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EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF ETHANOLIC ROOT EXTRACT OF "*VACCARIA PYRAMIDATA*" AGAINST CCL_4-INDUCED HEPATOTOXICITY IN WISTER RATS

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ABSTRACT

The present study was aimed to determine the hepatoprotective effects of ethanolic root extract of *vaccaria pyramidata* against CCl_4-induced hepatic injury in wistar albino rats. Pretreatment with Silymarin, ethanolic root extract of *vaccaria pyramidata* showed better protection against CCl_4 induced hepatotoxicity. Test indicated a reduction in elevated serum enzyme levels i.e. SGOT, SGPT & ALP significantly in test groups when compared with toxic control. Pretreatment with standard Silymarin, ethanolic extract significantly reduced the levels of direct & total bilirubin when compared to toxic control group. The rats pretreated with silymarin & ethanolic root extract of *vaccaria pyramidata* exhibited significant decreased in triglyceride levels when compared to the toxic group. This was also confirmed by the result of histopathological examination, which revealed dose dependent decrease in incidence and severity of histopathological changes.

KEYWORDS: *Vaccaria pyramidata* medik, Ethanolic extract, Carbon tetrachloride, Hepatoprotective activity.

INTRODUCTION

The hepatic ailment is one of the universal problems that unintentionally, leads to serious adverse effects. *Vaccaria pyramidata* is a rhizomatous beardless perennial plant which is used as anthelmintic, appetizer, depurative, diuretic, and in the treatment of liver complaints and oedema traditionally. Ayurveda, which means the Science of Life, is the oldest medical science in the Indian subcontinent and has been practiced since the 12th Century BC.[1] In Sanskrit, the word Ayurveda consists of the words *āyus*, meaning "longevity", and *Veda*, meaning "related to knowledge or science".[2] Ayurveda is not merely a system of medicine; rather it is a way of life. Its objective is to accomplish physical, mental, social and spiritual well-being by adopting preventive and promotive approaches as well as treating diseases with the holistic approach.[1] In the last few decades there has been an exponential growth in the field of herbal medicine. It is getting popularized in developing and developed countries owing to their effectiveness, minimal side effects in clinical experience and relatively low cost. Herbal drugs or their extracts are prescribed widely, even when their biological active compounds are unknown. Herbal drugs are significant source of hepatoprotective drugs. Mono and poly herbal preparations have been used in various liver disorders. According to one estimate, more than 700 mono and poly-herbal preparations in the form of decoction, tincture, tablets and capsules from more than 100 plants are in clinical use.[3] The major functions of the liver are carbohydrate, protein and fat metabolism, detoxification, secretion of bile and storage of vitamins. Thus, to maintain a healthy liver is a crucial factor for overall health and wellbeing. The liver is the largest organ in the body, contributing about 2 per cent of the total body weight, or about 1.5kg in the average adult human. The basic functional unit of the liver is the liver lobule, which is a cylindrical structure several millimeters in length and 0.8 to 2 millimeters in diameter. The human liver contains 50,000 to 100,000 individual lobules. The liver lobule, shown in cut-away format, is constructed around a central vein that empties into the hepatic veins and then into the vena cava. The lobule itself is composed principally of many liver cellular plates (two of which are shown in that radiate from the central vein like spokes in a wheel.[4] Each hepatic plate is usually two cells thick, and between the adjacent cells lie small bile canaliculi that empty into bile ducts in the fibrous septa separating the adjacent liver lobules. In the septa are small portal venules that receive their blood mainly from the venous outflow of the gastrointestinal tract by way of the portal vein. From these venules blood flows into flat, branching hepatic sinusoids that lie between the hepatic plates and then into the central vein. Thus, the hepatic cells are exposed continuously to portal venous blood. In addition to the hepatic cells, the venous sinusoids are lined by two other

types of cell: (1) Typical endothelial cells (2) Large Kupffer cells. Which are resident macrophages that line the sinusoids and are capable of phagocytizing bacteria and other foreign matter in the hepatic sinus blood.[5]

Fig 1: *Vaccaria pyramidata* Plant.

Fig 2: Flower & leaves.

