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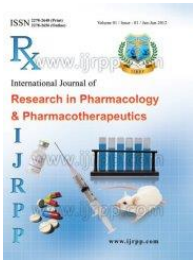

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Evaluation of Hepatoprotective activity of Jasminum Sambac in rats

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ABSTRACT

The present study was aimed to evaluate the hepatoprotective effect of *Jasminum Sambac* flower extract (JSFE) in acute experimental liver injury induced by Carbon tetrachloride (CCl_4), alcohol, paracetamol (PCM) and thioacetamide (TAA) in rats. In Ccl_4, alcohol, PCM and TAA models rats were treated with 10, 15, 5 and 7 days respectively. To induce the liver toxicity 24 hour after the last treatment CCl_4(2ml/kg. s.c), PCM (2g/kg, p.o.) and TAA (100mg/kg, s.c.) *was* administered where as for alcohol model alcohol (30% 1.5ml,*p.o.* twice a day) was given for 15 days. Rats were received different treatments such as silymarin (100 mg/kg), low and high doses of *Jasminum Sambac* flower extract (JSFE 100 and 500 mg/kg,) orally. The protective effect of prophylactic treatment was analysed by estimation of serum biomarkers like serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP) and bilirubin (total and direct) and by histopathological observation. The activities of serum biomarkers were significantly decreased in all treated groups compared with toxic control. It was concluded that high and the low dose of *Jasminum Sambac* flower extract (JSFE) demonstrated reduced serum biomarkers activity significantly which was supported by histopathological study.

Keyword: *Jasminum Sambac* seed extract, SGPT, SGOT, ALP, Bilirubin (Total and Direct).

INTRODUCTION

Man has been fascinated by nature since he evolved from his primitive ancestors, the apes. To start with, he hunted for food mainly by killing the wild animals, but if there was anything on which he could depend with any confidence towards its availability, it was the plant which provided him with food and they provided him with curative medicine and shelter. Because of this the primitive man was in love with the nature especially with plants because plants were the only source to fight with various diseases. From the plants they found various medicines and treatment practices to treat many diseases which put way for the modern treatment systems to save the human race. Today in this world traditional medicine plays a vital role in providing health care to large section of population, especially in developing countries [1]. Medicinal plants are the important source for the production of synthetic and herbal drugs. The medicinal plants or the herbal drugs are used in many ways by the human which are depicted in the given figure below.

The traditional systems of treatment such as Ayurveda, Unani, Siddha, western herbal medicine, traditional Chinese medicine and homeopathy use herbs for the treatment. Many researchers has prescribed about the importance of herbal medicine in the treatment of various diseases and because of the accessibility and cost effectiveness herbal treatment is still in practice by large number of practitioners [2]. The Importance of plants on human health began to arise in 1897, when Friedrich Bayer and Co. introduced synthetic acetyl salicylic acid (aspirin) to the world. Aspirin is a safer synthetic analogue of salicylic acid which is an active ingredient of willow bark, and was discovered independently as a remedy for aches and fever. Same like aspirin, digoxin (from foxglove), quinine (from cinchona bark), and morphine (from the opium poppy) are the other conventional drugs are obtained from the plants. As per WHO around 70 % of the word population rely on plant drugs than synthetic drugs. The herbal drugs are used by mankind in treating various disease conditions such as, malaria, chicken pox, cholesterol, heart diseases, lung diseases diarrhoea, psoriasis, skin disorders, fever, jaundice, asthma, diabetes etc. [3, 4]. Herbal medicine tends to have a greater demand as a primary health care system because of their lesser adverse effects, efficacy, safety etc. In the treatment of hepatic diseases a large number of plants has be proved their efficacy in reducing the risk of liver diseases.. Liver is a vital organ play a major role in metabolism and excretion of xenobiotics from the body. Liver cell injury caused by various toxic chemicals (certain anti-biotic, chemotherapeutic agents, carbon tetrachloride (CCl4), thioacetamide (TAA) etc.), excessive alcohol consumption and microbes is well-studied. The available synthetic drugs to treat liver disorders in this condition also cause further damage to the liver [5]. Hence, Herbal drugs have become increasingly popular and their use is wide-spread. Herbal medicines have been used in the treatment of liver diseases for a long time so the maintenance of a healthy liver is essential for the overall wellbeing of an individual. Liver injury induced by toxins is more common nowadays. Herbal remedies are focused in the pharmaceutical industry to evolve a safe route for liver disorders. Therefore, hepatoprotective natural products such as *Andrographic paniculata, Chamomile capitula, Silybum marianum, Coccinia grandis, Flacourtia indica, Wedelia calendulacea, Annona squamosa, Prostecheamichuacana, Ficuscarica, Lepidium sativum, Sargassum polycystum, Solanum nigrum, swertia chirata, Phyllanthus emblica, Curcuma longa, Picrorhiza kurroa, Azadirachta indica, Aegle marmelos, Cassia roxburghii, Orthosiphon stamineus, Jatropha curcas, Foeniculum vulgare, Trigonella foenum graecum, Eclipta alba, Garcinia mangostana Linn* is reviewed by many scientific studies and proved to have hepatoprotective activity [6]. Even though the modern medicine has proven its efficiency, there are hardly any reliable drugs that protect the liver from damage or help in regeneration of hepatic cell. Liver diseases are among the most serious ailment which can cause some of the serious pathological condition such as acute or chronic hepatitis (inflammatory liver diseases), hepatosis (non inflammatory diseases) and cirrhosis (degenerative disorder resulting in fibrosis of the liver) [7].

