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Evaluation of Hepatoprotective Activity of Hydroalcoholic Extract of Onion Peels Containing Protocatechuic Acid

Mrinalini C. Damle[1], Sachin V. Tembhurne[2], Swapnil R. Todkar[2]

[1] Department of Pharmaceutical Quality Assurance, All India Shri Shivaji Memorial Society's College of Pharmacy, Kennedy Road, Near RTO, Pune-411001.

[2] Department of Pharmacology, All India Shri Shivaji Memorial Society's College of Pharmacy, Kennedy Road, Near RTO, Pune-411001.

ABSTRACT

India is the second largest country for cultivation of onion. 19.90% of onion is cultivated in India. Onion that is Allium cepa family Amaryllidaceae used in daily diet for taste. Onions also have number of medicinal properties, such as hepatoprotection, anticancer, antifungal, antioxidant, antiulcer, anti-agging, anti-inflammatory, anticancer etc.[1-2] The dried scales or peels of onion also have the same medicinal constituents with same activity as raw onions. The onion peels contains flavonoids, such as anthocyanins, flavones (qurcertin and its derivatives), ferulic, Gallic, protocatechuic acids, sulphur, vitamins etc. In the present study determination of protocatechuic acid in onion peels extract has been performed using HPTLC. HPTLC separation was carried out on Merck TLC aluminium sheets precoated with silica gel $60F_{254}$ using Toluene: Ethyl acetate: Formic acid (6: 6: 1.2 v/v/v) as mobile phase.[3-11] Quantitative analysis was carried out in the absorbance mode at 258 nm. Hydroalcoholic extract was tested for hepatoprotective activity in wistar rats (either sex) by using CCl_4 as hepatotoxicity inducing agent and Silamyrin as standard. Hydroalcoholic extract shows hepatoprotective activity as indicated by decrease in the level of SGOT, SGPT, total protein, billirubin an which hepatotoxicity was induced by CCl_4 intraperitonial injection route to animal, from the result it may be concluded that onion peels extract may be used for hepatoprotective activity.

Keywords: Onion peel extract, Protocatechuic acid, HPTLC, hepatoprotection.

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***Address for Correspondence:**

Dr. Mrinalini C. Damle, Professor, Department of Quality Assurance, AISSMS College of Pharmacy, (Affiliated to Savitribai Phule Pune University), Maharashtra, India- 411001.

INTRODUCTION

There is an increasing interest in herbal remedies because of their effectiveness, less side effects in clinical experiments and relatively low cost. Protocatechuic acid (PCA) is widely distributed and present in most edible plants used in medicine. It is also a very common compound present in human diet, present in bran and brown rice (Oryza sativa L.) and onion (Allium cepa L.). Onion peels are the external part of onion bulb and having several folds, it is thin, light weight, strong often translucent paper. Onion peel contains numbers of phytoconstituent such as sulphur, qurcertin, protocatechuic acid, calcium, flavonoids, phenolic acid, etc[1,5].

PCA has been reported to possess antioxidant, antibacterial, anticancer, antiulcer, ant diabetic, ant ageing, antifibrotic, antiviral, anti-inflammatory, analgesic, antiatherosclerotic, cardio protective, hepatoprotective, neurological and nephroprotective activities. As per literature search there is no method for determination of protocatechuic acid in onion peel extract and evaluation of hepatoprotective activity in rats[6-9].

Liver is one of the largest organs in the human body and chief site for intense metabolism and excretion. Liver diseases are one of the major health problems in the world. These are caused by toxic chemicals, autoimmune disorders, infections and excess consumption of alcohol. The hepatotoxic chemicals can induce lipid peroxidation and oxidative damages. It is involved in almost all the biochemical pathways to growth, fight against the disease, nutrient supply, energy provision and reproduction.[10-14]

The hydroalcoholic extract of onion peels contains PCA as active constituent. There are many literature record indicating hepatoprotective activity of PCA. Despite of there is no report available on action of onion peel extract. For that reason the present study changed into undertaken to

evaluate the hepatoprotective activity of hydroalcoholic extract of onion peels in CCl_4 induced hepatotoxicity in wistar rats.

MATERIAL AND METHODS:

Procurement of drug: PCA was procured from Sisco Research Laboratories. Pvt Ltd.

Collection and Identification of plant Material:

Onion peels were collected from Talegaon Dhamdhere, Pune and authenticated by botanical survey of India, Pune (sample no. ST: 01)

Extraction method [12-13]

Peels were separated from onion bulb. Onion peels was sundried and crushed to obtain powder. This powder was weighed, added 70% ethanol with same quantity of 0.1M NaOH solution and kept into glass bottle for cold maceration. This mixture was placed for 72 hrs with intermittent shaking. After 72hrs the macerates were removed from bottle, filtered through whattman filter paper and filtrate was concentrated on waterbath.