Plant Profile

The plant *Vaccaria pyramidata Medik.* (Family: Caryophyllaceae) is grown as a wild flower .It has a medium greyish hairless annual with a regularly branching stem, oval leaves and small pink flower. It is found on arable fields, often on lime. It is found throughout India, as a weed. It is often grown in gardens, dry sandy plains near lakes, meadows, clay-solonetz places in steppes and solonetz meadows, marshes, ditches and wet grassy places. *Vaccaria pyramidata* is an annual plant growing to 0.6 m (2ft) flowers appear in July to August and the seeds ripe between Aug to September .The flower are hermaphrodite (have both male and female organs) and are self-pollinated by Lepidoptera. The plant is self-fertile.

Fig 3: Dried root (*Vaccaria pyramidata*).

The root is used for cough, asthma and other respiratory disorders, for jaundice, liver and spleen diseases (increases the bile flow). Mucilaginous sap is used in scabies. Saponins of the root showed haemolytic activity. Some others treditionals uses i. e. Anthelmintics, Appetizer, Depurative, Diuretic, Vermifuge. It is used with other herbs in the treatment of venereal infections, liver complaints and oedema.

The main chemical constituents i.e. Lanostenol, stigmasterol, beta-sitosterol, diosgenin, vaccaxanthone, Xanthones and an oliigosaccharide, vaccarose, have been isolated.

MATERIALS AND METHODS

The roots of the plant *vaccaria pyramidata medik* were collected from Punjab in the month of November 2015. The plant specimen was authenticated by Dr. Alok Lahri of National Botanical Research institute (Council of scientific and industrial research), Lucknow with reference no. CIF-RB-4-391dt., specification-NBRI-SOP-202.

Qualitative chemical investigations were conducted in order to identify various phytochemical constituents present in different extracts. The aspects of only positive tests were taken into consideration. (Alkaloid, Flavanoids, Cardice Glycosides, Saponin, Steroids, Tannins, Phenolics, Proteins and Amino Acids). The collected roots of "*vaccaria pyramidata* were ground to get coarse powder and then subjected to successive extraction using petroleum ether, chloroform, and ethanol with the help of Soxelet apparatus.

After extraction with chloroform the coarse powder of the roots of *vaccaria pyramidata* was dried and extracted with ethanol for 24 hrs. The liquid extract was then cooled and then placed on the water bath at temp. 40-50ºC until the entire solvent evaporated. The dried extract was weighed and the percentage yield was calculated with reference to the crude drug. The dried ethanolic extract was stored in a desiccator and subjected to various chemical tests to detect the presence of different phyto constituents like alkaloids, tannins, cardiac glycosides and traces of flavonoids etc.

Calculation of Percentage Yield

Percentage yield = Weight of extracts obtained/weight of crude drug*100

Healthy Wistar albino rats of both sexes 150–175 g used for the study. They were individually housed and allowed free access to standard pellet diet and water *ad libitum*. The surgical interventions were carried out under sterile conditions. The animals were kept under

standard laboratory conditions (12 hours light: 12 hours dark) at room temperature (20-25ºC) for a period of 10 days prior to experimentation. The relative humidity was maintained between 50%-60%. Body weights were checked weekly. The experimental protocol was approved by IAEC of the Institution.

After testing different doses of plant extract solution (5, 50, 300 and 2000mg/kg body weight) appropriate maximum tolerated safe dose was considered. $1/10^{th}$ and $1/5^{th}$ of the maximum tolerated safe dose was selected as treatment dose for further studies of pharmacological activities according to OECD, guidelines 425.

Wistar Albino rats weighing 150-175 g was used in the study. Females should be nulliparous and non-pregnant. Acute oral toxicity was performed as per OECD-425 guidelines. The animals were fasted over night with water *ad libitum*. The dosing was done in a stepwise procedure using the fixed doses of 5, 50, 300 and 2000 mg/kg. Further the groups of animals were dosed at higher or lower fixed doses, until and unless there was no signs of toxicity or mortality. The test substance was administered in a single dose by gavage using a stomach tube or a suitable intubation cannula. Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for 14 days.

Pharmacological Evaluation

CCl_4- induced hepatotoxicity study

Thirty Wistar albino rat of either group weighing 150-175 g were selected and divided in to five groups each group consisted of six animals.

Group I- Served as Vehicle control group, received olive oil orally.