Liver diseases are mainly caused by toxic chemicals (certain antibiotics, chemotherapeutics, peroxidised oil, aflatoxin, carbon-tetrachloride, chlorinated hydrocarbons, etc.), excess consumption of alcohol, infections and autoimmune/disorder [8]. In spite of the fact of tremendous advancement of modern medicine utility of such drugs in the therapy of hepatic diseases still under significant question mark due to less potency and toxicity profile [9]. Therefore, searching for effective and safe drugs for liver disorders are continues to be an area of interest. In the present scenario a number of formulations containing herbal extracts are in use for liver disorders, especially in countries like India, China, Nepal and Malaysia, in managing hepatic disorders [10,11].

Although their biologically active components are unknown, herbal drugs are prescribed widely because of their effectiveness, fewer side effects, and relatively low cost [12]. Due to lack of awareness of a satisfactory remedy for serious liver diseases and increasing doubt on the efficacy and safety of the currently used drugs or herbal formulations, there is a need to find effective and safe drugs or herbal medicines for liver disorders. *Jasminum Sambac* Linn. (Family-Oleaceae) commonly known as Motia or lily jasmine is a scandent or sub-erect shrub with young pubescent branches, broadly ovate or elliptic, opposite leaves, white, very fragrant flowers cultivated nearly throughout the tropical and subtropical parts of the world. Traditionally plant is

used in fever or cough, indolent ulcer, abdominal distension, diarrhoea, lowering the blood glucose level, regulating menstrual flow, to clean kidney waste, inflamed and blood shot eyes. Root, flowers, leaves act as lactifuge, arrest the secretion of milk in the puerperal state in case of threatened abscess [13]

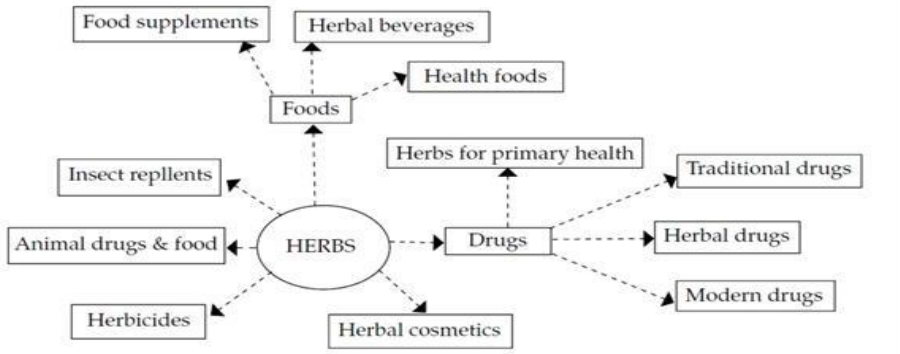

Figure 1: Utilization of herbal plants

MATERIALS AND METHODS

Experimental Animals

Rats of either sex weighing 170-250 g were housed at 25° ± 5°C, relative humidity 50± 5% in a well-ventilated animal house under 12:12 h light dark cycle. Institutional Animal Ethics Committee approved the experimental protocol. The animals were maintained under standard conditions in an animal house as per the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). The Institutional ethical committee approved the experimental protocol (KCP/IAEC-14/2011-12).

Preparation of *Jasminum sambac* Linn. Flower extract

The flowers were collected and dried under shade and were ground into coarse powder. The powdered material was subjected to soxhlation separately using soxhlet extractor. The extraction was carried out using ethanol as the solvent. The extraction was stopped after 16 cycles by observing the colour of the solvent. After extraction the solvent was removed by using rotary evaporator under reduced pressure. The extracted compound was concentrated by subjecting to evaporation to obtain a thick syrupy mass. The Obtained concentrated extract was kept in desiccators until further use [14].

Acute toxicity studies

The dose selection of *Jasminum sambac Linn*. Flower extract extract (JSFE) were based on acute toxicity studies, carried out according to OPPTS (Office of Prevention, Pesticide and Toxic Substance) guidelines following the limit test procedure.[2] Mice were divided into two groups of six each. Test dose of 2 g/kg body weight and 5 g/kg body weight were given orally to either group of mice. Mice were observed for 72 hours for mortality. $1/10^{th}$ and $1/50^{th}$ of the maximum safe dose corresponding to 500 mg/kg and 100 mg/kg body weight were selected as high and low doses respectively [15].

EXPERIMENTAL MODELS

Carbon Tetrachloride induced acute hepatitis in rats

The animals were divided into 5 groups consisting of six animals in each group. The animals were then subjected to either one of the following treatments for 10 days. At the end of treatment of animals for 10 days the animals of all groups simultaneously received CCl_4 (2 ml/kg, s.c.) at every 72 hrs. 24hrs after the administration of last treatment, the blood samples were collected by retro-orbital puncture method and isolated serum was subjected for the assay of marker enzymes such as serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP) and serum bilirubin

(Total and direct). Then the animals were sacrificed and the liver from each group were isolated and subjected for histopathological studies. Rats of either sex were divided into 5 treatment groups of six animals each and treated for 10 days according to the following treatment protocol [16].

Table 1: Treatment Protocol of Carbon tetrachloride Induced Acute Hepatitis In Rats

GROUPS	TREATMENT(10 DAYS)
Group - I (Normal control)	Vehicle (1% tween)
Group - II (Toxic control)	Vehicle + Ccl_4(2 ml/kg, s.c., every 74 hrs)
Group - III (Standard)	Silymarin (100 mg/kg/day, *p.o.*)+ Ccl_4(2 ml/kg, s.c., every 74 hrs)
Group -IV (Low dose)	JSFE (100 mg/kg/day, *p.o)* + Ccl_4(2 ml/kg, s.c., every 74 hrs)
Group - V (High dose)	JSFE (500 mg/kg/day, *p.o*) + Ccl_4

Alcohol induced acute hepatitis in rats [17,18]

The animals were divided into 5 groups consisting of six animals in each group. The animals were then subjected to either one of the following treatments for 15 days. At the end of treatment of animals for 15 days the animals of all groups simultaneously received 30% alcohol (1.5 ml/rat / twice a day). 24hrs after the administration of last treatment, the blood samples were collected by retro-orbital puncture method and isolated serum was subjected for the assay of marker enzymes such as serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP) and serum bilirubin (Total and direct). Then the animals were sacrificed and the liver from each group were isolated and subjected for histopathological studies. Rats of either sex were divided into 5 treatment groups of six animals each and treated for 15 days according to the following treatment protocol.

