A Comparative Study Among Selected Fruit Peel Extracts And Their Poly Herbal Mixture's Effects On CCL4-Induced Liver Toxicity

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ABSTRACT

Context: One of the leading causes of death in India is severe hepatic dysfunction. The primary goal of this study is to determine whether the polyherbal combination of chosen fruit peel extracts and hepatoprotective efficacy. Environment and Design: 1. With CCl4, induce hepatotoxicity 2. Investigation of the PHM and hepatoprotective properties of certain peel extracts. 3 Calculating different enzymes to determine liver function. Materials and Procedures After inducing hepatotoxicity, male Wistar albino rats (180-200 g) were separated into 12 groups and treated with PHM and specific extracts. Blood and liver samples were then taken and analysed on the 14th day. The experimental findings were reported as the mean of the statistical analysis (SEM). ANOVA was applied to the data where appropriate.Results and Discussion: All the selected plant extracts and PHM shown to prevent the leakage of SGOT, SGPT, and ALP in CCl4 induced hepatotoxicity. The order of potency was PHM>APMP>APCM>APMI>APAC. The APMP was the better hepatoprotective among the selected plant extracts. The activities might be due to the conditioning of hepatocytes by protecting the integrity of the membrane from CCl_4 induced leakage of serum markers into circulation. All the selected plant extracts and PHM shown to revert back the SOD, CAT and GSH to the normal values in CCl_4 induced rats in a dose dependent manner in liver homogenates.

Key words: Fruit peels, Hepatoprotective effects, hepatotoxicity, poly herbal mixture, non enzymatic antioxidants.

INTRODUCTION

The detoxification of xenobiotics, environmental contaminants, and chemotherapeutic drugs as well as the metabolism of several exogenous and endogenous substances is a major duty for the liver, the primary organ of metabolism and excretion. As a result, this organ may become exposed to ROS attack. Hepatocellular carcinoma, jaundice, non-alcoholic fatty liver disease, drug-induced hepatic problems, and other problems linked to cellular necrosis, increased tissue lipid peroxidation, decreased tissue antioxidant levels (GSH, SOD, CAT), elevated liver enzymes (SGOT, SGPT, ALP), and other markers (TB, cholesterol, etc.) are all attributed to oxidative stress [1]. One of the most prevalent diseases and a significant factor in morbidity and death in both India and the rest of the world are hepatic issues. Drug induced hepatotoxicity contributes more than 50% of acute liver injury cases; hence management of liver diseases is still a challenge to synthetic drugs. Therefore the search for newer, effective and safe alternatives to the current drugs is on surge. Herbal medicines which are known to play a vital role in the management of various ailments including liver disorders are found to be the safer, cheaper and beneficial alternatives to toxic modern medicines [2]. Fruit by products such as seeds, peels usually been discarded and currently pose a serious disposal problem in food and agricultural industries[3]. Therefore extensive researches on utilizing fruit peel wastes are being carried out worldwide [4]. The peel was found to contain much higher beneficial compounds that possessed antioxidant capacities compared to other fruit parts [5]. The preliminary phytochemical analysis of fruit peels of *Ananas comosus* revealed the presence of alkaloids, proteins, tannins, and glycosides etc which are responsible for pharmacological activities [6]. Peels of apple are rich sources of bioactive molecules that reflect high antioxidant activity [7]. Studies have shown that peels of *Cucumis melo* exhibihted properties that can promote antioxidative reactions and anticancer effects. The presence of phenolic compounds possibly explains the antioxidant and antiproliferative potential [8]. Polyherbal mixtures are also claimed to posses' beneficial activities in the treatment of diseases. The phytochemical analysis of various extracts of kiwi fruit peels showed presence of alkaloids, phenols and tannins and showed significant level of inhibition in free radical scavenging assays [9].

MATERIALS AND METHODS

Chemicals

Silymarin obtained from Merck chemical company, Mumbai. All other chemicals and kits used for this study were analytical grade.