Selection of Analytical Wavelength

For the selection of the analytical wavelength, the Protocatechuic acid solution was prepared by dissolving 10 mg in 10 ml (1000µg/ml) of methanol and further diluted with methanol to produce (10 µg/ ml) its absorbance was taken on UV visible spectrophotometer in range of 200-400 nm. The compound absorb at 258nm which is selected as an analytical wavelength.

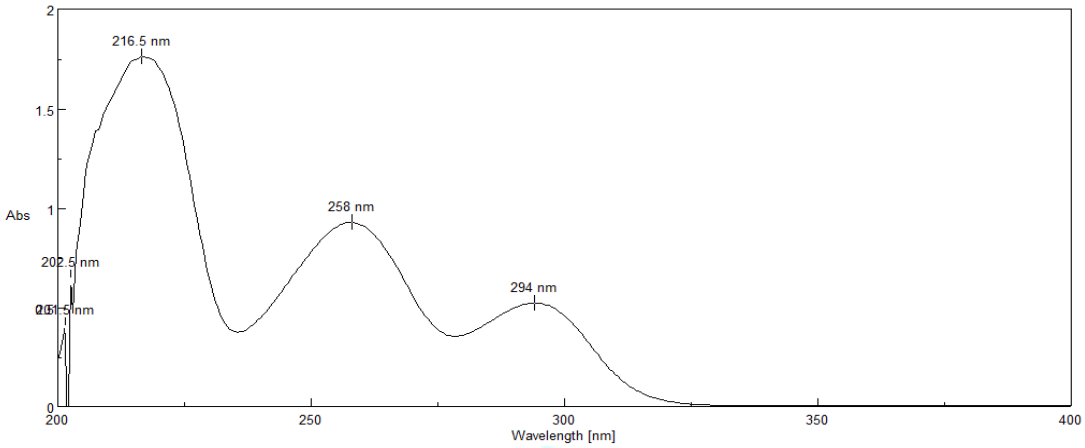


Figure 1: UV Spectrum of protocatechuic acid(10µg/ml)

HPTLC analysis[13-16]

The chromatographic separation was carried on aluminium plates precoated with silica gel $60F_{254}$ in (10 cm × 10 cm with 250 µm layer thickness). Sample was applied on the plate with band width of 6 mm width using Camag 100 µL sample syringe (Hamilton, Switzerland) with Linomat 5 applicator (Camag, Switzerland). A solution of 100µg/ ml in methanol of PCA was prepared and then spotted using Camag Linomat V sample applicator. The optimised mobile phase comprises of Toluene: Ethyl acetate: Formic acid (6:6:1.2, v/v/v). CAMAG twin trough glass chamber was used for linear ascending development of TLC plate with 15 min saturation time for mobile phase, migration distance was 90 mm. Densitometric scanning was performed using Camag TLC scanner 3 at 258 nm, operated by win CATS software (version 1.4.3, Camag),slit dimensions were 4.00 × 0.45 mm and deuterium lamp was used as a radiation source.

The identification of the protocatechuic acid in sample densitogram was confirmed by compairing the Rf value with that obtained from pure marker. The identity of protocaechuic acid in the hydro alcoholic extract was confirmed by comparing the UV absorption spectra with that of standard using a CAMAG TLC scanner 3.

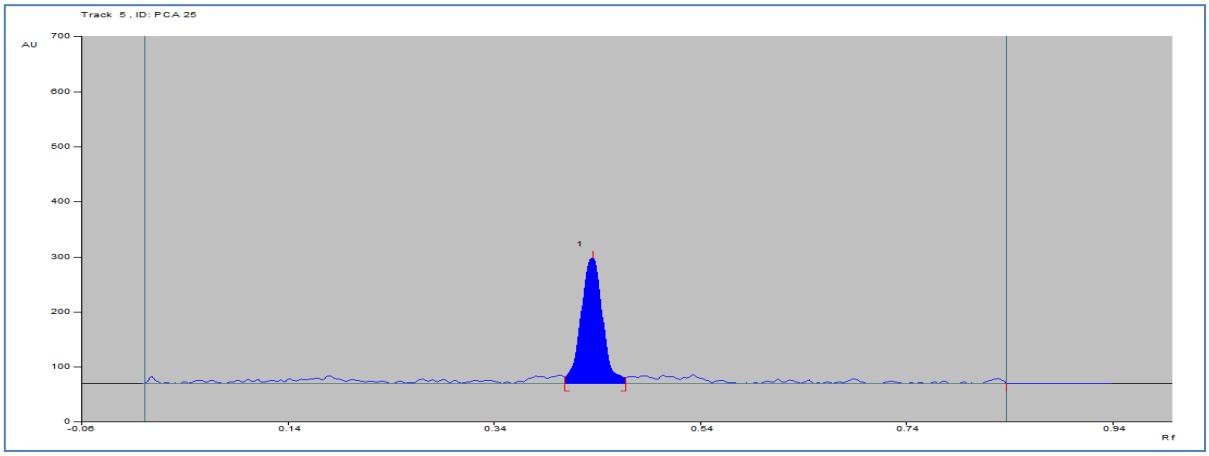


Figure 2: HPTLC densitogram of protocatechuic acid (Rf =0.52)