Group II- Served as Negative control group, administered olive oil orally for 1-13 days, and 14th day, CCl_4: olive oil (1:1) v/v mixture was given at the dose of 1.25 ml/kg body weight orally.

Group III- Served as Positive control group received Silymarin suspended in olive oil at the dose of 100mg/kg body weight orally for 1-13 days, and 14th day, CCl_4: olive oil (1:1)v/v mixture was given at the dose of 1.25 ml/kg body weight orally.

Group IV- Served as Test group-1, received ethanolic extract of *vaccaria pyramidata medik* at the dose of 200 mg/kg body weight orally for 1-13 days, and 14th day CCl_4: olive oil (1:1) v/v mixture was given at the dose of 1.25 ml/kg body weight orally.

Group V- Served as Test group-2, received ethanolic extract of *vaccaria pyramidata medik* at the dose of 400 mg/kg body weight orally for 1-13 days, and 14th day, CCl_4: olive oil (1:1) v/v mixture was given at the dose of 1.25 ml/kg body weight orally.

In the present study, the hepatoprotective activity of test drugs was evaluated by Biochemical estimation of serum. After 24 hrs. of CCl_4 administration, blood was collected directly from the retro orbital plexus using non-Heparinized Hematocrit capillaries in previously labeled eppendrof tubes and blood was allowed to clot for 30 min. at room temperature, for further biochemical assays. Serum was separated from the blood by centrifugation at 2500 rpm for 10 min. at 4ºC. The supernatant containing the serum was collected and stored at 2-4ºC in refrigerator for further study. The separated serum was used for the estimation of biochemical parameters such as SGOT/AST, SGPT/ALT, ALP, Total and Direct Bilirubin and triglycerides. After the blood collection completed, all animals were sacrificed and their livers were collected, and accurately weighed. Collected liver was preserve in 10% formalin solution for further studies.

Animals were sacrificed and liver were isolated and washed with saline and then weighed by using an electronic balance. The liver weights were expressed with respect to its body weight i.e. gm/100gm. After recording the weight, all the livers were determined for their individual volumes, using water displacement method. The liver was placed in a measuring cylinder containing a fixed volume of distilled water or saline and the volume displaced was recorded.

Percent inhibition will be calculated by applying the following formula

$$\% \text{ inhibition} = (AB_{Control} - AB_{Negative\ Control}) = AB_{Value} \div AB_{Negative\ Control} \times 100$$

Blood was collected from the retro orbital plexus of the animals and serum was allowed to coagulate at 37ºC for 30 minutes, and subjected to centrifugation at 2500 rpm. Serum samples were stored at 2-8ºC until further use. The enzyme assay was used to determine SGOT, SGPT, ALP, Total Bilirubin, Direct Bilirubin and Triglyceride levels using commercially available enzyme kits.

After 24 hrs. of ccl4 administration animals were anaesthetized using ether and 1 ml of blood was collected by cardiac puncture. The blood was allowed to clot and centrifuged at 2500 rpm for 10 mintues. The serum was separated and used for assay of alanine phosphatase (ALP), SGOT, SGPT,Total and Direct Bilirubin, Triglycerides, by standard methods using enzyme assay kits adopted to micro lab, semi acto analyzer. The animal was excised, washed with phosphate buffer and dried with tissue paper. It was weighed and transferred to a 10% formalin fixative solution for 48 hrs. The liver tissue were processed for paraffin embedding and sections (5 µm thick) were taken in a microtome. After staining with hematoxylin and eosin, slides were examined under microscope for histropathological changes.

RESULTS AND DISCUSSION

The data will be expressed as Mean ± SEM (standard error of Mean) (n=6). Statistically difference between the groups will be analyzed by using analysis of variance (ANOVA) and post hoc, Dunnet's test. Where, * represents significant at p<0.05, ** represents highly significant at p< 0.01, and *** represents very significant at p<0.001. All p values are compared with negative control.

Vaccaria pyramidata dried root was extracted using different solvents i.e. petroleum ether, chloroform and ethanol and then obtained extracts were subjected to various phytochemical studies. Then the ethanolic extract of *Vaccaria pyramidata* root was subjected for toxicity studies. Ethanolic extract was administered up to dose (2000mg/kg b.w) and extract did not produce any mortality, thus 1/10th (200mg), 1/5th (400mg) dose & were selected for the hepatoprotective evaluation. In order to conclude the hepatoprotective potentions, animals (Wister albino rats of either sex) were subjected to Carbon tetra chloride induced hepatotoxicity and liver weight, volume, estimation of serum biochemical parameters like SGOT, SGPT, Direct bilirubin, Total Bilirubin, Triglyceride and histopathological investigation of liver tissues were performed.