Preparation of peel extracts and PHM

Fresh fruits were purchased from local market and they are washed under running tap water and their peels were separated. They were shade dried separately over a month. The dried peels were powdered using a mechanical grinder. Each of the samples was approximately weighed and subjected to extraction by cold maceration with water (80 parts and 20 parts ethanol) for 72hrs. After 72hrs the sample were filtered and concentrated. The obtained aqueous extracts were powdered and stored for further use. Poly herbal mixture was prepared by taking equal quantities of the selected fruit peel extracts

APAC: Aqueous peel extract of *Annona squmosa*
APMI: Aqueous peel extract of *Mangifera indica*
APCM: Aqueous peel extract of *Cucumis melo*
APMP: Aqueous peel extract of *Malus pumila*
PHM: Poly Herbal Mixture

Preliminary phytochemical Analysis

The aqueous peel fraction of *Ananas comosus* showed presence of alkaloids, phenolics compounds, saponins, triterpenes, carbohydrates and tannins. The aqueous fraction of *Mangifera indica* showed presence of phenolics, triterpenes and steroids. The *Cucumis melo* aqueous fraction showed presence of flavanoids, saponins, carbohydrates, steroids and tannins. *Malus pumila* were tested for phytochemical screening resulted that the aqueous fraction showed presence of glycosides, flavanoids, terpenoids and steroids. (Table 1)

Experimental animals

Animal were obtained from the Mahaveer laboratories, Hyderabad. Albino Wistar rats (180-200 g) of male were used in the present study. The animals were housed under standard environmental conditions (23±1ºC) with relative humidity of 50±10% and maintain 12:12 dark and light cycle, maintained with free access to water and *ad libitum* standard laboratory diet (70% carbohydrates, 25% proteins, 5% lipids (Hindustan liver Bangalore). After randomization before the experiment, the rats were acclimatized for a period of two weeks. The animal housing, handling and all the experimental procedures were carried out in strict compliance with the Institutional Animal Ethics Committee regulations (Reg. No. 516/01/A/CPSCEA).

Acute toxicity studies (OECD 423)

Acute toxicity studies were performed on the aqueous extract following OECD guidelines (420). The dosages for the pharmacological studies were selected as $1/5^{th}$ and $1/10^{th}$ of the highest dose (2000 mg/kg) administered.

Experimental protocol

Induction of hepatotoxicity

The rats were fasted for 18 h prior to the experiment with water *ad libitum*.0.5 ml/kg body weight of carbon tetrachloride (1:1 ratios of CCl4 in olive oil) was given twice a week for a period of 14 days.

Experimental Design

After the induction of CCl4, the rats were grouped in to twelve different groups of each containing six animals.

Group 1: Served as control rats received distilled water and fed on normal diet.
Group 2: Served as Disease control rats
Group 3: Received silymarin 50 mg/kg (p.o.) for 14 days
Group 4: Treatment with aqueous peel extract of *Ananas comosus* (APAC) (200 mg/kg)
Group 5: Treatment with aqueous peel extract of *Ananas comosus* (APAC) (400 mg/kg)
Group 6: Treatment with aqueous peel extract of *Mangifera indica* (APMI) (200 mg/kg)
Group 7: Treatment with aqueous peel extract of *Mangifera indica* (APMI) (400 mg/kg)
Group 8: Treatment with aqueous peel extract of *Cucumis melo* (APCM) (200 mg/kg)
Group 9: Treatment with aqueous peel extract of *Cucumis melo* (APCM) (400 mg/kg)
Group 10: Treatment with aqueous peel extract of *Malus pumila* (APMP) (200 mg/kg)
Group 11: Treatment with aqueous peel extract of *Malus pumila* (APMP) (400 mg/kg)
Group 12: Treatment with poly herbal mixture (PHM) (200 mg/kg)

Collection of blood

The treatment for a period of 14 days once daily with selected fruit peel extracts. On the 14th day, blood samples were collected from all animals by puncturing retro-orbital plexus under mild ether anaesthesia, later animals were sacrificed and liver tissues were collected.

Isolation of liver

Liver was carefully excised and washed in ice cold normal saline and pressed between filter paper pads and a portion of liver (one animal of each group) was preserved in 10% neutral formalin for histopathology studies.