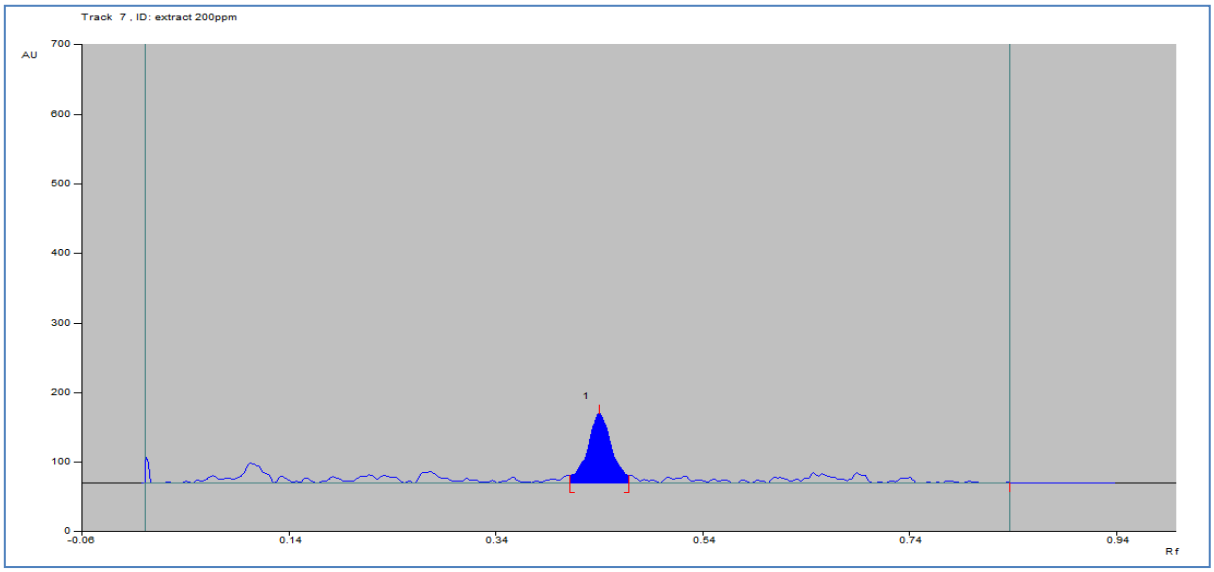


Figure3: Typical HPTLC densitogram obtained from extract showing peak at Rf = 0.52

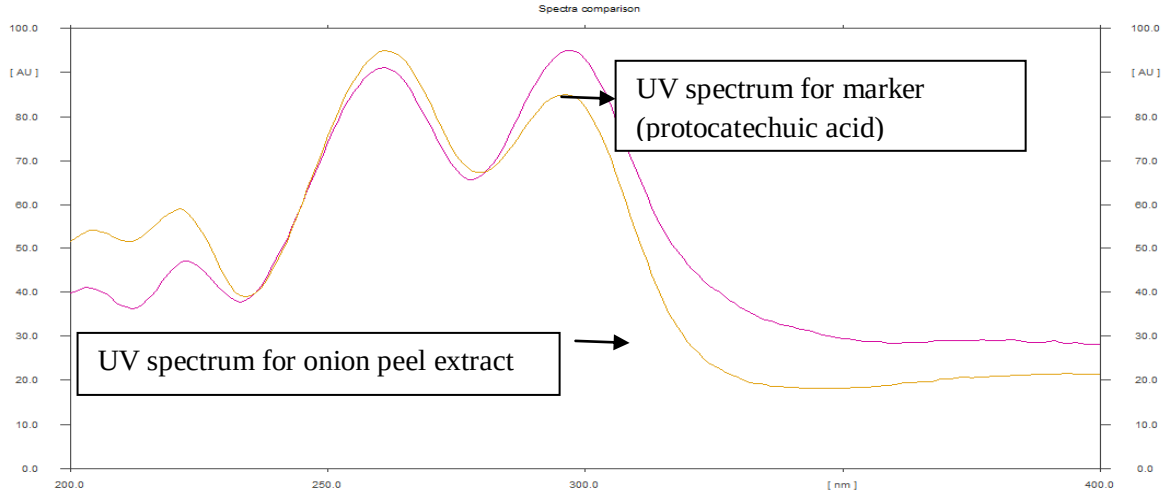


Fig.4. UV spectrum match of protocatechuic acid marker and onion peel extract at Rf. 0.52

Regression data obtained from calibration curve demonstrated excellent linear relationship over 100-500ng/ band concentration range. The linear regression equation was found to be

y = 12.436x +176.82 having correlation coefficient 0.9949.

