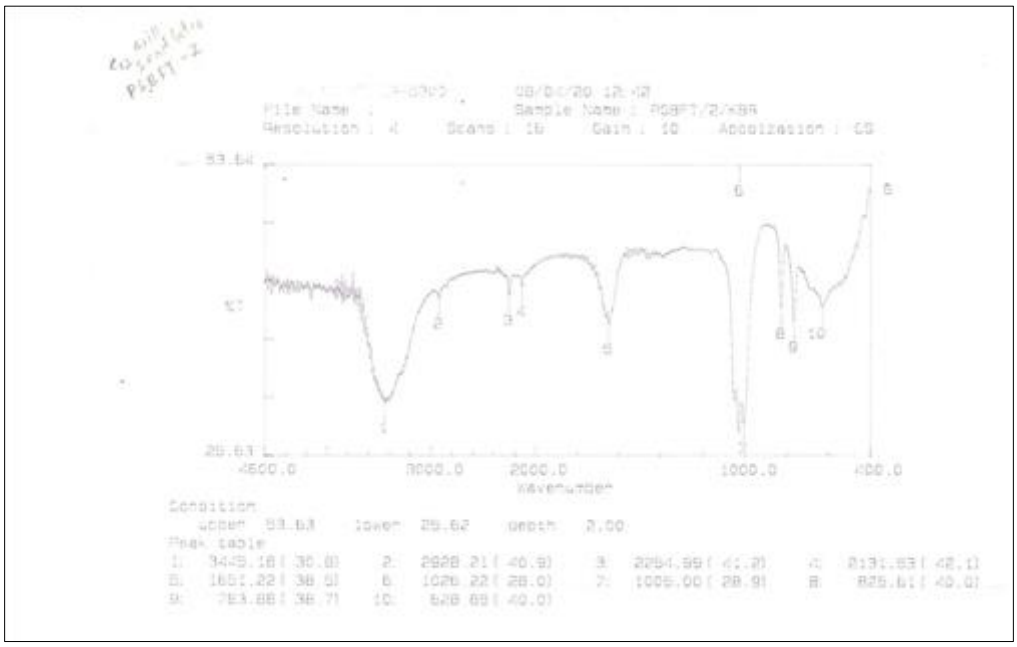

Figure 2 IR spectrum of the phytoconstituent Tridecan-1-ol.

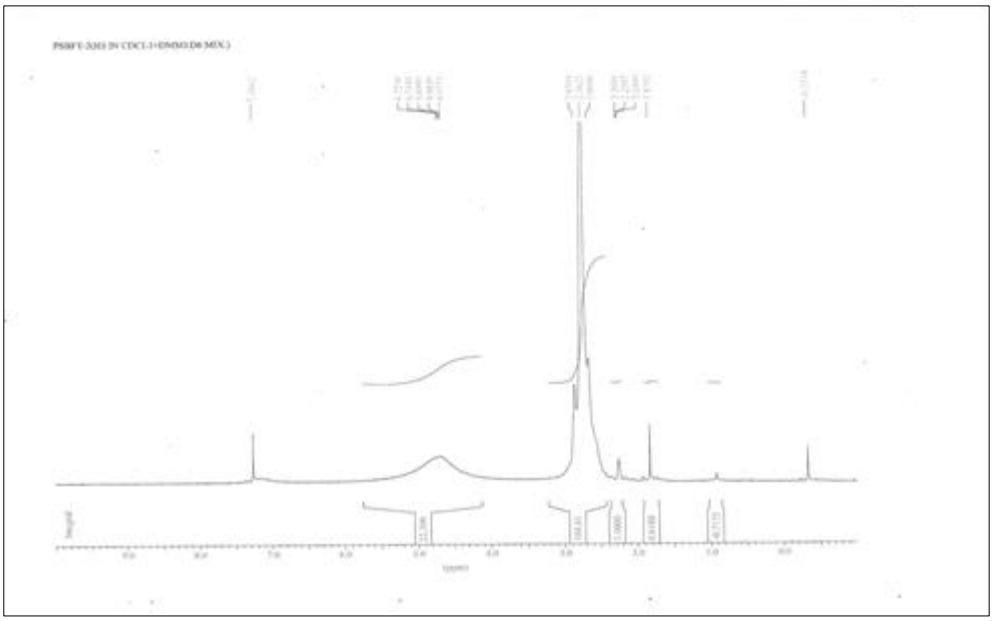

Figure 3 H^1 NMR of the phytoconstituent Tridecan-1-ol.