Assessment of biochemical parameters

The blood samples were centrifuged at 3000 rpm (micro centrifuge) for 10 min to separate serum. The serum samples thus collected were used for the estimation of serum glutamate oxaloacetate transaminase (SGOT)**,** serum glutamate pyruvate transaminase (SGPT), alkaline phosphate (ALP), bilirubin (TB) [10-12]. A 10% w/v liver homogenate was prepared with 0.1M phosphate buffer (p^H 7.4)and centrifuged at 3000rpm for 10 min at 4 ^{0}C and supernatant was used for the further estimation of GSH [10], SOD, CAT levels [13,14]. All these methods were performed by following the standard methods of international Federation of clinical chemistry (IFCC). All the parameters were estimated using standard diagnostic kits.

Histopathological studies

Collected liver tissues were processed and embedded in paraffin wax. Sections of 5-6µm in thickness was cut and stained in hematoxylin and eosin dye, were observed microscopically for histopathologicla changes [15].

Statistical analysis

The data of the biochemical parameters were expressed as mean ± SEM. Results were analyzed statistically using one way analysis of variance (ANOVA) followed by DUNNETT's multiple comparison test. The minimum level of significance was fixed at $p < 0.05$.

Results

The aqueous peel extracts of the selected fruits *Ananas comosus*, *Mangifera indica*, *Cucumis melo*, *Malus pumila* showed significant hepatoprotective action. The results were summarized in tables 2 to 8. At the end of 14 days treatment, blood samples of disease control group showed significant increase in the serum levels of biochemical parameters as compared to normal control group animals, where as blood samples of animals treated with different doses (200, 400 mg/kg) of selected fruit peel extracts showed significant decrease in the levels of SGOT, SGPT, ALP, total bilirbin when compared with disease control group. Similarly the liver homogenate showed significant increase in SOD, CAT, GSH levels as compared to the control group.

Table 1 Phytoconstituents present in selected aqueous peel extracts

Phytoconstituents	*Ananas comosus*	*Mangifera indica*	*Cucumis melo*	*Malus pumila*
Alkaloids	+	-	-	-
Glycosides	_	-	-	+
Phenolics	+	+	+	+
Carbohydrates	+	-	+	-
Amino acids	_	-	-	-
Saponins	_	-	+	-
Tannins	+	-	+	-

Table 2 Effect of selected plant extracts and polyherbal mixture on SGOT levels in CCl4 induced hepatotoxicity in rats.

Groups	SGOT (mg/dL)						Mean±SEM
	R1	R2	R3	R4	R5	R6	
Control	74	65	78	67	75	78	72.83±2.27
Disease Control	125	129	133	121	141	135	130.66±2.94
Silymarin	85	74	67	88	69	70	75.50±3.62*
APAC (200 mg/kg)	108	111	94	101	106	97	102.83±2.71*
APAC (400 mg/kg)	97	91	99	87	92	96	93.66±1.82*
APMI (200 mg/kg)	100	106	111	94	89	84	97.33±4.19*
APMI (400 mg/kg)	99	94	85	87	89	91	90.83±2.07*
APCM (200 mg/kg)	106	102	109	100	92	89	99.66±3.19*
APCM (400 mg/kg)	88	95	89	80	92	87	88.50±2.07*
APMP (200 mg/kg)	110	106	94	97	87	93	97.83±3.51*
APMP (400 mg/kg)	87	93	80	86	82	99	87.83±2.89*
PHM	68	86	71	80	77	63	74.16±3.43*

*P<0.05** significance followed by one way ANOVA followed by DUNNETT's multiple comparison test.

Table 3 Effect of selected plant extracts and polyherbal mixture on SGPT levels in CCl_4-induced hepatotoxicity in rats.

Groups	SGPT (mg/dL)						Mean±SEM
	R1	R2	R3	R4	R5	R6	
Control	38	42	33	41	39	37	38.33±1.30
Disease Control	77	75	80	68	88	76	77.33±2.67
Silymarin	37	47	49	44	38	40	42.50±2.01*
APAC (200 mg/kg)	61	59	66	52	66	64	61.33±2.18*
APAC(400 mg/kg)	52	62	54	58	56	49	55.16±1.62*
APMI (200 mg/kg)	56	59	55	59	60	50	56.50±1.52*
APMI (400 mg/kg)	49	52	49	54	59	52	52.50±1.52*
APCM (200 mg/kg)	52	64	69	62	54	49	58.33±3.19*
APCM (400 mg/kg)	42	49	58	57	52	47	50.83±2.49*
APMP (200 mg/kg)	56	52	58	59	60	50	55.83±1.64*
APMP (400 mg/kg)	49	51	41	49	54	52	49.33±1.83*
PHM	38	43	49	52	51	45	46.33±2.18*

*P<0.05** significance followed by one way ANOVA followed by DUNNETT's multiple comparison test.