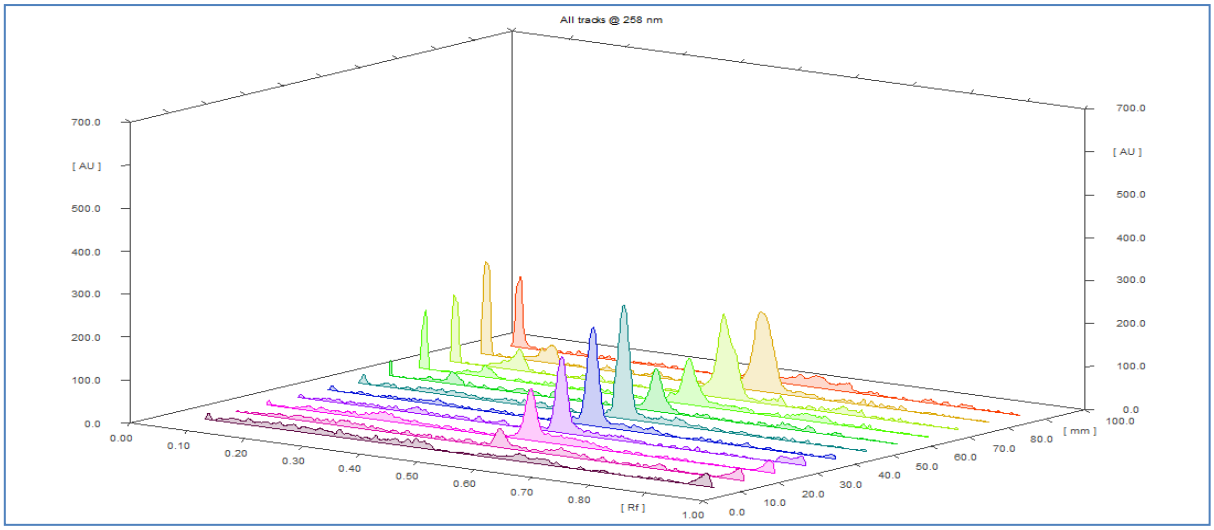


Fig.5. 3D densitogram of protocatechuic acid (track 1 methanol, track 2 to 6 std. protocatechuic acid 100-500ng/band, track 7-8 and 9-10 onion peel extract 2000 and 4000ng/band)

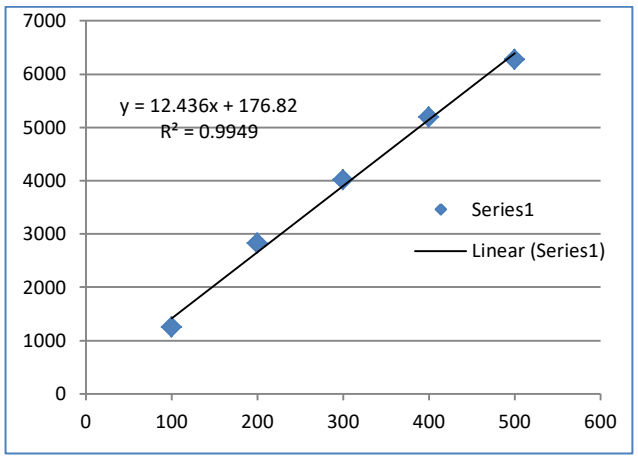


Fig.no. 6: Calibration curve of PCA (100-500ng/band)

With the help of above statistical data, the content of protocatechuic acid was determined in the hydroalcoholic onion peel plant extract which was found to be 6.5%. The HPTLC data obtained for the quantification of protocatechuic acid is summarized in Table 1. HPTLC analysis was carried out to determine the content and to quantify the protocatechuic acid present in onion peel extract.