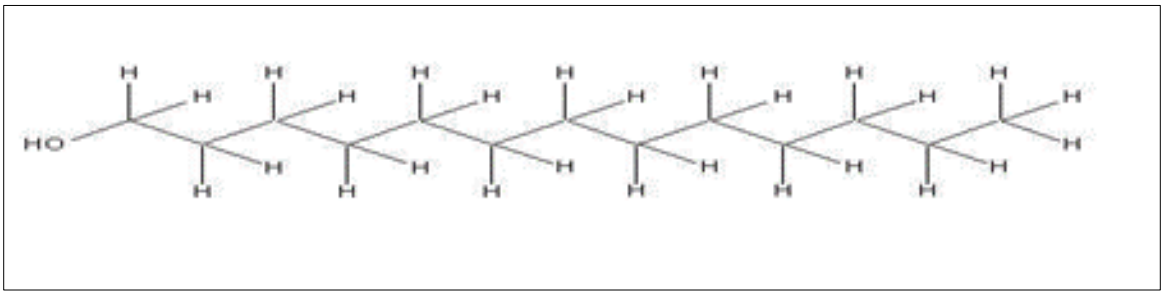

Figure 4 Structure of phytoconstituent tridecan-1 ol

3.2. Hepatoprotectivity of Tridecan-1-ol

Acute toxicity study experiment revealed that the Petroleum ether extract and its isolated phytoconstituent Tridecan-1-ol did not showed the symptoms of toxicity and mortality at the concentrations of 500 and 150mg/kg per body weight respectively. The results of hepatoprotective effect of petroleum extract and tridecan-1-ol on CCl_4 intoxicated rats are shown in Table1.

Table 1 Hepatoprotective activity of pet ether crude extract and it's phytoconstituent Tridecan-1-ol from F. trinervia.

Sl. No	Samples	T.Bil (mg/dL)	T.Prot (mg/dL)	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)
1	control	0.36±0.08	7.40±0.36	209.41±13.57	76.31±9.11	116.86±9.57
2	CCl4	2.24±0.29**	4.08±0.27**	1194.08±73.60**	321.67±27.93**	403.73±8.66**
3	Silymarin + CCl_4	0.40±0.14*	7.34±0.42*	217.64±23.8*	78.81±8.05*	118.21±9.7*
4	Petether extract+ CCl_4	0.47±0.16*	7.10±0.25*	211.81±16.28*	80.80±5.71*	126.14 ±7.27*
5	Tridecan-1-ol+CCl_4	0.45 ±0.12*	6.94±0.49*	253.57±19.48*	87.07±16.09**	149.13±12.05*

Values are the mean±S.E.M. of six rats. Symbols represent statistical significance; * P < 0.01; ** P < 0.001, as compared to control group.

The CCl_4 intoxicated rats displayed hyper bilirubinemia (2.24mg/dL), a common sign of hepatic jaundice. The rise in the levels of ALT (1194.08U/L), AST(321.67 U/L) and ALP(403.73IU/L) activities may be due to a disturbance in the secretary activity or in the transport of metabolites or may be due to altered synthesis and it depicts the intensity of jaundice. Concomitment administration of petroleum ether extract and its constituent tridecan-1-ol to CCl_4 intoxicated rats elicited immediate recovery in the levels of above hepatic function tools. The constituent Tridecan-1-ol exhibited significant hepatoprotectivity (P<0.01) by restoring the levels of serum bilirubin (0.45mg/dL), ALT(253.57U/L), AST(87.07 U/L) and ALP(149.13U/L) with subsequent increase in the levels of serum protein (6.94mg/dL). The hepatoprotective effect of phytoconstituents isolated from the medicinal plants were also reported by many investigators [18][19][20]&[21]. The attainment of these liver function marker enzymes towards a near- normalcy in the animals treated with petroleum ether extract and its isolated phytoconstituent Tridecan-1-ol confirms the hepatoprotective effect. The results were found comparable to a commercial hepatoprotective drug silymarin which is a composite name of three flavonoids isolated from tender *Silybum marinum*[22][23]. Many investigator's used silymarin as a positive control for the evaluation of hepatoprotective effect of phytoconstituents.

4. Discussions

There are many plants have been using to treat jaundice in folklore and ethnically. In various medical systems and indigenous system of medicine several plants and phyto constituents were known to act as potent hepatoprotective drugs for healing jaundice and their therapeutic property was evaluated by many investigators using animal models. In the present research one of the ethno medically significant hepatoprotective plant in southern India *Flaveria trinervia* chosen for the isolation of phytoconstituent and evaluation of hepatoprotective efficacy. As a result we isolated phytoconstituent Tridecan-1 ol from *Flaveria trinervia* and evaluated the hepatoprotective activity. The results of histological studies of the liver tissue also supported the hepatoprotective property of Tridecan-l-ol. Histology of the liver sections of control animals (GroupI) showed normal hepatic cells with well preserved cytoplasm, prominent nucleus, nucleolus and visible central veins (Fig.5A.) The liver sections of CCl_4- intoxicated rats exhibited intense centri lobular necrosis, vacuolization, macro vesicular fatty changes showed massive fatty accumulation in the hepatocytes, and broad infiltration of the lymphocytes and the loss of cellular boundaries (Fig.5B.). The liver sections of silymarin treated animals showed normal hepatic architecture(Fig.5C.).