Table 4 Effect of selected plant extracts and polyherbal mixture on ALP levels in CCl_4 induced hepatotoxicity in rats.

Groups	ALP (mg/dL)						Mean±SEM
	R1	R2	R3	R4	R5	R6	
Control	192	175	166	181	172	166	175.33±4.06
Disease Control	277	281	269	274	281	269	275.16±2.22
Silymarin	190	182	189	177	187	204	188.16±3.73*
APAC (200 mg/kg)	222	219	227	207	229	231	222.50±3.59*
APAC (400 mg/kg)	212	202	208	218	197	204	206.83±3.06*
APMI (200 mg/kg)	218	246	230	244	231	201	228.33±6.88*
APMI (400 mg/kg)	194	210	201	192	189	195	196.83±3.09*
APCM (200 mg/kg)	211	214	205	216	201	208	209.16±2.30*
APCM (400 mg/kg)	204	184	192	172	190	201	190.50±4.75*
APMP (200 mg/kg)	222	231	204	214	222	216	218.16±3.72*
APMP (400 mg/kg)	190	184	175	192	184	177	183.66±2.76*
PHM	182	179	164	177	176	184	177.00±2.87*

*P<0.05** significance followed by one way ANOVA followed by DUNNETT's multiple comparison test.

Table 5 Effect of selected plant extracts and polyherbal mixture on bilirubin levels in CCl4 induced hepatotoxicity in rats.

Groups	Bilirubin (mg/dL)						Mean±SEM
	R1	R2	R3	R4	R5	R6	
Control	0.76	0.72	0.79	0.65	0.62	0.700	0.70±0.02
Disease Control	1.87	1.81	1.77	1.94	1.82	1.630	1.80±0.04
Silymarin	0.63	0.74	0.81	0.75	0.82	0.670	0.73±0.03*
APAC (200 mg/kg)	1.12	1.03	0.99	1.07	1.09	1.17	1.07±0.02*
APAC (400 mg/kg)	0.91	0.99	0.84	0.89	0.94	1.00	0.92±0.02*
APMI (200 mg/kg)	0.84	0.87	0.91	0.81	0.94	0.970	0.89±0.02*
APMI (400 mg/kg)	0.81	0.93	0.78	0.88	0.74	0.820	0.82±0.02*
APCM (200 mg/kg)	0.85	0.90	0.84	0.98	0.91	0.950	0.90±0.02*
APCM (400 mg/kg)	0.90	0.76	0.81	0.77	0.79	0.740	0.79±0.02*
APMP (200 mg/kg)	0.94	0.93	0.84	0.86	0.88	0.940	0.89±0.01*
APMP (400 mg/kg)	0.81	0.74	0.77	0.67	0.72	0.680	0.73±0.02*
PHM	0.62	0.74	0.66	0.70	0.79	0.810	0.72±0.03*

*P<0.05** significance followed by one way ANOVA followed by DUNNETT's multiple comparison test.

Table 6 Effect of selected plant extracts and polyherbal mixture on SOD levels in CCl_4 induced hepatotoxicity in rats.