Table 2: Treatment Protocol of Alcohol Induced Acute Hepatitis In Rats

GROUPS	TREATMENT(15 DAYS)
Group - I (Normal control)	Vehicle (1% tween)
Group - II (Toxic control)	Vehicle + 30% alcohol (1.5ml, *p.o*/rat, twice a day)
Group - III (Standard)	Silymarin (100 mg/kg/day, *p.o.*)+ 30% alcohol (1.5ml, p.o/rat, twice a day)
Group -IV (Low dose)	JSFE (100 mg/kg/day, *p.o)* + 30% alcohol (1.5ml, *p.o* /rat, twice a day)
Group - V (High dose)	JSFE (500 mg/kg/day, *p.o*) + 30% alcohol (1.5ml, *p.o*/rat, twice a day)

Thioacetamide (TAA) Induced Liver Necrosis in Rats [19]

The animals were divided into 5 groups consisting of six animals in each group. The animals were then subjected to either one of the following treatments for 7 days. The TAA (100 mg/kg, *s.c.*) was administered after dilution with distilled water. 48 hrs after the administration of TAA, blood samples were collected by retro-orbital puncture method and serum was used for assay of marker enzymes such as serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP) and serum bilirubin(Total and direct). Then the animals were sacrificed and the liver from each group were isolated and three liver were subjected for histopathological studies. Rats of either sex were divided into 5 treatment groups of six animals each and treated for 7 days according to the following treatment protocol.

Table 3: Treatment Protocol of Thioacetamide (TAA) Induced Liver Necrosis in Rats

GROUPS	TREATMENT(7 DAYS)
Group - I (Normal control)	Vehicle (1% tween)
Group - II (Toxic control)	Vehicle + TAA (100 mg /kg/day, *s.c.*) on seventh day.
Group - III (Standard)	Silymarin (100 mg/kg/day, *p.o.*) + TAA (100 mg /kg, *s.c.*) on seventh day.
Group -IV (Low dose)	JSFE (100 mg/kg/day, *p.o*) + TAA (100 mg /kg, *s.c*) on seventh day.
Group - V (High dose)	JSFE (500 mg/kg/day, *p.o*) + TAA (100 mg /kg, *s.c*) on seventh day.

Paracetamol (PCM) induced liver toxicity in rats

The animals were divided into 5 groups consisting of six animals in each group. The animals were then subjected to either one of the following treatments for 5days. The paracetamol (2 g/kg, *p.o.*) was administered in three divided dose on fifth day. 48hrs after the administration of PCM, blood samples were collected by retro-orbital puncture method and serum was used for the assay of marker enzymes such as serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP) and serum bilirubin(total and direct). Then the animals were sacrificed and the liver from each group were isolated and three subjected for histopathological studies. Rats of either sex were divided into 5 treatment groups of six animals each and treated for 5 days according to the following treatment protocol.

Table 4: Treatment Protocol of Paracetamol (PCM) Induced Liver Toxicity in Rats

GROUPS	TREATMENT(5 DAYS)
Group - I (Normal control)	Vehicle (1% tween)
Group - II (Toxic control)	Vehicle + PCM (2 g/kg, *p.o.*) on fifth day
Group - III (Standard)	Silymarin (100 mg/kg/day, *p.o.*) + PCM (2 g/kg, *p.o.*) on fifth day
Group -IV (Low dose)	JSFE (100 mg/kg/day, *p.o*) + PCM (2 g/kg, *p.o.*) on fifth day
Group - V (High dose)	JSFE (500 mg/kg/day, *p.o*) + PCM (2 g/kg, *p.o.*) on fifth day

Statistical analysis

Results are expressed as mean ± SE. Statistical significance was assessed using One-way Analysis of variance (ANOVA) followed by Tukey-Karmer multiple comparison tests. $P<0.05$ was considered significant.

RESULTS

Preparation of *Jasminum sambac* flower extract

The extract of *Jasminum sambac* flower extract (JSFE) was prepared and yield was found to be 32%.

Phytochemical investigation

Table 5: Phytochemical investigation for various compounds

Sl. No.	TEST	INFERENCE	RESULT
1.	**Test for alkaloids**		
1.a	Hager's test	No Yellow colour	-
1.b	Mayer's test	No cream precipitate	-
1.c	Dragendroff's test	No orange precipitate	-
1.d	Wagner's test	No red-brown precipitate	-
2.	**Test for carbohydrates**		

2a	Molish test	Violet colour	+
2b	Fehling's test	Break red colour	+
2c	Borfoed's test	Red colour	+
2d	Benedict's test	Red colour	+
3.	**Test for steroids, triterpenoids and glycosides**		
3a	Liebermann-buchard test	No Redish- violet colour	-
3b	Salkowski test	No Red colour	-
3c	Baljet test	No Orange colour	-
3d	Keller killani test	No Red colour	-
4.	**Test for saponins**		
4.a	Froth test	1 cm foam	+
4.b	Haemolytic test	No precipitate	+
5	**Test for tannins**		
5.a	Ferric chloride test	Blue colour	+
5.b	Lead acetate test	Yellow colour	+
6	**Test for proteins and Amino acids**		
6.a	Millon's test	Red colour	+
6.b	Biuret test	Violet colour	+
6.c	Ninhydrin test	Violet colour	+
7	**Test for flavanoids**		
7.a	Ferric chloride test	Blackish red colour	+
7.b	Lead acetate test	Yellow precipitate	+
8	**Test for specific flavonoids**		
8.a	Test for carotenoids	Deep blue colour	+
9	Test for phenolic compound	Radish brown colour	+

Toxicity studies

The acute oral toxicity study was performed according to the OPPTS guidelines (Office of Prevention, Pesticide and Toxic Substance) following the limit test procedure. The animals were fasted overnight prior to the experiment. Test dose of 2 g/kg and 5 g/kg were given orally to mice. Both doses were found to be safe. Hence $1/10^{th}$ and $1/50^{th}$ of the maximum safe dose corresponding to 500 mg/kg and 100 mg/kg orally were selected as high and low doses respect.

