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IN VIVO HEPATOPROTECTIVE AND *EX-VIVO* ANTISPASMODIC ACTIVITY OF EXTRACT OF *PSIDIUM CATTLEIANUM* SABINE LEAVES IN WISTAR ALBINO RATS

Elamaran Tamil Jothi*[1], Joseph Paily[1], T. Savitha[1], C. K. Amritha[1]

[1]*Department of Pharmacology, Devaki Amma Memorial College of Pharmacy, Malappuram, Kerala, India.

ABSTRACT

The objective of the present study was to evaluate the hepatoprotective and ex-vivo antispasmodic activity of extract of *Psidium cattleianum* Sabine leaves in Wistar albino rats. The *in-vivo* hepatoprotective activity of hydroalcoholic extract of *P. cattleianum* (HAEPC) was screened by hepatotoxicity was induced by Carbon tetrachloride (0.1ml/kg b.wt with CMC (1:1), i.p) in Wistar albino rats. The study duration was 14 days. Silymarin (100mg/kg b.wt p.o) was used as the standard. The effects of HAEPC were evaluated at doses of 200 and 400mg/kg. *Ex-vivo* antispasmodic activity also performed on excised rat ileum in which Atropine was used as standard. In *in-vivo* study, there is a significant increase in the serum parameters such as SGPT, SGOT, ALP and total bilirubin were seen in rats treated with Carbon tetrachloride (0.1ml/kg/day) in negative control when compared with normal control. The increased levels of these parameters were significantly reduced in groups treated with different doses of extracts (200mg/kg and 400mg/kg). CCl4 induced liver damage is associated with the increased levels of lipid peroxidation which is significantly reduced in case of hydro alcoholic extract of *Psidium cattleianum* Sabine leaves treated rats. The catalase, glutathione peroxidase levels are elevated by administration of hydro alcoholic extract of *Psidium cattleianum* Sabine leaves. The extract also showed an antispasmodic activity on excised rat ileum with increasing concentration. Results obtained suggests that the hydroalcoholic extract of *Psidium cattleianum* Sabine leaves exhibits a significant hepatoprotective activity in a dose dependent manner and also possess an antispasmodic activity with increasing concentration.

KEYWORDS

Psidium cattleianum, Hepatoprotective activity, Antispasmodic activity, Carbon tetrachloride, Silymarin and Atropine.

Author for Correspondence:
Elamaran Tamil Jothi,
Department of Pharmacology,
Devaki Amma Memorial College of Pharmacy,
Malappuram, Kerala, India.
Email: tamilcologist@gamil.com

INTRODUCTON

The liver is the largest solid organ in the body. Liver act as giant chemical processing plant in the body and center in the regulation of body homeostasis. Hepatotoxicity refers to liver dysfunction or liver damage that is associated with an overload of drugs or xenobiotic. Chemicals that

cause liver injury are called hepatotoxins. There literally thousands of chemicals that could be toxic to the liver and a few examples of these chemicals include: carbon tetra chloride, thioacetamide, galactosamine, alcohol, paracetamol, antitubercular drugs (rifampicin, isoniazid and pyrazinamide), azathioprine, ranitidine etc[1].

Carbon tetrachloride is a well-known hepatotoxic industrial solvent. It is a simple molecule, which causes centrilobular hepatic necrosis and fatty liver. It is a very lipid soluble compound and is consequently well distributed through the body, but despites its major toxic effect is on the liver[2].

Chronic administration or exposure of CCl_4 causes liver cirrhosis, liver tumors and kidney damage. Toxicity is depend on metabolic activation by CYP2E1[3].

An antispasmodic is a pharmaceutical drug or other agent that suppresses muscle spasms. Certain types antispasmodics is used for smooth muscle relaxation, especially in tubular organs of gastrointestinal tract. The effective is for preventing spasm of the stomach, intestine and urinary bladder.

Muscle cramps are common in patients with liver disease and it influence quality of life. The exact mechanism is unclear, although a number of pathophysiological events unique to liver disease may contribute.

Modern medicines have limited success in providing effective cure and there is a severe need to develop new drugs capable of healing toxic liver damages[4] and antispasmodic activity.

Psidium cattleianum Sabine, Myrtacae, is native to Atlantic coast of Brazil. This low profile plant is an untapped source of therapeutically relevant phytochemicals[5]. Several studies reported the presence of flavonoids, saponins, cardiac glycosides, triterpenoids, sesquiterpine, phenolic compounds, anthraquinones, tannins, catechins, vitamin C, monosaccharides, fats, fixed oils, volatile oil in various extracts of *P.cattleianum* Sabine leaves[6-8].