The ethanolic root extract of *Vaccaria pyramidata* (200 mg/kg b.w.) and (400mg/kg b.w.) showed reduction of wet liver weight and volume significantly at p<0.05.

Pretreatment with Silymarin, ethanolic extract of *Vaccaria pyramidata* showed good protection against CCl_4 induced toxicity to liver. Test indicated a significant reduction in elevated serum enzyme levels i.e. SGOT, SGPT and ALP with extract treated as compared to toxic control animals. It also significantly reduced levels of direct and total bilirubin when compared to toxic control group. However triglyceride levels significantly decreased. Histopathological studies also revealed protection against hepatocellular necrosis with dilation of sinusoids and congestion in central vein within the lesion area.

Table no. 1: Biochemical parameters of different groups for ccl_4 induced hepatotoxicity.

Group	Treatment	Dose	SGPT level (U/L)	SGOT levels (U/L)	ALP levels (U/L)	Direct bilirubin levels(mg/dl)	Total bilirubin levels(mg/dl)
I	Control	1.25 ml/kg	37.88±3.08***	94.29±1.79***	31.84±2.70***	0.1945±0.028***	0.2989±0.049***
II	Negative Control	1.25ml/kg CCl_4, p.o.	273.66±16.45	352.30±1.80	123.91±6.22	1.0106±0.053	1.0151±0.169***
III	Standard	100mg/kg Sylimarine+ CCl_4 ,p.o.	55.62±1.41***	151.43±2.70***	59.32±3.61***	0.3856±0.023***	0.3003±0.050
IV	Low dose	200 mg/kg *vaccaria pyramidata* + CCl_4, p.o.	96.75±5.16***	285.76±1.38***	84.38±3.55***	0.6897±0.037***	0.3557±0.0592***
V	High dose	400 mg/kg *vaccaria pyramidata* + CCl_4, p.o.	88.29±6.11***	258.56±2.36***	78.47±4.89***	0.8510±0.023*	0.5186±0.086***

Table no. 2: Triglyceride levels in ccl_4 induced hepatotoxicity rats.

Group	Treatment	Dose	Triglycerides levels(mg/dl)	Wet Liver weight (gm/100gm)	Liver volumes (ml/100gm)
I	Control	-	25.587±0.028***	3.35±0.17***	4.00±0.13
II	Negative Control	1.25 ml/kg CCl_4, p.o.	128.6±1.237	6.21±0.36	6.96±0.35
III	Standard	100mg/kg Sylimarine+ CCl_4 ,p.o.	30.983±0.654***	4.20±0.18***	4.96±0.19
IV	Low dose	200mg/kg *vaccaria pyramidata* + CCl_4, p.o.	40.780±2.922***	4.78±0.31**	5.53±0.29
V	High dose	400 mg/kg *vaccaria pyramidata* + CCl_4, p.o.	58.480±1.981***	4.98±0.19**	5.83±0.12

Values are mean ± SEM (n=6) one way ANOVA .Where, * represents significant at p<0.05, ** represents highly significant at p< 0.01, and * represents very significant at p<0.001. All values are compared with negative control.**

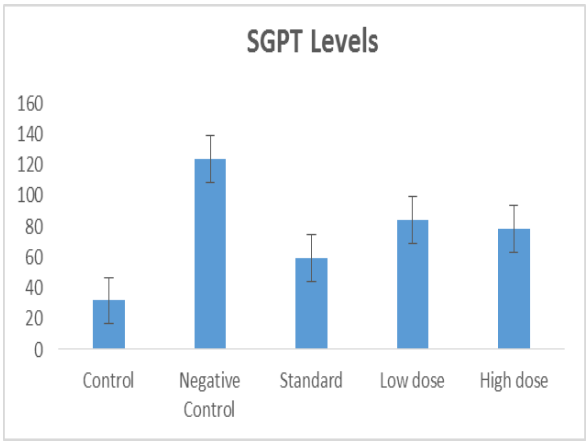


Figure 1: Graph representing the effect of ethanolic extract of *Vaccaria pyramidata* roots on SGPT levels in CCl_4- induced hepatotoxic rat.