Carbon tetrachloride induced acute hepatitis

Effect on Serum glutamate oxaloacetate transaminase (SGOT)

In Carbon terachloride induced acute hepatitis model toxic control group showed extremely significant (P<0.001) increase in SGOT level when compared with vehicle control. Other prophylactically treated group such as standard (silymarin) and high dose of JSFE showed an extremely significant (P<0.001) decrease where as for low dose it was found to be moderately significant (P<0.01) compared with toxic control group.

Effect on Serum Glutamate Pyruvate Transaminase (SGPT)

SGPT level of toxic control group showed an extremely significant (P<0.001) increase compared to vehicle control while, other prophylactically treated group such as standard (silymarin) low and high dose of JSFE showed an extremely significant (P<0.001) decrease compared to toxic control group.

Effect on Alkaline phosphatase (ALP)

Serum Alkaline phosphatase (ALP) levels of toxic control group showed an extremely significant (P<0.001) increase compared to vehicle control while, other prophylactically treated group such as standard (silymarin) low and high dose of JSFE showed an extremely significant (P<0.001) decrease compared to toxic control group

Effect on Total and Direct bilirubin

Toxic control group showed extremely significant (P<0.001) increase in Total and Direct bilirubin levels compared to vehicle control group. Other

treated groups such as standard (silymarin) and high dose of showed an extremely significant (P<0.001) decrease in Total and Direct bilirubin values where as for low dose it was found to be significant (P<0.05) and moderately significant (P<0.01) respectively for Total Bilirubin and Direct Bilirubin values compared with toxic control group.

Table 6: Effect of Silymarin and JSFE on serum SGOT, SGPT, ALP, Bilirubin (Total and Direct) in Carbon tetrachloride induced acute hepatitis in rats.

Treatment	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	Total Bilirubin	Direct Bilirubin
Vehicle control	92.66 ± 2.99	79.33 ± 8.64	31.28 ± 3.18	0.29 ± 0.09	0.12 ±0.04
Toxic control (Ccl_4)	296.52 ± 1.84*	384.72 ± 1.37***	251.30 ± 1.62***	3.98 ± 0.02***	0.26 ± 0.03***
Silymarin(100 mg/kg)	103.32 ± 3.36$^{*\#\#\#}$	129.51 ± 5.47$^{***\#\#\#}$	159.26 ± 5.28$^{***\#\#\#}$	0.36 ± 0.013$^{\#\#\#}$	0.19 ± 0.054$^{\#\#\#}$
JSFE-100	172.6 ± 4.315$^{***\#\#}$	225.23± 6.15$^{***\#\#\#}$	343.46 ± 23.2$^{***\#\#\#}$	0.72 ± 0.01 $^{*\#}$	0.49 ± 0.01$^{**\#\#}$
PVSE-500	149.23 ± 5.47 $^{\#\#\#}$	175.68 ± 4.11$^{***\#\#\#}$	230.26 ± 7.26$^{***\#\#\#}$	0.45 ± 0.01 $^{\#\#\#}$	0.28 ± 0.03 $^{\#\#\#}$

All values are mean ± SEM, n=6 *P< 0.05, **P< 0.01, ***P< 0.001 when compared to normal control, $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P< 0.001 compared to toxic control.

Figure 1: Haematoxylin and eosin (H&E) stained section of liver in Carbon tetrachloride induced acute hepatitis. Photographed at magnification 400X.0

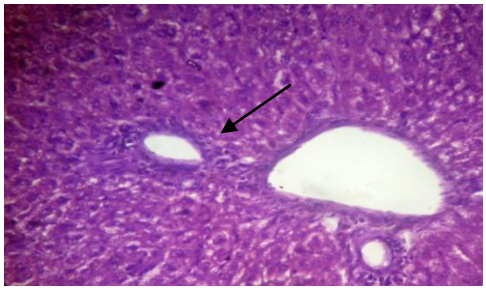

Figure 1a: Normal control. **1.**Normal texture liver tissue.

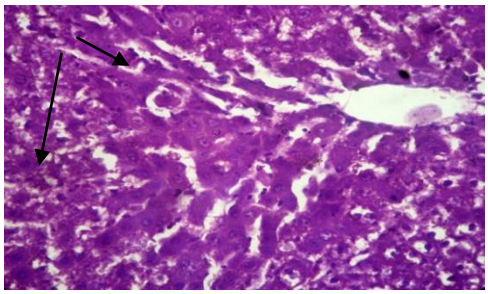

Figure 1b: Toxic control. **1.**Moderate to severe tissue degranulation.

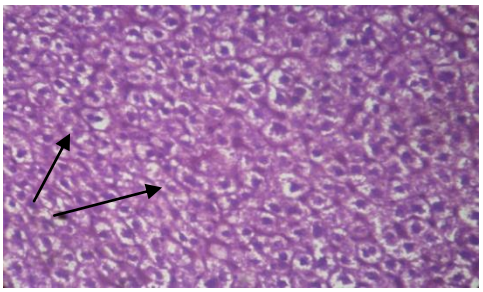

Figure 1c: Standard drug. **1.**Mild liver tissue degranulation.