According to the data reported in ethnobotaniacal studies, *P. cattleianum* has been used as antihaemorrhagic, antispasmodic, antidiarrhoeal, antidiabetic etc[9].

MATERIAL AND METHODS

Plant materials

Fresh leaves of *Psidium cattleianum* Sabine was collected from Wayanad, Kerala. The voucher specimen was deposited in the Herbarium of Department of Botany, University of Calicut (No. 148219).

Preparation of extract

Hexane, chloroform and 70% aqueous ethanol extracts of *P. cattleianum* Sabine was prepared by successive solvent extraction in soxhlet apparatus. The filtrates obtained was distilled and concentrated under reduced pressure at low temperature and finally freeze dried.

In vivo pharmacological evaluation

Carbon tetrachloride induced hepatotoxicity

Study design

The study consisted of 30 Albino Wistar rats equally divided into five groups designated as group 1 (normal control group), group 2 (Negative control group), group 3 (standard group), group 4 and 5 served as treatment group. Following CCl_4 administration, standard group was administered with Silymarin at a dose of 100mg/kg per oral and treatment groups 4 and 5 were administered leaves extracts with dose of 200mg/kg (low dose) and 400mg/kg (high dose) per oral for 15 days. On 15th day blood was collected by retro orbital puncture under mild anesthesia (Thiopentone sodium). The serum was separated and used for the estimation of hepatic biochemical markers. The animals were then sacrificed, liver excised and weighed and further used for histopathological studies[10].

Estimation of biochemical parameters

Biochemical parameters like SGOT, SGPT, ALP, Total Bilirubin were estimated by commercial kits (Pathozyme Diagnostic) as per the manufactures instructions[11-16].

Estimation of oxidative stress markers

Catalase

The assay mixture consisted of 2 ml phosphate buffer (0.1 M, $_p$H 7.4), 1 ml H_2O_2 and 40µl of enzyme extract. Absorbance is measured at 240 nm.

Glutathione peroxidase

A known volume of homogenate was added to incubation medium (o.4 ml of buffer, o.2 ml of

sodium azide, 0.2 ml of EDTA, 0.2 ml of hydrogen peroxide and 0.2 ml of reduced glutathione) and made up to 2 ml with water. Incubated at 37ºC for 90 and 180 min and terminated by adding 1 ml of precipitating agent. Centrifuged and to the supernatant 60 ml of disodium hydrogen phosphate was added.1 ml of DTNB added prior to the colourimetric analysis. The absorbance measured at 412 nm.

Histopathology

Excised liver were fixed in 10% normal buffered formalin. They were subjected to tissue processing by dehydration through ascending ethanol series, clearing in toluene and embedding completely in paraffin wax into blocks. The blocks were serially sectioned at 5µm thickness using semi-automatic rotary microtome and were mounted on microscope slides. The slides were stained with H and E stains and then observed under light microscope connected to camera to capture images[17-20].

***Ex- vivo* anti spasmodic activity**

Evaluation of Antispasmodic activity of HAEPC on isolated rat ileum preparation against acetyl choline as spasmogen. Rats were sacrificed by cervical displacement followed by exsanguinations. The ileum was dissected out, immersed in Tyrode's solution. Respective segments of 2-3cm long were mounted in a 25ml tissue organ bath maintained at 37ºC. Concentration dependent responses of acetylcholine (10µg/ml) were recorded (with dose of 0.1ml, 0.2ml, 0.4ml, 0.8ml, 1.6ml). Then, same concentration dependent responses of Acetylcholine (10µg/ml) + HAEPC (10µg/ml) were recorded. The same concentration dependent responses of Acetylcholine (10µg/ml) + Atropine as a standard antispasmodic agent (10µg/ml) was recorded. Lastly, the concentration dependent response of HAEPC (10µg/ml) alone was recorded. Compare the percentage response of acetyl choline along with atropine and HAEPC in each concentration response curve[21].

Statistical analysis

The data obtained are statistically analysed using Instat and Graph Pad Prism softwares. The differences between groups (P value) are considered significant at $P<0.05$.

RESULTS AND DISCUSSION

***In-vivo* hepatoprotective activity**

Hepatoprotective activity of hydro alcoholic extract of *Psidium cattleianum* Sabine leaves on carbon tetrachloride induced hepatotoxic rats was evaluated by determining various biochemical parameters. There was a significant increase in the serum parameters such as SGPT, SGOT, ALP and total bilirubin were seen in rats treated with Carbon tetrachloride (0.1ml/kg/day) in negative control when compared with normal control. The increased levels of these parameters were significantly reduced in groups treated with different doses of extracts (200mg/kg and 400mg/kg) when compared to negative control and restored near to the level of Silymarin (standard) treated group.