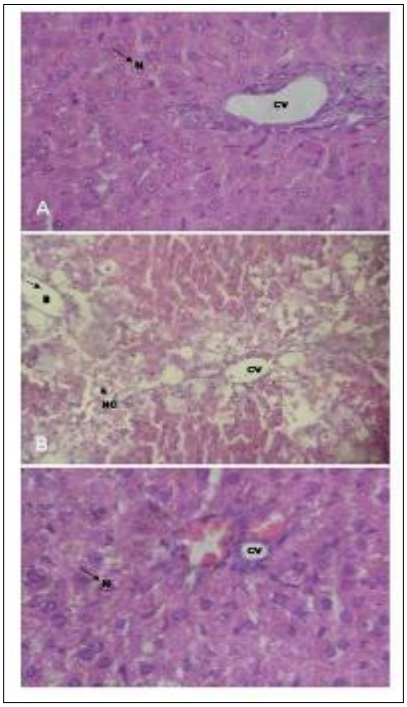

Figure 5 A Liversectionsofcontrolratsshowingnormalhepaticcellswithwell-preservedcytoplasmwell broughtoutcentralvein(CV)andprominentnucleus(N) ; Fig. **5B** LiversectionofCCl4treatedratsshowingmassive fatty lobulation, necrosis (NC),ballooning (B) degeneration, and the loss of cellular boundaries. **Fig 5C** Liver section of rats treated with CCl_4 and silymarin showing regeneration of hepatocytes with prominent nucleus (N)

The histological architecture of liver sections of the rats treated with Tridecan-1-ol exhibited significant liver protection against CCl_4 injury as evident by the presence of normal hepatic cords, absence of necrosis, fatty infiltration (Fig.5D.) and its hepatic architecture is almost similar to silymarin treated group. Liver sections from rats treated with petroleum ether extract showed moderate with appreciable histological architecture (Fig.5E). The bacterio static effect of Tridecan-l-ol was reported by Gilbertson *et al.,* (1984) [24] and Atsushi*etal.,* [25]. Tridecan-1-ol has antipest property[26].Some wild tomatoes are resistant to mites due to the presence of tridecan-1-ol on exudates of leaf glandular trichomes which are toxic to *A. lycopersici* [27][28][29]. The present investigation revealed the hepatoprotective effect of Tridecan-l-ol against CCl_4induced toxic hepatitis.

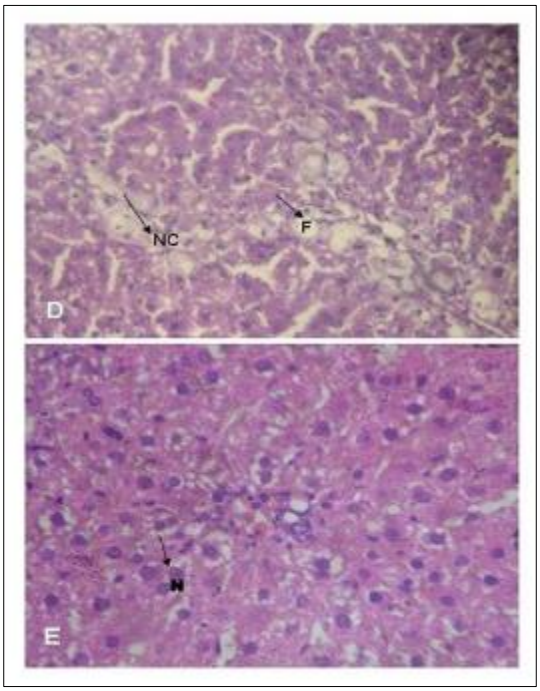

Figure 5D Liver section of rats treated with CCl_4 and petroleum ether extract of *Flaveria trinervia* showing regeneration of hepatocytes, moderate fatty lobulation(F) and necrosis. **Figure 5E** Liver section of rats treated with CCl_4 and Tridecan-1-ol showing: well brought out central vein, hepatic cell with well-preserved cytoplasm, arrow shows the prominent nucleus(N)

5. Conclusion

To conclude, the constituent Tridecan-1-ol isolated from the petroleum ether extract of *F. trinervia* is a novel phytoconstituent afforded protection against hepato toxicity. The protection against the injurious effects of carbon tetrachloride that may result from the interference with cytochromeP_{450}, resulting in the hindrance of the formation of hepatotoxic free radicals. The study also supported the traditional claim of *Flaveria trinervia* as a potent herb for treating jaundice.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors of this manuscript have no conflicts of interest/ Competing Interests they may have with publication of the manuscript or an institution or product that is mentioned in the manuscript.

Statement of ethical approval

'The present research work contain studies performed on animals. The Institutional Animal Ethical Committee (Reg.No.NGSMIPS/IAEC/DEC-2020/217) permitted the study.

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