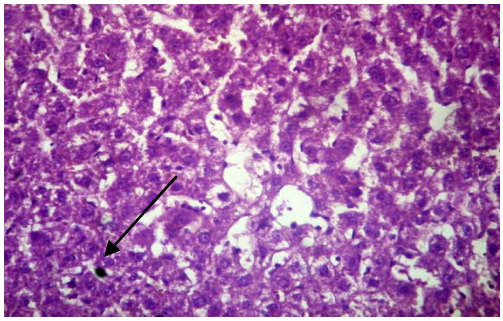

Figure 1d: JSFE-100. **1.**Moderate degranulation of liver tissue.

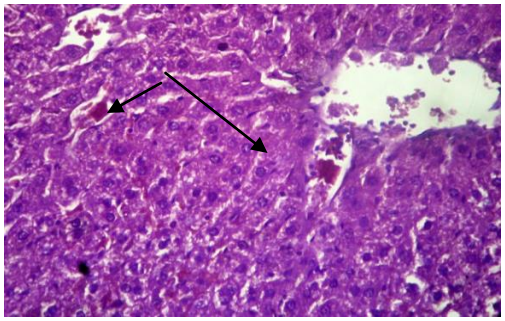

Figure 1e: JSFE-500. **1.**Mild liver tissue degranulation.

ALCOHOL INDUCED ACUTE HEPATITIS

Effect on Serum glutamate oxaloacetate transaminase (SGOT)

In Carbon terachloride induced acute hepatitis model toxic control group showed extremely significant (P<0.001) increase in SGOT level when compared with vehicle control. Other prophylactically treated group such as standard (silymarin) and high dose of JSFE showed an extremely significant (P<0.001) decrease where as for low dose it was found to be moderately significant (P<0.01) compared with toxic control group.

Effect on Serum Glutamate Pyruvate Transaminase (SGPT)

SGPT level of toxic control group showed an extremely significant (P<0.001) increase compared to vehicle control while, other prophylactically treated group such as standard (silymarin) low and high dose of JSFE showed an extremely significant (P<0.001) decrease compared to toxic control group.

Effect on Alkaline phosphatase (ALP)

Serum Alkaline phosphatase (ALP) levels of toxic control group showed an extremely significant (P<0.001) increase compared to vehicle control while, other prophylactically treated group such as standard (silymarin) low and high dose of JSFE showed an extremely significant (P<0.001) decrease compared to toxic control group

Effect on Total and Direct bilirubin

Toxic control group showed extremely significant (P<0.001) increase in Total and Direct bilirubin levels compared to vehicle control group. Other treated groups such as standard (silymarin) and high dose of showed an extremely significant (P<0.001) decrease in Total and Direct bilirubin values where as for low dose it was found to be significant (P<0.05) and moderately significant (P<0.01) respectively for Total Bilirubin and Direct Bilirubin Values compared with toxic control group.

Table 7: Effect of Silymarin and JSFE on serum SGOT, SGPT, ALP, Bilirubin (Total and Direct) in Alcohol induced acute hepatitis in rats

Treatment	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	Total Bilirubin	Direct Bilirubin
Vehicle control	92.66 ± 2.99	79.33 ± 8.64	31.28 ± 3.18	0.29 ± 0.09	0.12 ± 0.04
Toxic control (Alcohol)	228.53 ± 7.84***	334.72 ± 5.37***	496.30 ± 6.62***	0.98 ± 0.02***	0.66 ± 0.03***
Silymarin(100 mg/kg)	105.32 ± 3.40$^{*\#\#\#}$	129.51 ± 5.97$^{***\#\#\#}$	159.26 ± 5.28$^{***\#\#\#}$	0.36 ± 0.01$^{\#\#\#}$	0.19 ± 0.05$^{\#\#\#}$
JSFE-100	172.6 ± 4.35$^{***\#\#}$	225.33± 7.15$^{***\#\#\#}$	323.46 ± 25.1$^{***\#\#\#}$	0.72 ± 0.01 $^{*\#}$	0.49 ± 0.01$^{**\#\#}$
JSFE-500	149.23 ± 5.47 $^{\#\#\#}$	175.68 ± 4.11$^{***\#\#\#}$	230.26 ± 7.26$^{***\#\#\#}$	0.45 ± 0.01 $^{\#\#\#}$	0.27 ± 0.02 $^{\#\#\#}$

All values are mean ± SEM, n=6 *P< 0.05, **P< 0.01, ***P< 0.001 when compared to normal control, $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P< 0.001 compared to toxic control.

Figure 2: Haematoxylin and eosin (H&E) stained section of liver in Alcohol induced acute hepatitis. Photographed at magnification 400X.0

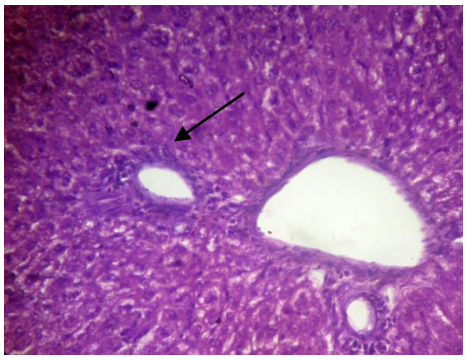

Figure 2a: Normal control. **1.**Normal texture liver tissue.

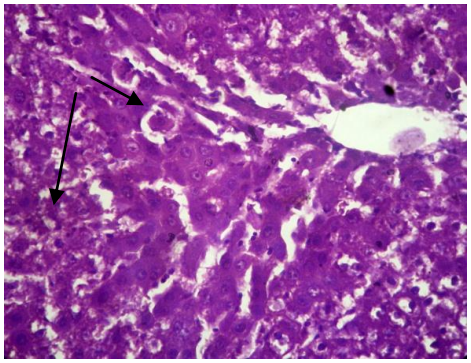

Figure 2b: Toxic control. **1.**Moderate to severe tissue degranulation.