Groups	SOD						Mean±SEM
	R1	R2	R3	R4	R5	R6	
Control	3.12	3.06	2.64	2.98	3.10	3.12	3.00±0.07
Disease Control	0.89	1.02	1.10	1.16	1.12	0.94	1.03±0.04
Silymarin	2.56	2.22	2.34	2.49	2.46	2.51	2.43±0.05*
APAC (200 mg/kg)	1.72	1.85	1.79	1.80	1.62	1.77	1.75±0.02*
APAC (400 mg/kg)	2.09	2.11	1.99	2.00	2.07	1.94	2.03±0.02*
APMI (200 mg/kg)	1.86	1.78	1.84	1.64	1.67	1.90	1.78±0.04*
APMI (400 mg/kg)	2.13	2.21	2.31	2.27	2.06	2.16	2.19±0.03*
APCM (200 mg/kg)	1.94	2.11	2.03	1.99	2.06	2.13	2.04±0.02*
APCM (400 mg/kg)	2.13	2.30	2.16	2.21	2.24	2.35	2.23±0.03*
APMP (200 mg/kg)	1.94	1.86	1.78	2.00	2.06	1.92	1.92±0.04*
APMP (400 mg/kg)	2.23	2.42	2.13	2.16	2.34	2.27	2.25±0.04*
PHM	2.34	2.49	2.44	3.16	2.64	2.99	2.67±0.13*

*P<0.05** significance followed by one way ANOVA followed by DUNNETT's multiple comparison test.

Table 7 Effect of selected plant extracts and polyherbal mixture on CAT levels in CCl_4 induced hepatotoxicity in rats.

Groups	CAT						Mean±SEM
	R1	R2	R3	R4	R5	R6	
Control	1.36	1.49	1.38	1.41	1.29	1.34	1.37±0.02
Disease Control	0.20	0.16	0.25	0.30	0.29	0.19	0.23±0.02
Silymarin	0.89	0.94	0.85	0.93	0.84	0.96	0.90±0.02*
APAC (200 mg/kg)	0.39	0.41	0.52	0.49	0.54	0.37	0.45±0.02*
APAC (400 mg/kg)	0.87	0.67	0.84	0.84	1.03	0.84	0.84±0.04*
APMI (200 mg/kg)	0.46	0.59	0.39	0.50	0.48	0.57	0.49±0.0.3*
APMI (400 mg/kg)	0.92	0.89	0.86	0.94	0.99	0.87	0.91±0.02*
APCM (200 mg/kg)	0.49	0.46	0.54	0.61	0.57	0.49	0.52±0.02*
APCM (400 mg/kg)	0.94	0.84	0.89	0.84	0.92	1.03	0.91±0.02*
APMP (200 mg/kg)	0.66	0.64	0.57	0.54	0.52	0.61	0.59±0.02*
APMP (400 mg/kg)	0.84	0.88	1.12	0.82	0.94	0.94	0.92±0.04*
PHM	1.20	1.34	0.94	1.06	1.31	0.92	1.12±0.07*

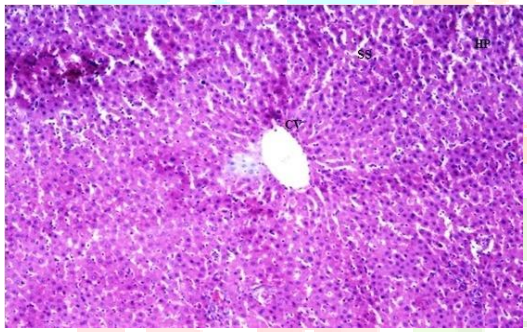
*P<0.05** significance followed by one way ANOVA followed by DUNNETT's multiple comparison test

Table 8 Effect of selected plant extracts and polyherbal mixture on GSH levels in CCl_4 induced hepatotoxicity in rats

Groups	GSH						Mean±SEM
	R1	R2	R3	R4	R5	R6	
Control	18.65	21.16	20.46	19.87	22.16	23.16	20.91±0.66
Disease Control	13.64	15.23	14.94	14.26	15.06	14.94	14.67±0.24
Silymarin	20.64	21.94	19.64	20.64	21.06	20.46	20.73±0.30*
APAC (200 mg/kg)	17.2.3	17.13	16.94	17.64	17.01	16.49	16.90±0.20*
APAC (400 mg/kg)	18.94	20.16	18.14	19.64	19.12	19.47	19.24±0.28*
APMI (200 mg/kg)	17.86	17.94	17.64	18.64	17.26	18.22	17.92±0.19*
APMI (400 mg/kg)	21.61	20.64	19.64	18.64	18.94	19.00	19.74±0.47*
APCM (200 mg/kg)	19.67	18.23	19.19	18.91	19.06	18.22	18.88±0.23*
APCM (400 mg/kg)	21.89	19.69	18.93	19.97	19.46	20.19	20.02±0.41*
APMP (200 mg/kg)	22.22	19.29	19.87	20.03	19.88	20.11	20.23±0.41*
APMP (400 mg/kg)	21.03	22.22	20.68	21.67	18.98	19.42	20.66±0.51*
PHM	22.26	21.19	20.23	21.67	22.44	23.06	21.80±0.41*