Table no.1: HPTLC data for Protocatechuic acid

Sr. No	Validation Parameters	Protocatechuic acid
1	Linearity Equation (r^2) Range	y = 12.43x + 176.8 R^2 = 0.994 100-500 ng/ band
2	Precision	(% RSD)
	a) Intra day	1.44
	b) Interday	1.11
		0.85
		1.13
3	Accuracy	% Recovery
	80%	99.84
	100	100.22
	120%	99.80
4	Limit of Detection	3.74 ng/band
5	Limit of Quantitation	11.35ng/band
6	Specificity	Specific
7	Robustness	Robust
8	Solution stability	Stable

Hepatoprotective activity[15-24]

Wister rats of either sex weighing around 150 to 200 gm housed in standard conditions of temperature (22 ± 2ºC), relative humidity (55 ± 5%), and light (12hrs light/dark cycles) were used. They were feed with standard pellet diet and water ad libitum. The experimental protocol was approved by Institutational Animal Ethical Committee as per the guidelines of CPCSEA, Government of India (protocol no. CPCSEA/IAEC/QA-02/01-2K18)

Experimental design

A total of 30 rats were divided into 5 groups of 6 rats each. Group I served as normal control and received only the vehicle, Group II received CCl_4 1 mL/kg (1:1 of CCl_4 in olive oil) i.p. once daily for 7 days. Group III received CCl_4 1 mL/kg (1:1 of CCl_4 in olive oil) i.p. and silymarin as standard drug 100 mg/kg orally (p.o.) for 7 days. Groups IV, V, were administered hydro alcoholic onion peels extract 300 and 600 mg/kg body weight p.o.[15], as low and high dosage respectively and dose of 1 mL/kg i.p. of CCl4 (1:1 of CCl4 in olive oil) for 7 days.

All rats were sacrificed by cervical dislocation 24 hrs after the last treatment. Just before sacrifice, blood was collected from the retro-orbital sinus plexus under mild ether anaesthesia. Collected blood was allowed to clot and serum was separated at 3500 rpm for 15 min to carry out further biochemical investigations such as SGPT, SGOT, total protein and billirubin. Liver was dissected out and used for histopathological examination.

Histopathological examination of Liver

After decapitation, liver from each rat was collected, rinsed in 0.9% saline and fixed in 10% formalin. After fixation, these tissues were trimmed and processed. Tissue processing was done to dehydrate in ascending grade of alcohol, clearing in xylene and embedded in paraffin wax. Paraffin wax embedded tissue were sections at 3-5µm thickness with rotary microtome. Slides were stained with hematoxylin and eosin stain. The prepared slides were examined under microscope.

Statistical analysis:

Results were expressed as Mean ± S.E.M. The data was analysed by using one way ANOVA (analysis of variance) followed by Tukey-Kramer's test and $p < 0.05$ was considered as statistically significant.

RESULTS

In the present investigations rats treated with chronic dose of CCL_4 developed significant hepatic damage which was observed through a significant increase in the concentration of SGOT, SGPT and total billirubin. While there was substantial reduction in the content of the total protein. The treatment with hydro alcoholic extract of onion peels in CCL_4 treated group result in decreased in hepatic injury to a considerable extent which was reflected by the ability of the extract to lower the elevated serum enzyme levels. The increased level of SGOT, SGPT, and total billirubin in serum is indicative of cellular leakage and loss of functional integrity of cell membrane in liver. With respect to that extract mediated reduction in the level of SGOT, SGPT and total billirubin towards the respective normal values is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by CCL_4. This effect is in agreement with commonly accepted view that serum levels of transaminase return to normal level with healing of hepatic parenchyma and the regeneration of hepatocyte.

