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Evaluation of Hepatoprotective Effect of *Sesamum indicum* Linn. Seed Extract against Paracetamol Induced Hepatotoxicity in Rats

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ABSTRACT
The present study was aimed to assess the hepatoprotective activity of ethanolic extract of *Sesamum indicum* Linn. seeds against Paracetamol-induced liver damage in rats. Paracetamol-induced liver damage was produced by the treatment of Paracetamol (2g/kg/day, p.o) on three consecutive days for seven days. Other groups of rats were pretreated with two doses of *Sesamum indicum* (400mg/kg and 700mg/kg) and silymarin (25mg/kg, bw, p.o.) 30 min prior to Paracetamol ingestion. Liver damage was assessed using various biochemical parameters viz. Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyurvate Transaminase (SGPT), Alkaline phosphatase (ALP), Acid phosphatase (ACP), Total Protein, Albumin and Total Bilirubin along with histopathological examination of liver tissue. There was a significant increase in serum enzymatic levels of Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyurvate Transaminase (SGPT), Alkaline phosphatase (ALP), Acid phosphatase (ACP) and Total Bilirubin with a decrease in Total Protein and Albumin level, in Paracetamol treated animals, reflecting liver injury. Pretreatment with two different doses (400mg/kg and 700mg/kg) of *Sesamum indicum* produced significant reversal in the above biochemical parameters and reduced histopathological scores of fatty degeneration, centrilobular necrosis with significant evidence of regeneration. The results of the study indicate that the extract of *Sesamum indicum* possesses significant protection against Paracetamol-induced hepatocellular injury.

Keywords: *Sesamum indicum* Linn, Paracetamol, Hepatoprotective, Histopathology.

INTRODUCTION

Liver is the vital organ in the body that is concerned with detoxification and disposition of toxic substances. [1] Since it is involved in the biochemical conversions of various endogenous and exogenously administered substances, there is possibility of generating various highly reactive species of free radicals. In spite of these free radicals generating, hepatotoxins like Paracetamol overpower the protective mechanism of the liver and cause hepatic damage. [2] Therefore, herbal and other indigenous sources have been adequately explored for the safe and effective hepatoprotective action. Earlier studies reported that medicinal herbs play a protective role against Paracetamol induced liver damage.

The herb *Sesamum indicum* Linn. (Pedaliaceae) is commonly known as Til in Hindi, Seman sesamin in Pharm, Sesame in French, Sesamo in Spanish, Wijen in Japnanese and found in the warmer region of Africa, Asia and Australia. [3] About six species are recorded in India of which *Sesamum indicum* is widely cultivated. Literature survey has revealed that sesame oil act as laxative, demulcent and has got emollient properties. [4] The alcoholic extract of *Sesamum indicum* is reported for antitumor effect. It is also useful in burns, scald and poultices of the seed is applied to the ulcer. Powdered seed are used in the amenorrhea and dysmenorrheal. [5] Chemically the presence of two lignans, sesamin and sesamolin in addition to glycerides of oleic and linoleic acid has been reported. [6] A perusal of literature revealed its hepatoprotective effects on Paracetamol-induced liver damage remains to be studied. Here, we report the hepatoprotective effect of ethanolic extract of *Sesamum indicum* on Paracetamol-induced liver damage in wistar rats.

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MATERIALS AND METHODS

Plant material

Fresh seeds of the plant *Sesamum indicum* Linn. was purchased from the crude drug market of Udaipur city and was authenticated by Head, Department of Pharmacognosy, B. N. College of Pharmacy, Udaipur (Rajasthan). The voucher specimen of the plant (01/2007) has been deposited in the herbarium of the institute.

Preparation of extract: The powdered drug was extracted with aqueous ethanol (80%v/v) by a cold maceration process.

The extract was then concentrated and the solvent was removed completely under reduced pressure. The yield of the extract was found to be 15 % (w/w). [7]

Animals

The young healthy albino rats of Wistar strains of either sex, weighing between 200 to 225 g. were used for the experiment. They were housed in the cages under the laboratory standard condition (23±2°C, humidity 60-70%, 12 hr light/dark cycles) and given standard pellet diet. Water was give *ad libitum*. The study was permitted by the institutional animal ethical committee at the B. N. College of Pharmacy, Udaipur with reg. no. 870/ac/05/CPSEA.