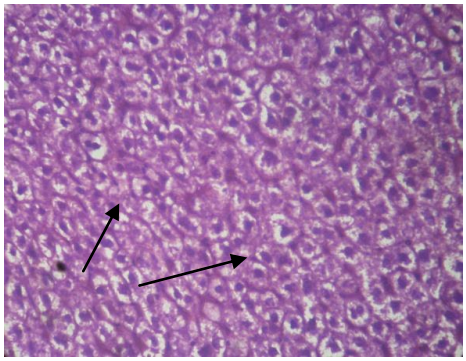

Figure 2c: Standard drug. **1.**Mild liver tissue degranulation.

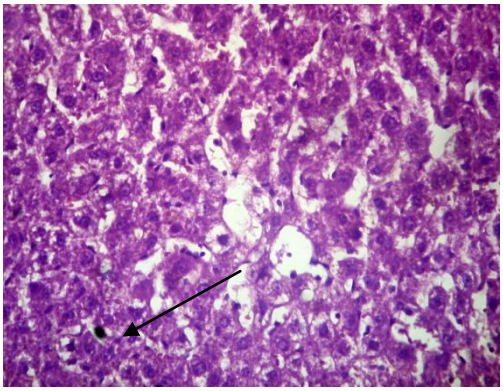

Figure 2d: JSFE-100. **1.**Moderate degranulation of liver tissue.

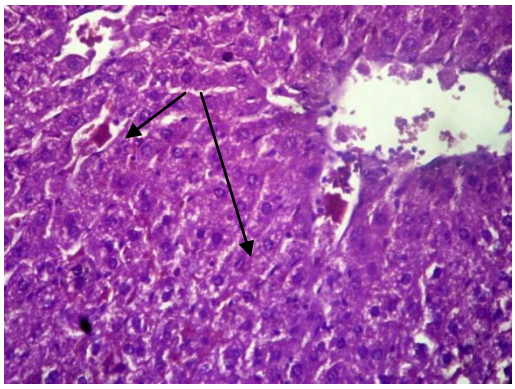

Figure 2e: JSFE-500. **1.**Mild liver tissue degranulation.

PARACETAMOL (PCM) INDUCED LIVER TOXICITY

Effect on Serum glutamate oxaloacetate transaminase (SGOT)

Toxic control group of Paracetamol (PCM) induced liver toxicity showed extremely significant (P<0.001) increase in SGOT level when compared with vehicle control. Other prophylactically treated group such as standard (silymarin) low and high dose of JSFE showed an extremely significant (P<0.001) decrease compared with toxic control group.

Effect on Serum Glutamate Pyruvate Transaminase (SGPT)

SGPT level of toxic control group showed an extremely significant (P<0.001) increase compared to vehicle control while, other prophylactically treated group such as standard (silymarin) low and high dose of JSFE showed an extremely significant (P<0.001) decrease compared to toxic control group

Effect on Alkaline phosphatase (ALP)

Serum Alkaline phosphatase (ALP) levels of toxic control group showed an extremely significant (P<0.001) increase compared to vehicle control while, other prophylactically treated group such as standard (silymarin) low and high dose of PVSE showed an extremely significant (P<0.001) decrease compared to toxic control group

Effect on Total and Direct bilirubin

Toxic control group showed extremely significant (P<0.001) increase in Total and Direct bilirubin levels compared to vehicle control group. Other treated groups such as standard (silymarin) showed

an extremely significant (P<0.001) decrease in total and direct bilirubin values whereas high dose showed moderately significant(P<0.01) for total bilirubin and extremely significant(P<0.001) for direct bilirubin and low dose showed moderately significant(P<0.01) decrease compared with toxic control group.

Table 8:Effect of Silymarin and JSFE on serum SGOT, SGPT, ALP, Bilirubin (total and direct) in PCM induced liver toxicity in rats

Treatment	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	Total Bilirubin	Direct Bilirubin
Vehicle control	92.66 ± 2.99	79.33 ± 8.64	31.28 ± 3.18	0.29 ± 0.09	0.12 ± 0.04
Toxic control(PCM)	213.44 ± 5.25***	323.18 ± 7.23***	505.94 ± 11.23***	0.93 ± 0.04***	0.70 ± 0.06***
Silymarin (100mg/kg)	99.25 ± 4.21$^{###}$	105.19 ±5.26$^{*###}$	153.24 ± 4.17$^{***###}$	0.39 ± 0.07$^{###}$	0.19 ± 0.13$^{###}$
JSFE-100	151.09 ± 9.19$^{***###}$	210.17 ± 4.38$^{***###}$	304.23 ± 8.49$^{***###}$	0.69 ± 0.02$^{##}$	0.45 ± 0.03$^{##}$
JSFE-500	109.23 ± 5.31$^{###}$	139.20 ± 4.19$^{*###}$	213.32 ± 4.16$^{***###}$	0.47 ± 0.02$^{##}$	0.24 ± 0.01$^{###}$

All values are mean ± SEM, n=6 *P< 0.05, ***P< 0.001 when compared to normal control, $^{##}$P<0.01, $^{###}$P< 0.001 compared to toxic control.

Figure 3: Haematoxylin and eosin (H&E) stained section of liver in PCM induced liver toxicity. Photographed at magnification 400X.