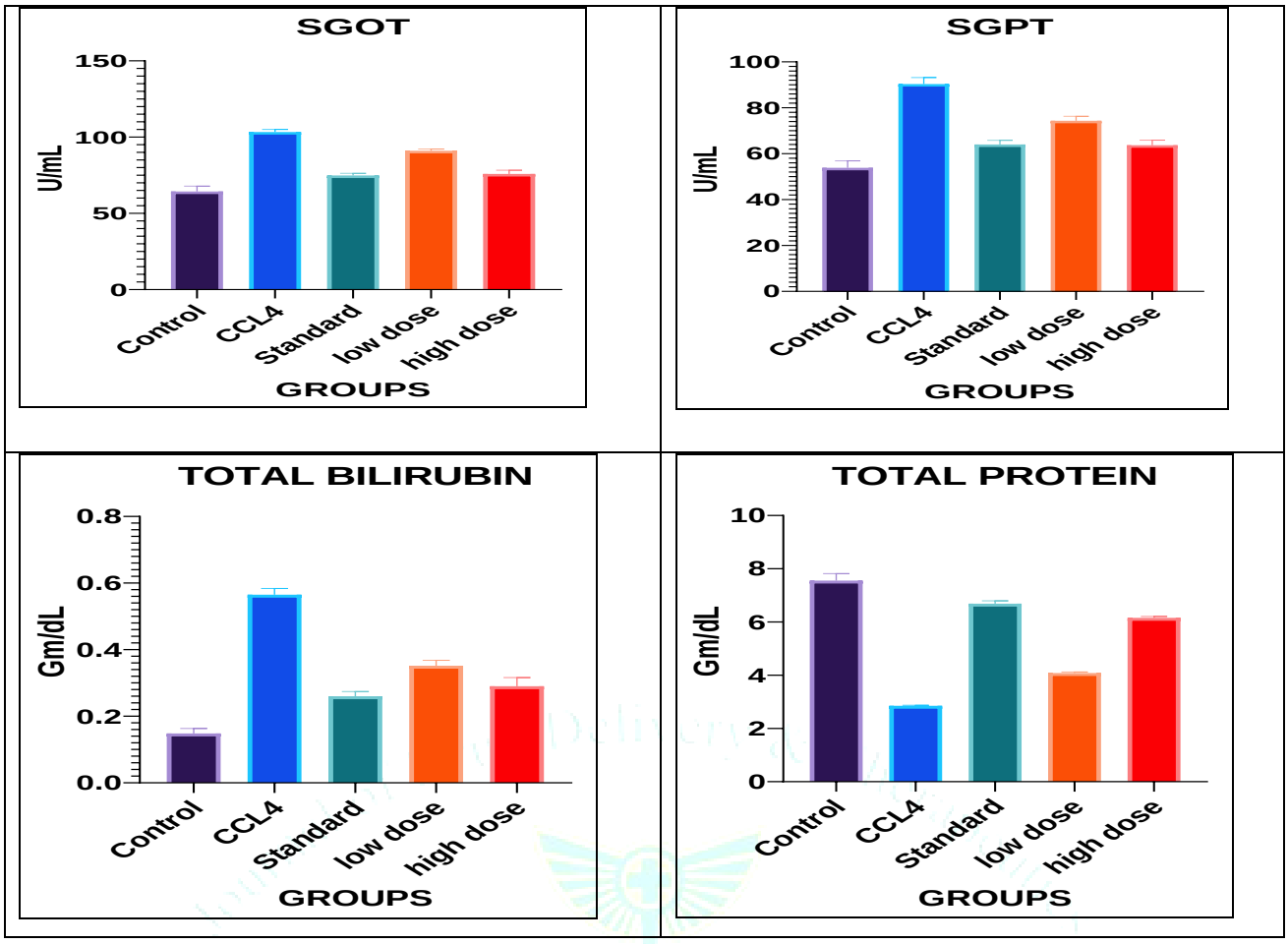
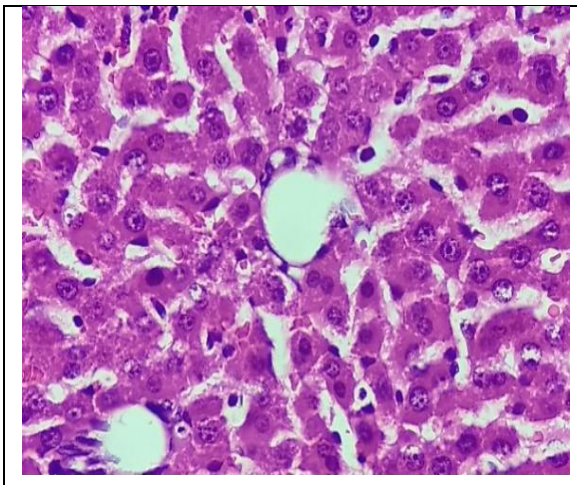


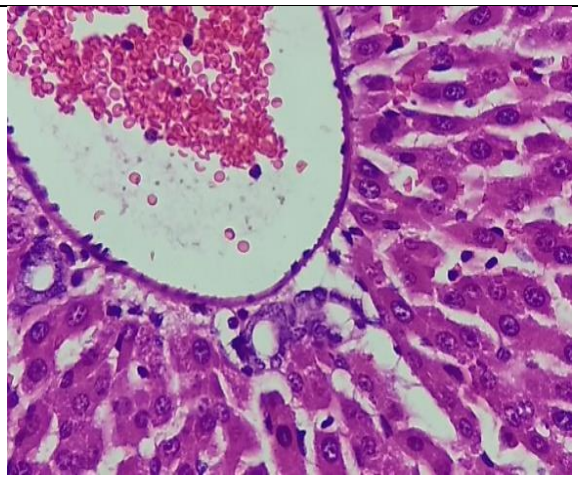
Fig.no.7: Effect of hydroalcoholic extract of *onion peels* on SGOT, SGPT, Toal protein and Total Bilirubin. Values are considered as Mean ± S.E.M. (n = 6), one way ANOVA followed by the Tukey- Kramer's Test.