*P<0.05** significance followed by one way ANOVA followed by DUNNETT's multiple comparison test

Histopathological studies



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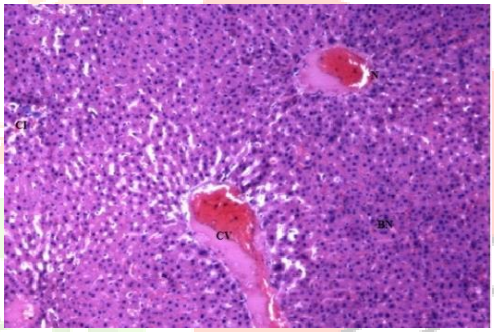

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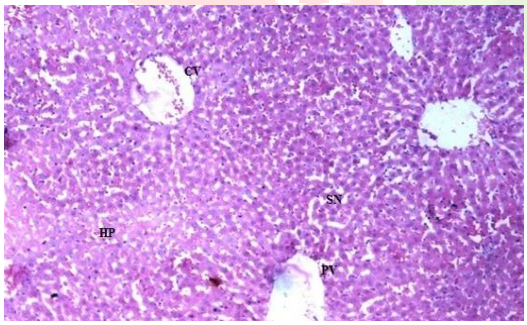

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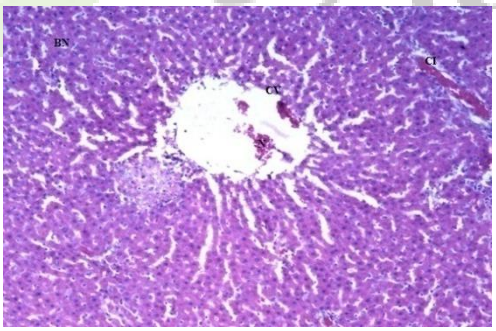

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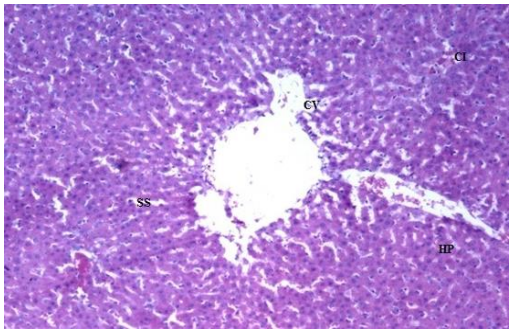

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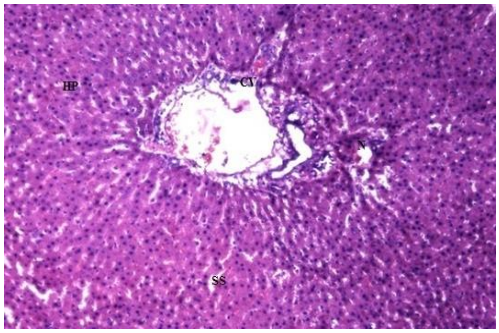

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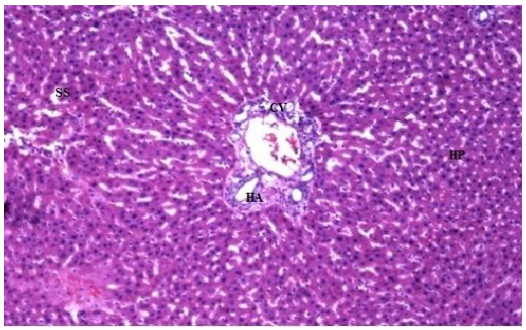
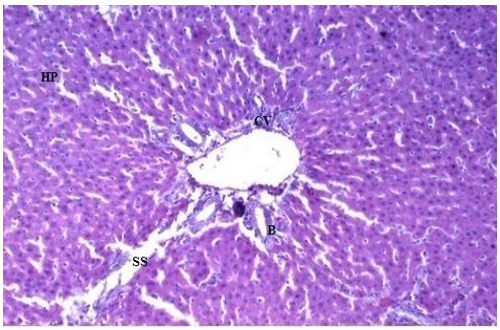
g) 

h) 