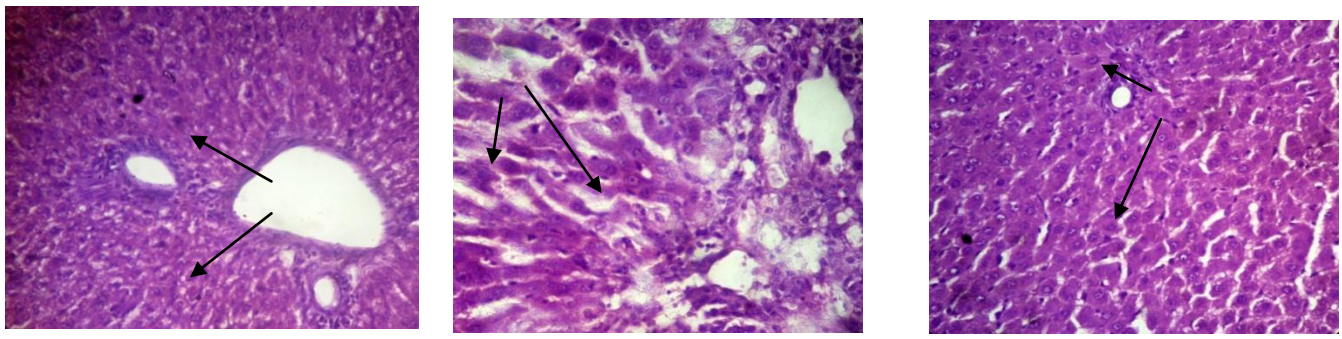

Fig. 3a: Normal control **1.**Normal tissue

Fig. 3b: Toxic control.**1.**Severe tissue degranulation and necrosis.

Fig. 3c: Standard drug. **1.** Mild texture of liver tissue. moderate liver tissue degranulation.

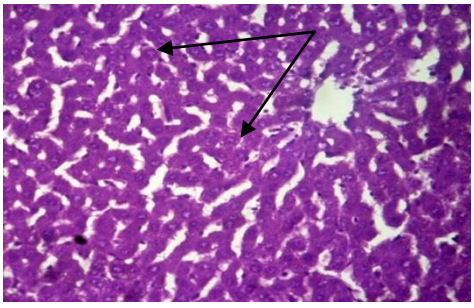

Fig. 3d: JSFE-100. **1.** Moderate

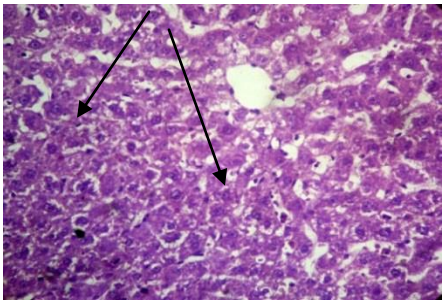

Fig. 3e: JSFE-500. **1.** Mild liver

THIOACETAMIDE (TAA) INDUCED LIVER NECROSIS

Effect on Serum glutamate oxaloacetate transaminase (SGOT)

Toxic control group of Paracetamol (PCM) induced liver toxicity showed extremely significant (P<0.001) increase in SGOT level when compared with vehicle control. Other prophylactically treated group such as standard (silymarin) and high dose of PVSE showed an extremely significant (P<0.001) decrease where as for low dose it was found to be moderately significant (P<0.01) compared with toxic control group.

Effect on Serum Glutamate Pyruvate Transaminase (SGPT)

SGPT level of toxic control group showed an extremely significant (P<0.001) increase compared to vehicle control while, other prophylactically treated group such as standard (silymarin) low and high dose of JSFE showed an extremely significant (P<0.001) decrease compared to toxic control group

Effect on Alkaline phosphatase (ALP)

Serum Alkaline phosphatase (ALP) levels of toxic control group showed an extremely significant (P<0.001) increase compared to vehicle control while, other prophylactically treated group such as standard (silymarin) low and high dose of JSFE showed an extremely significant (P<0.001) decrease compared to toxic control group

Effect on Total and Direct bilirubin

Toxic control group showed extremely significant (P<0.001) increase in total and direct bilirubin levels compared to vehicle control group. Other treated groups such as standard (silymarin) and high dose of JSFE showed an extremely significant (P<0.001) decrease in total and direct bilirubin values whereas low dose showed extremely significant (P<0.001) for total protein and moderately significant (P<0.01) for direct bilirubin compared with toxic control group.

Table 9: Effect of Silymarin and JSFE on serum SGOT, SGPT, ALP, Bilirubin (Total and Direct) in TAA induced liver necrosis in rats.

Treatment	SGOT(U/L)	SGPT (U/L)	ALP (U/L)	Total Bilirubin	Direct Bilirubin
Vehicle control	92.66 ± 2.99	79.33 ± 8.64	40.16 ± 3.25	0.25 ± 0.06	0.15 ± 0.02
Toxic control (TAA)	322.26 ± 6.10***	659.66 ± 13.11***	609.23 ± 6.37***	4.16 ± 0.11***	0.87 ± 0.05***
Silymarin(100 mg/kg)	103.46 ± 5.91$^{###}$	109.07 ± 6.27$^{###}$	164.42 ± 5.24$^{***###}$	0.59 ± 0.6$^{*###}$	0.27 ± 0.02$^{###}$
JSFE-100	224.16 ±8.31$^{***##}$	296.42 ± 5.23$^{***###}$	316.4 ± 6.27$^{***###}$	1.86 ± 0.08$^{***###}$	0.59 ± 0.06$^{**##}$
JSFE-500	143.47 ± 4.63 $^{*###}$	169.36 ± 5.22$^{**###}$	239.46 ± 4.14$^{***###}$	0.99 ± 0.24 $^{*###}$	0.22 ± 0.02 $^{###}$

All values are mean ± SEM, n=6 *P< 0.05, **P< 0.01, ***P< 0.001 when compared to normal control, $^{##}$P<0.01, $^{###}$P< 0.001 compared to toxic control.