Histopathological Examination

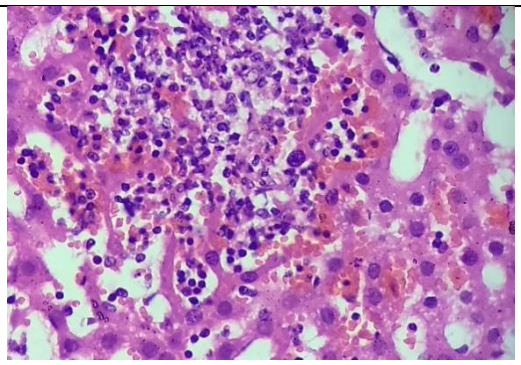
Microscopic examination of liver treated with CCL_4 showed mild hepatocellular necrosis with infiltration of inflammatory cells when compared with control group. Severity of hepatocellular necrosis with infiltration of inflammatory cells was decreased in animals treated with Standard and Extract given at 300 mg/kg body weight and animals treated with Extract at 600 mg/kg did not show any hepatic changes when compared with control group.



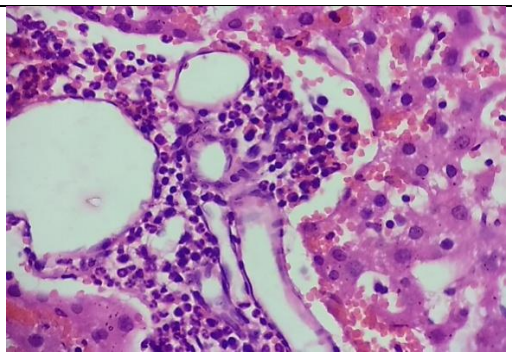
Control; Liver; Male: Showing normal hepatic parenchyma. 40 X, H & E



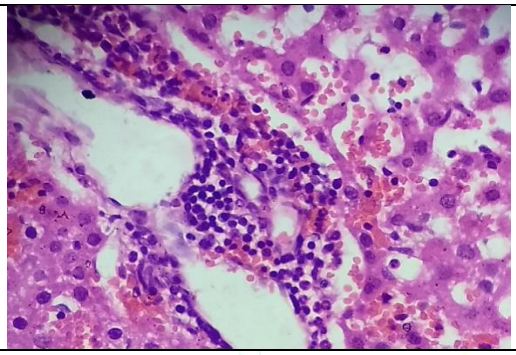
Control; Liver; Female: Showing normal hepatic parenchyma. 40 X, H & E



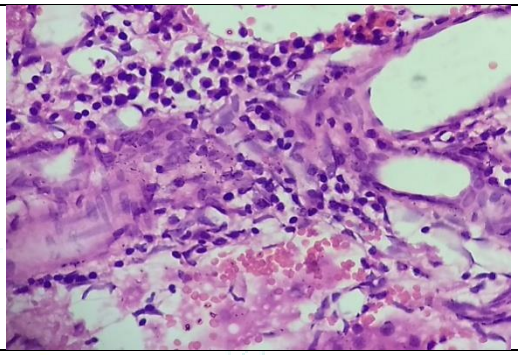
CCl_4; Liver; Male: Showing hepatocellular necrosis with infiltration of inflammatory cells (Mild). 40 X, H & E



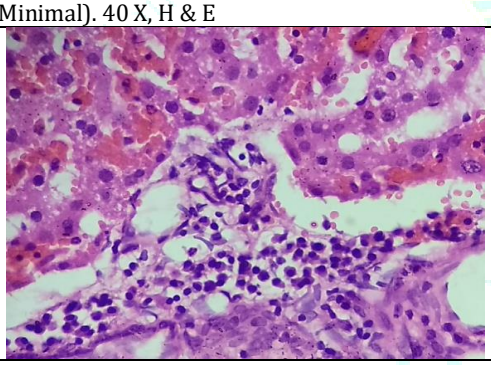
CCl_4 ; Liver; Female: Showing hepatocellular necrosis with infiltration of inflammatory cells (Mild). 40 X, H & E



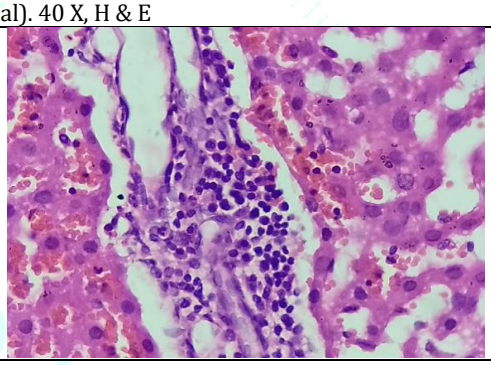
Standard (Silymarin); Liver; Male: Showing hepatocellular necrosis with infiltration of inflammatory cells (Minimal). 40 X, H & E