Phytochemical studies

The extract was subjected for phytochemical study. [8]

Acute toxicity studies

The acute toxicity study for ethanolic extract of *Sesamum indicum* seed were performed using Wistar rats. The animals were fasted overnight prior to the experiment and maintained under standard conditions. The extract was administered orally in increasing dose and found safe up to dose of 2000mg/kg.

Paracetamol induced toxicity

Healthy Wistar rats of either sex weighing between 200 to 225 g. were randomly divided into five groups of six rats each. The animals from group I served as the control and received the vehicle 5% gum acacia at a dose of 1ml/kg/day, p.o. for 7 days. Group II animals were similarly treated as group I. Group III animals received Silymarin (microlab) at a dose of 25 mg/kg/day, p.o. for 7 days. Group IV-V animals were treated with two different doses (400mg/kg and 700mg/kg) of *Sesamum indicum* for 7 days respectively. On the 7th day Paracetamol (Indian pharmaceutical company, Bombay) suspension in a dose of (2g/kg/kg/day, p.o.) [9] was administrated to all the rats except rats of group I, 30 min after the administration of silymarin and extract of *Sesamum indicum.* The animals were sacrificed 48 hr after Paracetamol administration by mild anaesthesia. The blood sample was collected, allowed to clot and serum was separated at 2500 rpm for 15 min. and biochemical investigations were carried out. Liver was dissected out and used for histopathological studies. [10]

Biochemical parameters

The biochemical parameters like serum enzymes Serum Glutamate Oxaloacetate Transaminase (SGOT), [11] Serum Glutamate Pyurvate Transaminase (SGPT), [11] Alkaline phosphatase (ALP), [12] Acid phosphatase (ACP), [12] Total Protein, Albumin [13] and Total Bilirubin [14] were assayed using enzyme kits (Merck Ltd.).

Histopathological studies

The liver specimen obtained from control and treated groups of animals were fixed in 10% buffered formalin for 24 h. Section were prepared and then stained with haematoxylin and eosin for photomicroscopic observation of liver histological architecture.

Statistical analysis

The data was expressed as mean ± SD. Statistical differences between means were determined by one-way ANOVA followed by Scheff's / Dunnet's test. Value of $P< 0.05$ was considered as statistically significant.

RESULTS

Phytochemical studies

The extract of *Sesamum indicum* subjected for phytochemical study showed the presence of lignans, proteins, amino acids, carbohydrates and lipids.

Acute toxicity studies

No mortality observed with oral administration of *Sesamum indicum* even at the highest dose (2000mg/kg). Both the doses of *Sesamum indicum* had no toxic effect on the normal behavior of the rats.

Biochemical parameters

Administration of Paracetamol (2g/kg//day, p.o.) induced a marked increase in the serum level of Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyurvate Transaminase (SGPT), Alkaline phosphatase (ALP), Acid phosphatase (ACP), and Total Bilirubin, whereas there was a significant decrease in the level of Total Protein and Albumin as compared to normal control indicating acute hepatocellular damage. Pre-treatment of the rats treated with two different doses (400mg/kg and 700mg/kg) of *Sesamum indicum* showed prior to paracetamol administration showed a significant reduction in the level of Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyurvate Transaminase (SGPT), Alkaline phosphatase (ALP), Acid phosphatase (ACP), and Total Bilirubin , whereas increase in level of Total Protein and Albumin almost comparable to the silymarin (25mg/kg) treated group.

Histopathological studies

The hepatoprotective effect of *Sesamum indicum* was confirmed by histopathological examination of liver tissue of control and treated animals. The histological architecture of Paracetamol-treated liver section showed fatty degeneration, intense centrilobular necrosis, vacuolization, ballooning of parenchymal cells. The rats treated with ethanolic extract of *Sesamum indicum* and silymarin showed a good sign of protection against the toxicant to considerable extent as it was evident from the formation of normal hepatic cords, less fatty degeneration, absence of necrosis and vacuoles.