There was an increase in the level of lipid peroxidation in liver reflects hepatocellular damage. In the present study, CCl4 induced liver damage is associated with the increased levels of lipid peroxidation which is significantly reduced in case of hydro alcoholic extract of *Psidium cattleianum* Sabine leaves treated rats. The decreased enzymatic activity would result in cell injury. The catalase, glutathione peroxidase levels are elevated by administration of hydro alcoholic extract of *Psidium cattleianum* Sabine leaves to CCl4 intoxicated rats suggesting that it has the ability to restore the enzyme activity towards normalization in CCl4 damaged liver.

Histolopathology of Liver

In the microphotographs of liver damage was done after staining slides of liver tissue with Haematoxylin and Eosin. The histopathological studies observed in the photomicrographs of liver for treated and control groups. Treatment with the extract decreased the extent of fatty liver and necrosis caused by the hepatotoxic agent CCl4. Based on the results obtained from the histopathology, the Hydro alcoholic extract of *Psidium cattleianum* Sabine leaves shows a significant hepatoprotective activity.

***Ex-vivo* antispasmodic activity**

Evaluation of effect of anti spasmodic activity of HAEPC on isolated rat ileum preparation against acetyl choline as spasmogen. There was a

contraction (spasm) produced on excised rat ileum when Acetylcholine alone used and it increased with increase in dose. Treatment of anti-cholinergic drug Atropine (Standard antispasmodic agent) along with Acetylcholine produced a decrease in response showing antispasmodic activity in a dose dependent manner. Treatment of Hydro alcoholic extract of *Psidium cattleianum* Sabine leaves along with Acetylcholine produced a reduction in responses, hence the extract showed an antispasmodic activity with increasing concentrations.

Liver has a great role in the regulation of physiological processes in our body. It is having so many vital functions such as metabolism, secretion and storage. Furthermore, detoxification of a variety of drugs, chemicals and xenobiotics occurs in liver. So liver diseases are among the most serious health ailments nowadays. The conventional drugs used in the treatment of liver diseases are sometimes inadequate and may lead to serious adverse effects. In India, there are a number of folk medicinal plants and their formulations are used for liver disorders. Though, *Psidium cattleianum* Sabine leaves has been reported to possess a wide variety of pharmacological activities such as Antioxidant, Anticancer, Anti-inflammatory, Analgesic, Antimicrobial, Antifungal etc. There exists no scientific evidence on its Hepatoprotective activity using CCl4 as hepatotoxin and antispasmodic activity. Thus, in the present study, Hydro alcoholic extract of *Psidium cattleianum* Sabine leaves evaluated as an alternative cure on CCl4 induced hepatotoxicity and muscle spasm. Hexane, Chloroform and 70% Aqueous Ethanol extracts are prepared by successive solvent extraction of *Psdium cattleianum* Sabine leaves powder in Soxhlet apparatus. The phytochemical screening of *P. cattleianum* Sabine leaves extracts possess the presence of various chemical constituents. Among that the Hydro alcoholic extract showed a positive result for all three tests for phenols and flavonoids. Phenolic compounds and flavonoids are the major targeting active constituents for hepatoprotective activity. It also possessed higher percentage yield in the extraction process. The hydro alcoholic fractions obtained was subjected to evaluation for hepatoprotective and *ex-vivo* Antispasmodic activity. From the results, it was observed that the Hydroalcoholic extract of *Psidium cattleianum* Sabine leaves at concentration of 400 mg/kg showed better hepatoprotective activity than 200mg/kg and the extract showed an antispasmodic activity with increasing concentrations. Administration of carbon tetrachloride to rats increased the levels of serum parameters like SGPT, SGOT, ALP and Total bilirubin levels. Lipid peroxidation is also increased. The Catalase and Glutathione peroxidase enzyme levels were significantly decreased in groups treated with HAEPC at 200mg/kg and 400mg/kg dose respectively. Reduction in the levels of SGPT, SGOT, ALP, and Lipid peroxidase towards the normal value upon extract treatment (HAEPC 200mg/kg and 400mg/kg) is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damages caused by CCl4.The extract also possess an antispasmodic activity. There was a contraction (spasm) produced on excised rat ileum when Acetylcholine alone used and it increased with increase in dose. Treatment of anti-cholinergic drug Atropine (Standard antispasmodic agent) along with Acetylcholine produced a decrease in response showing antispasmodic activity in a dose dependent manner. Treatment of Hydroalcoholic extract of *Psidium cattleianum* Sabine leaves along with Acetylcholine produced a reduction in responses when compared with Acetylcholine alone. So the extract showed an antispasmodic activity with increasing concentrations.