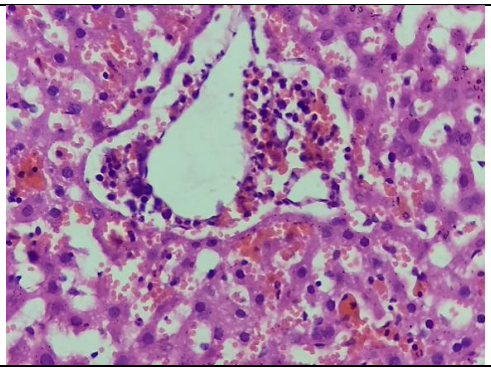
Standard (Silymarin); Liver; Female: Showing hepatocellular necrosis with infiltration of inflammatory cells (Minimal). 40 X, H & E



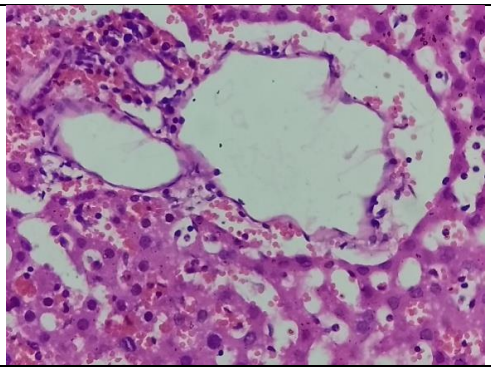
Low Dose; Liver; Male: Showing hepatocellular necrosis with infiltration of inflammatory cells (Minimal). 40 X, H & E



Low Dose; Liver; Female: Showing hepatocellular necrosis with infiltration of inflammatory cells (Minimal). 40 X, H & E



High Dose; Liver; Male: Showing normal hepatic parenchyma. 40 X, H & E



High Dose; Liver; Female: Showing normal hepatic parenchyma. 40 X, H & E

DISCUSSION:

CCl_4 is a hepatotoxin used extensively for inducing liver injury in various experimental models to elucidate the mechanisms underlying hepatotoxicity. CCl_4 mediated hepatotoxicity is developed from the biotransformation of CCl_4 by cytochrome P450 2E1 to the trichloromethyl free radical (•CCl3). Hepatocyte damage is characterised by different hepatic marker enzymes (SGPT, SGOT) and the levels of total billirubin and total protein. When liver cells

are damaged, these enzymes leak into the bloodstream from liver tissue and produce markedly elevated serum levels. Both SGOT and SGPT are associated with liver parenchymal cells. SGPT is found predominantly in the liver with negligible quantities found in heart, kidneys and skeletal muscles, whereas SGOT is found in liver, cardiac muscles, skeletal muscles, brain, kidney and red blood cells. Thus SGPT is a more specific indicator of liver intoxication as levels of SGOT may also be increased in diseases affecting other organs.

On the other hand, billirubin levels are related to the functions of hepatic cell. Elevation in level is due to increased synthesis, in presence of increased biliary pressure. Our experiment showed that rats intoxicated with CCL_4 develop a significant liver necrosis which was evidenced by increased activities of hepatic marker enzymes (SGPT, SGOT) and the levels of total billirubin, whereas levels of total protein were decreased due to liver injury. The result of this study showed that after administration with *onion peels extract* the activities of the serum marker enzymes SGPT, SGOT and the levels of total billirubin and total protein were restored to normal level, thus indicating *onion peels extract* preserved the structural integrity of hepatocellular components and protected the liver from the harmful effect of this hepatotoxin.

The above inferences were further confirmed by histopathological studies. The result of the histopathology showed that CCl_4 administration caused severe acute liver damage in rats which is characterised by hepatic cell necrosis, ballooning degeneration, fatty changes or inflammatory cell infiltration and other histological manifestations, which were consistent with previous findings. After treatment with *onion peels extract* hepatic injury caused by CCl_4 administration was significantly prevented and the structure of hepatocytes was almost restored to normal. The results of the different biochemical parameters and histopathological findings are co-related well with each other, which indicated that *onion peels extract* possesses significant hepatoprotective effect in CCl_4-intoxicated liver injury in rats.

CONCLUSION:

The present study demonstrated that the hydroalcoholic extract obtained from onion peels contains Protocatechuic acid as active phytoconstituent and has significant hepatoprotective activities against CCl_4-induced hepatotoxicity in Wistar albino rats. The hepatoprotective activity of onion peels extract due to the presence of protocatechuic acid in the extract which was determined by HPTLC method. Therefore, the present study shows that there is a prospective future in the use of Onion peel as a source of natural medicine for curing hepatic diseases.

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