The histopathological feature, as shown in Figure a indicated the normal liver lobular architecture and cell structure of the liver and central vein in the normal control rats. In CCl_4 treated severe hepatocellular necrosis along with cellular infiltration, presence of bi nucleated cells in rats (Figure b). Treatment with Silymarin showed protection over the necrosis of the liver hepatocytes by preventing oxidation in liver cells (Figure c). In APAC treated rats with lesser vacuolar degeneration and hepatic necrosis was observed (Figure d). There was a lesser protection of cellular damage of hepatocytes around central vein was observed with APMI treatment in CCl_4 induced necrosis (Figure e). APCM treatment showed mild hepatocellular degeneration, necrosis, less inflammatory cell infiltration and well preserved hepatocytes (Figure f). The APMP treatment showed partial protection of hepatocellular infiltration, preserved central vein; hepatocytes in normal architecture (Figure g). PHM treated group showed hepatocytes, central vein and marked amelioration of hepatocellular necrosis (Figure h).

Discussion

The aqueous extracts of the selected fruit peel extracts showed potential reduction in CCl_4 induced oxidative stress. The liver damaging effect of CCl_4 is explained by its ability to produce trichloromethyl free radicals and reactive oxygen species (ROS) after being metabolized by cytochrome P450. These metabolites initiate a lipid peroxidation chain reaction and eventually lead to many chronic diseases including liver injury. Due to liver injury caused by CCl_4 overdose, the transport function of the hepatocytes gets disturbed resulting in the leakage of the plasma membrane [16], thus causing an increase in serum enzyme levels. The SGOT, SGPT and ALP are important serum enzymes usually help to detect chronic liver diseases by monitoring their concentrations.

At the same time the antioxidant defense system must be able to balance the oxidative stress and antioxidant levels which otherwise leads to events that deregulates the cellular functions leading to hepatic necrosis [17]. Among all the treatment groups the PHM was found to be more potent than standard Silymarin.

The order of potency was PHM>APMP>APCM>APMI>APAC in CCl_4 induced hepatotoxicity. (Table and Figures 2 to5,). The APMP was the better hepatoprotective among the selected plant extracts. The activities might be due to the conditioning of hepatocytes by protecting the integrity of the membrane from CCl_4 induced leakage of serum markers into circulation.

The SOD, CAT and GSH levels were decreased in CCl4 induced hepatotoxicity. This is considered as a main mechanism for centrilobular hepatic necrosis, leading to acute liver failure [18]. All the selected plant extracts and PHM shown to revert back the protein, SOD, CAT and GSH to the normal values in CCl4 induced rats in a dose dependent manner in liver homogenates. The order potency was PHM>APMP>APCM>APMI>APAC in CCl4 induced hepatotoxicity (Table and Figures 6 to 8). Among all the treatment groups the PHM was found to be more potent than standard Silymarin.

The activity might be due to the scavenging activity of superoxide, hydroxyl and hydrogen peroxide free radicals by the selected plant extracts. The preliminary phytochemical studies suggest that the antioxidant and hepatoprotective activity of the selected peel extracts might be due to the presence of phenolic compounds such as flavanoids, tannins, anthocyanidins. The present study indicates the selected fruit peel extracts and poly herbal mixture can be a potent hepatoprotective agent due to their antioxidant and anti-inflammatory actions.

Conclusion

From the results obtained it is evident that the selected fruit peel extracts and PHM are potent antioxidants, anti-inflammatory and hepatoprotective agents. Their activity can be attributed to several mechanisms. Hence further studies are encouraged to establish their clear mechanism of action. In conclusion the selected fruit peel extracts and poly herbal mixture can be a potent hepatoprotective agent due to their antioxidant and anti-inflammatory actions.

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