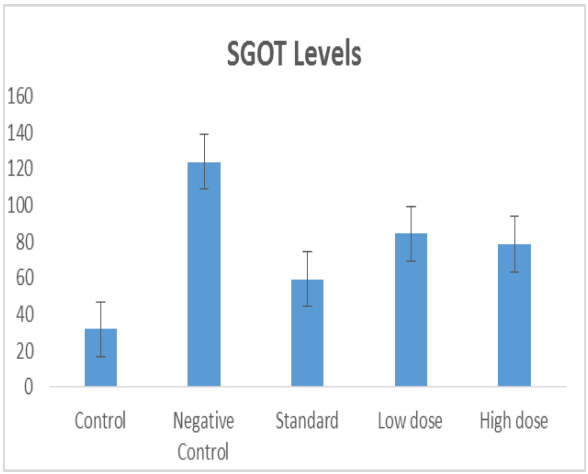


Figure 2: Graphical representation of the effect of ethanolic extract of *Vaccaria pyramidata* roots on SGOT levels in CCl_4 -induced hepatotoxic rats.

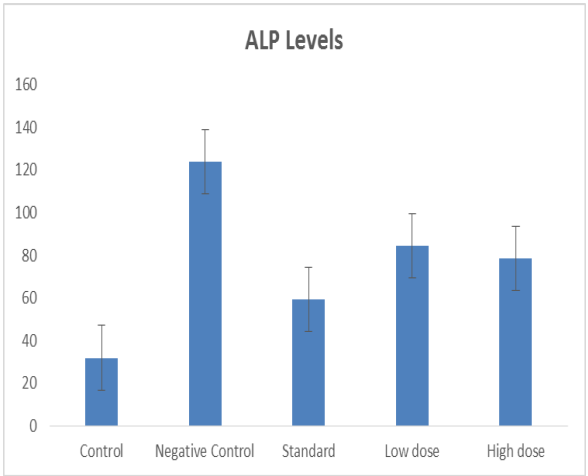


Figure 3: Graph representing the effect of ethanolic extract of *Vaccaria pyramidata* roots on Alkaline Phosphatase levels in CCl_4 -induced hepatotoxic rats.

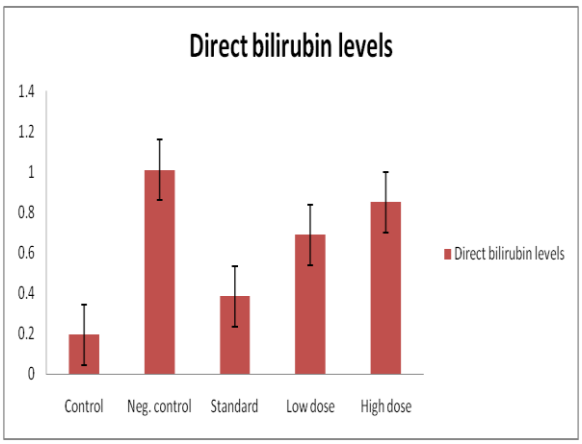


Figure 4: Graph representing the effect of ethanolic extract of *Vaccaria pyramidata* roots on Direct Bilirubin levels in CCl_4- induced hepatotoxic rats.

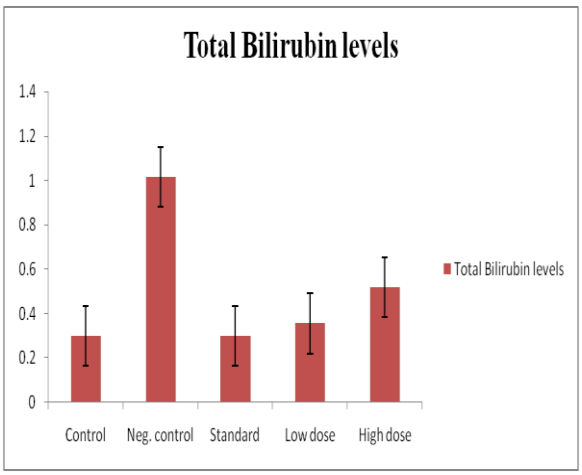


Figure 5: Graph representing the effect of ethanolic extract of *Vaccaria pyramidata* roots on Total Bilirubin levels in CCl_4- induced hepatotoxic rats.