Values are expressed as mean ± SEM. One way ANOVA comparison between negative group and control group and between negative group and treatment groups (Tukey's Method). The data are considered significant if *p<0.05, **p<0.01, ***p< 0.001, ns-non significant.

Values are expressed as mean ± SEM. One way ANOVA comparison between negative group and control group and between negative group and treatment groups (Tukey's Method). The data are considered significant if *p<0.05, **p<0.01, ***p< 0.001, ns-non significant.

Values are expressed as mean ± SEM. One way ANOVA comparison between negative group and control group and between negative group and treatment groups (Tukey's Method). The data are considered significant if *p<0.05, **p<0.01, ***p< 0.001, ns-not significant.

Table No.1: Effect of HAEPC liver enzyme markers against CCl_4 induced hepatotoxicity in rats

S.No	Groups	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	Total Bilirubin (mg/dL)
1	Control	49.118±2.504	25.645±1.253	123.463±1.125	0.45±0.020
2	Negative (CCl4, 0.1ml/kg)	75.391±1.157***	45.613±1.952***	192.951±2.596***	1.11±0.081***
3	Standard (Silymarin, 100 mg/kg)	52.716±2.213***	29.046±1.367***	128.266±1.261***	0.51±0.021***
4	HAEPC (200 mg/kg)	67.260±1.155*	38.991±1.254*	168.11±1.849***	0.75±0.049***
5	HAEPC (400 mg/kg)	58.965±1.072***	35.181±0.760***	154.341±1.964***	0.47±0.047***

Table No.2: Effect of HAEPC on oxidative stress enzyme markers against CCl_4 induced hepatotoxicity in rats

S.No	Groups	LPO (nmol/g tissue)	CATALASE(U/mg)	GLUTATHIONE PEROXIDASE (U/mg protein)
1	Control	7.86±0.31	45.82±0.98	55.92±0.1521
2	Negative (CCl4, 0.1ml/kg)	39.07±0.86***	17.83±0.50***	21.32±0.8121***
3	Standard (Silymarin, 100 mg/kg)	10.82±0.58***	42.09±0.31***	46.18±0.3812***
4	HAEPC (200 mg/kg)	17.31±0.42***	35.69±0.40***	39.46±0.3515***
5	HAEPC (400 mg/kg)	13.005±0.35***	36.87±0.47***	44.11±0.9620***

Table No.3: Determination of relative liver weight

S.No	GROUP	RELATIVE LIVER WEIGHT (Mean ± SEM)	
1	Control	2.576	± 0.1750
2	CCl4 (1 ml/kg)	6.495	± 0.5255**
3	CCl4 (0.1 ml/kg) + Silymarin (100 mg/kg)	3.667	± 1.155**
4	CCl4 (0.1 ml/kg) + hydro alcoholic extract of *Psidium cattleianum* Sabine (200mg/kg)	5.002	± 0.1572**
5	CCl4 (1 ml/kg) + hydro alcoholic extract of *Psidium cattleianum* Sabine (400mg/kg)	4.532	±0.1154**

Table No.4: Evaluation of effect of antispasmodic activity of HAEPC on isolated rat ileum preparation against acetylcholine as spasmogen

CONCENTRATION (µg)	PERCENTAGE RESPONSE (%)		
	Acetylcholine (10µg/ml)	Acetylcholine (10µg/ml) + Atropine (10µg/ml)	Acetylcholine (10µg/ml) + HAEPC (10µg/ml
1	26	15	21
2	38	18	23
3	64	24	31
4	85	35	41
5	100	39	44