DISCUSSION

The present study was aimed to investigate the possible hepatoprotective activity of *Jasminum sambac* Linn. Flower extract (JSFE) during hepatic damages induced by various toxic substances like Alcohol, Paracetamol (PCM) and Thioacetamide (TAA) in rat liver. Alcohol is mainly metabolized in the liver through a series of chemical reactions known as oxidation reactions. In the alcohol metabolism pathway, known as alcohol dehydrogenase pathway, the enzyme alcohol dehydrogenase converts alcohol to a toxic intermediate substance, acetaldehyde by removing two atoms of hydrogen from each alcohol molecule. Then a second enzyme, aldehyde dehydrogenase, quickly converts acetaldehyde to acetate by again removing hydrogen and adding oxygen. A secondary pathway of alcohol metabolism is microsomal ethanol-oxidizing system (MEOS). MEOS is activated by long-term heavy alcohol consumption. The MEOS pathway involves the enzyme cytochrome P450 2E1 or CYP 2E1 that strips

hydrogen away from alcohol to produce acetaldehyde. In both of these pathways, more markedly in the MEOS pathway-oxidation reactions spawn highly unstable free oxygen radicals. As discussed in results both the extract JSFE-100 and JSFE-500 reversed hepatic damage by its antioxidant activity and decreased serum biomarker enzymes. The results suggested that JSFE act dose dependently against liver damage. The findings were also supported by the results of histopathological studies. Antioxidant potential which is the prime reason of the activity may be due to presence of flavonoids. In case of PCM induced toxicity, the liver damage is due to its toxic metabolite. Paracetamolis normally eliminated mainly as sulfate and glucoronide. Upon administration of toxic doses of paracetamol the sulfation and glucoronidation routes become saturated and hence, higher percentage of paracetamol molecules is oxidized to highly reactive N-acetyl-p-benzo quinine imine (NAPQI) by cytochrome-450 enzymes. Semi Quinone a radical obtained by one electron reduction of NAPQI, can covalently bind to macromolecules of cellular membrane and increases the lipid peroxidation resulting in the tissue damage. Higher dose of paracetamol and NAPQI can alkylate and oxidize intracellular Glutathione (GSH) and protein thiol group, which results in the depletion of liver GSH pool subsequently, leads to increased lipid peroxidation and liver damage. Significant hepatic damage due to paracetamol is evident from the fact that there is elevation in the levels of various biochemical markers of hepatic damage like Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT), bilirubin, and Alkaline Phosphate (ALP). Decreased level of enzymatic antioxidants is a clear manifestation of excessive formation of free radical during the metabolism of the PCM and activation of lipid peroxidation system. In this model also the documented results proved that JSFE is able to provide hepatoprotection by reducing the elevated level of biomarkers. The probable mechanism of this action may be due to free radical scavenging activity like earlier model which provides protection to liver by scavenging reactive molecule produced by PCM metabolism. The hepatoprotective effect was supported by histological changes produced by the experimental animal of different groups. The mechanism behind in thioacetamide induced hepatic toxicity is thought to be associated with its toxic metabolites which interfere with the movement of RNA from the nucleus to cytoplasam which may cause membrane injuries. Thioacetamide reduce the number of viablehepatocytes as well as rate of oxygen consumption. Hepatic damage associated with toxic metabolite of thioacetamide is evident from the increased level of biomarkers and reduced level of antioxidant enzyme system such as SOD and catalase in the liver tissue homogenate. It also decreases the volume of bile and its content. Following the same trend like earlier models JSFE had proven significant protection by decreasing the increased level of serum biomarkers. The possible reason may be same antioxidant activity like earlier models which may neutralize reactive metabolite of TAA. The hepatoprotective effect was once again supported by histological changes produced by different groups In all experimental models of the present study, both high and low dose (500 and 100 mg/kg p.o.) reported significant level of protection. The predicted reason is the antioxidant property due to the presence of saponins, flavonoids such as quercetin which has been witnessed as the chief chemical constituent justifying the therapeutic potential. Some of the reported activities of *Jasminum sambac* linn antidiabetic, anticancer and antilipidemic may be attributed to the antioxidant property of the plant. However, further studies are required to understand the exact mechanism behind the hepatoprotective effect of *Jasminum sambac* linn. The probable mechanisms of flavonoids are it directly scavenges free radicals by oxidizing itself by radicals, resulting in a more stable, less reactive radicals. Flavonoids interfere with nitric oxide synthase activity which is useful to release nitric oxide for maintaining the dilation of blood vessels. Flavonoids scavenged peroxynitrite radicals produced by reaction of nitric oxide with free radicals. Flavonoids inhibit xanthine oxidase which involved in metabolism of xanthine to uric acid. Xanthine oxidase is a source of oxygen free radicals. In the reperfusion phase (reoxygenation), xanthine oxidase reacts with molecular oxygen, thereby releasing superoxide free radicals. Flavonoids decrease the number of immobilized leukocytes. Immobilization of leukocytes to the endothelial wall leads to generation of oxygen derived free radicals. Specific flavonoids are known to chelate iron,

thereby removing a casual factor for development of free radicals.

CONCLUSION

From the findings obtained from the present study it can be concluded that the flower extract of *Jasminum sambac* Linn(JSFE)in both low and high doses (100 and 500 mg/kg,*p.o*) shown a marked protective effect against Alcohol, paracetamol (PCM) and Thioacetamide (TAA) induced hepatotoxicity in rats by evaluating the physical, biochemical, functional and histological parameters. The finding of the present study proved that there is a significant dose dependent hepato protective activity by JSFE in all models. The hepatoprotective effect of the flower extract is assumed may be due to the presence of flavonoids and saponins which are responsible for the scavenging of free radicals and also due to the individual and combined action of phytoconstituents present in it. To put in a nut shell, the present study confirms the potential hepatoprotective activity of *Jasminum sambac* linn flower extract. As stated earlier, further study of the isolated compoundsfrom the extracts with more specific models will throw some more light. A large number of plants are being reported to have hepatoprotective activity. A formulation containing these herbal drugs might be the right medicine we are looking for the management of hepatic diseases.

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