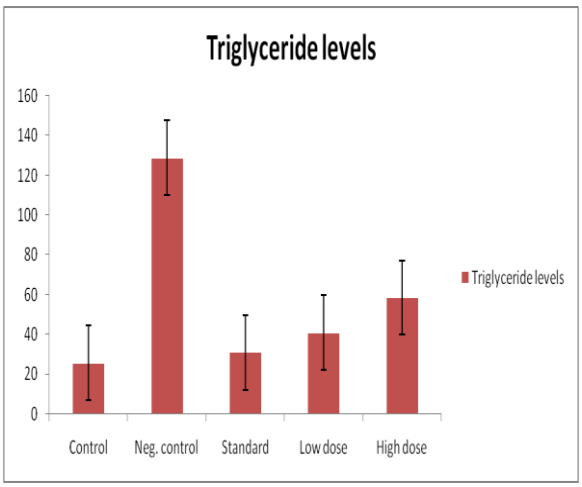


Figure 6: Graph representing the effect of ethanolic extract of *Vaccaria pyramidata* roots on Serum Triglycerides levels in CCl_4- induced hepatotoxic rats.

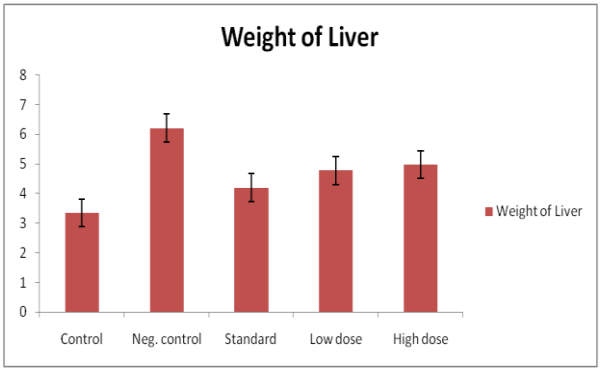


Figure: 7 Graph representing the effect of ethanolic extract of *Vaccaria pyramidata* roots on Wet liver weights in CCl_4 induced hepatotoxic rats

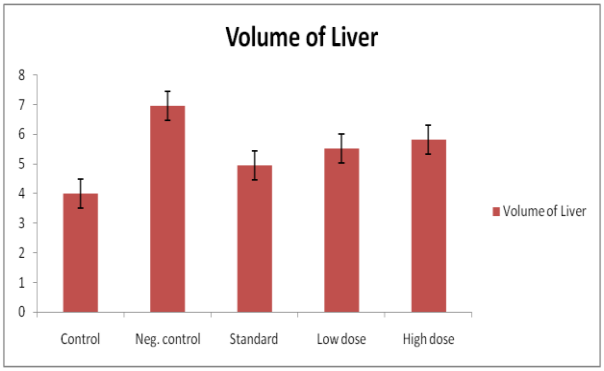
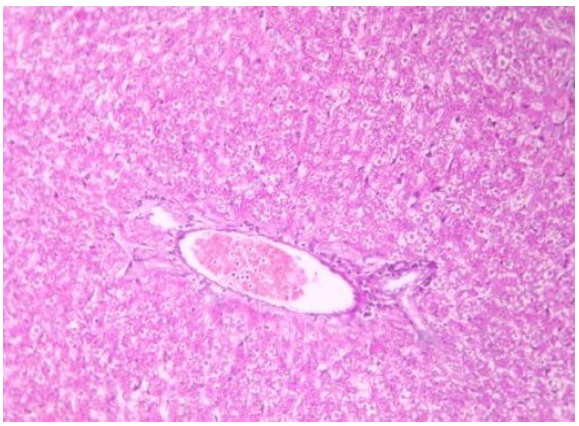
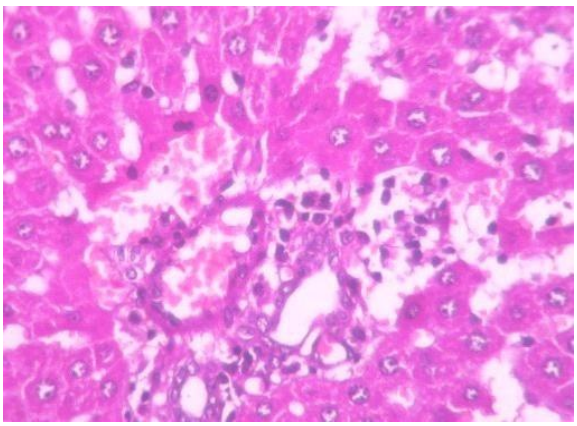