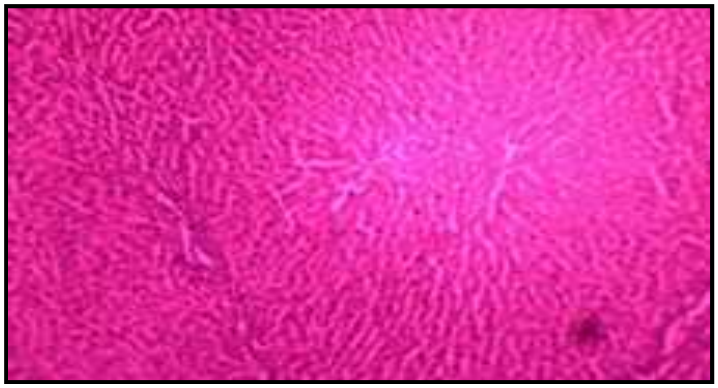

Figure No.1: Microphotographs showing histopathology of liver in normal group

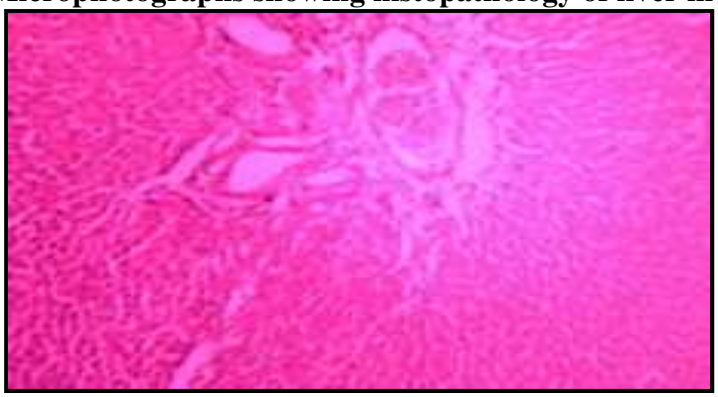

Figure No.2: Microphotographs showing histopathology of liver in CCl4 group

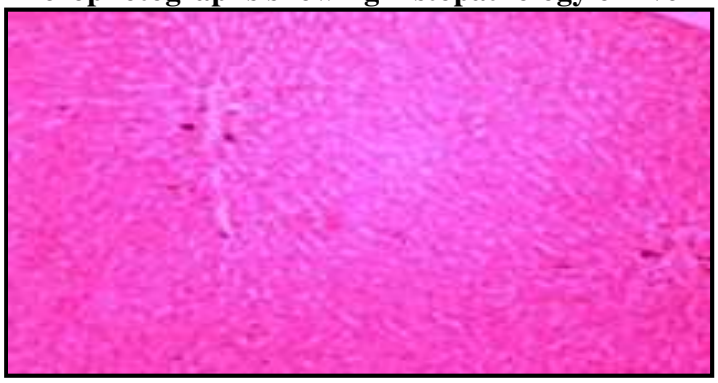

Figure No.3: Microphotographs showing histopathology of liver in Silymarin group

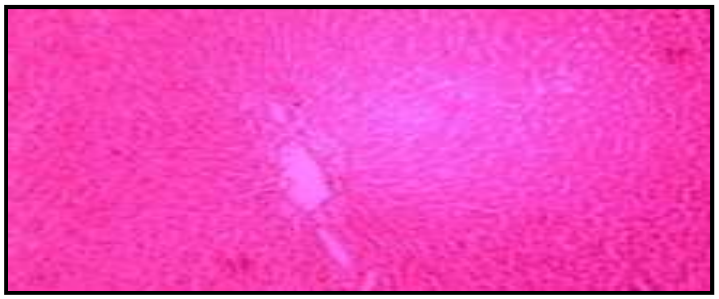

Figure No.4: Microphotographs showing histopathology of liver in HAEPC (200mg/kg) group

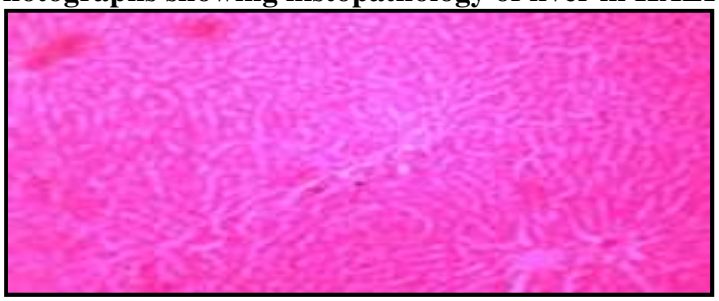

Figure No.5: Microphotographs showing histopathology of liver in HAEP (400mg/kg)

CONCLUSION

The study revealed that the Hydro alcoholic extract of *Psidium cattleianum* Sabine leaves was better choice for hepatoprotective and Antispasmodic activity. Thus it can be concluded that HAEPC possess significant hepatoprotective potential. It is a proven antioxidant. The hepatoprotective activity of extract might be due to the antioxidant property of leaves and presence of phytochemical constituents such as Flavonoids, Glycosides, Saponins, Phenolic compounds, Tannins, Triterpenoids, Amino acids and Proteins, Carbohydrates, Gum and mucilage and Vitamin C. The Flavonoids isolated from *Psidium cattleianum* Sabine leaves are Reynoutrin, Guajavarin, Quercetin, Morin, Myricetin, Luteolin, and Kaempferol have powerful antioxidant and radical scavenging activities as proven by ALP, DPPH, ABTS and ORAC assays. Further studies required to conclude the mechanism of action of the extract.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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