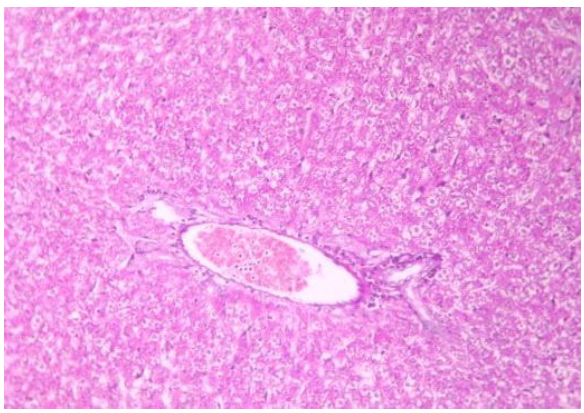
Figure: 8 Graph representing the effect of ethanolic extract of *Vaccaria pyramidata* roots on Wet liver volume in CCl_4 induced hepatotoxic rats



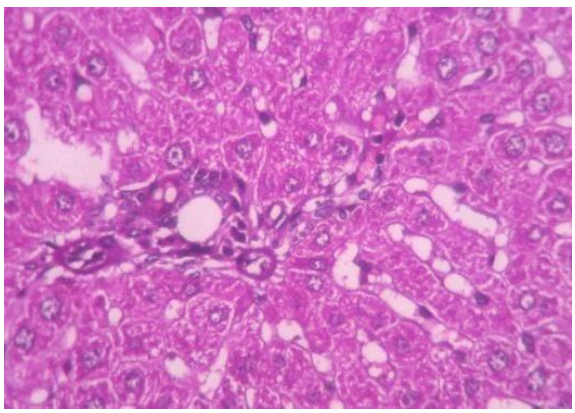
Control



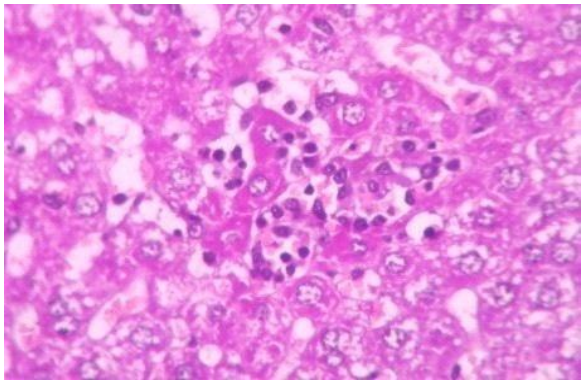
Negative Control (CCl_4)



Standard(Silymarin100mg/kg)



Low Dose (200mg/kg)



High Dose (400mg/kg)

CONCLUSION

The phytochemical investigation of Ethanolic extract of *vaccaria pyrimadata medik* have shown the presence of Flavonoid. Ethanolic extract of *vaccaria pyrimadata medik* offers protective effect against ccl_4 – induced hepatotoxicity in experimental rats. An acute oral toxicity study of ethanolic extract of *vaccaria pyramidata* roots was done on Wistar albino rats, 200 and 400 b.w. extract dose were selected for the in- vivo study. The study, revealed that the group treated with CCl_4 showed dramatic elevation in serum SGOT, SGPT, ALP, (total and direct) bilirubin levels and triglycerides levels. The liver tissues also showed histopathological changes, confirming hepatotoxicity by CCl_4.

The administration of ethanolic extract of *vaccaria pyramidata* and Silymarin prevented CCl_4 induced elevation in different biochemical parameters indicating the hepatoprotective activity of the extract against CCl_4 induced hepatotoxicity. This was also confirmed by the result of histopathological examination, which revealed dose dependent decrease in incidence and severity of histopathological changes.

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