DISCUSSION

Liver is a versatile organ in the body concerned with regulation of internal chemical environment. Therefore damage to the liver inflicted by a hepatotoxic agent is of grave consequences. [15]

The present study reveals the potential hepatoprotective activity of ethanolic extract of *Sesamum indicum* seeds against hepatic injury produced by paracetamol in rats. Paracetamol is a common antipyretic agent, which is a safe in therapeutic dose but can produce fatel hepatic necrosis in man, rats and mice with toxic doses. [16] It is employed as an experimental toxic agent. [17] It is metabolized in the liver to excretable glucuronide and sulphide conjugates. [18] However hepatotoxicity of paracetamol has been attributed to the formation of toxic metabolites when a part of paracetamol is activated by hepatic cytochrome P-450 [19] to a highly reactive metabolite N-Acetyl-p-benzoquinoneimine. [20] An obvious sign of hepatic injury is leakage of cellular enzyme into plasma [21-22] due to the disturbance caused in the transport function of hepatocytes. [23] When liver cell plasma is damaged, a variety of enzymes located in the cytosol is released in to the blood strem. Their estimation in the serum is useful quantitative marker for the extent and type of hepatocellular damage. [24] In the present investigation, the dose of Paracetamol used (2.5g/kg.), caused liver injury in

Table 1: Effect of *Sesamum indicum* (SI) Linn. on different parameters in the Serum of Paracetamol (Pcm) treated rats

TREATMENT	Biochemial Parameters			
	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	ACP (U/L)
Control (LP only)	73±2.19	88.5±3.83	39.5±1.64	29.5±2.73
Pcm only	120±3.28^{+++}	124.5±3.83^{+++}	85±3.28^{+++}	68±3.28^{+++}
Pcm + Silymarin	84.5±2.73***	92±2.19***	46.5±1.64***	33±1.09***
Pcm+SI(400mg/kg)	93.5±3.83***	95.5±2.73***	50.5±2.73***	44±3.28***
Pcm+SI(700mg/kg)	90±2.19***	93.5±1.64***	49±2.19***	41±2.19***

All values are represented as **Mean ± SD (n=6)**

P value: +++ < 0.001 When compared with control untreated animals.

***<0.001 When compared with carbon tetrachloride induced hepatotoxic rats models

Table 2: Effect of *Sesamum indicum* (SI) Linn. on different parameters in the serum of Paracetamol (Pcm) treated rats

TREATMENT	Biochemial Parameters		
	TOTAL PROTEIN (g/dl)	TOTAL ALBUMIN (g/dl)	TOTAL BILIRUBIN (mg/dl)
Control (LP only)	7.69±0.279	4.44±0.230	0.44±0.049
Pcm only	6.10±0.164^{+++}	3.86±0.109^{+++}	1.38±0.087^{+++}
Pcm + Silymarin	7.24±0.131***	4.26±0.153***	0.89±0.054***
Pcm+SI(400mg/kg)	6.95±0.219**	4.00±0.087**	0.98±0.023***
Pcm+SI(700mg/kg)	7.03±0.131***	4.18±0.115***	0.94±0.044***

All values are represented as **Mean ± SD (n=6)**

P value: +++ < 0.001 When compared with control untreated animals.

<0.01;*<0.001 When compared with carbon tetrachloride induced hepatotoxic rats models

rats, [25] treated with an overdose of Paracetamol developed significant hepatic damage, which was observed by a substantial increase in the concentration of serum hepatic enzymes. As saying the activities of these marker enzymes and *Sesamum indicum* helps to assess the liver function.

Pretreatment of rats with two doses of *Sesamum indicum* (400mg/kg and 700mg/kg) for 21 days before administration resulted in a significant reduction of Paracetamol-induced elevation of serum enzymes markers, almost comparable to the effect of silymarin, the positive control used. Silymarin is a known hepatoprotective compound obtained from silybum marianum. It is reported to have protective effect of plasma membrane of hepatocytes. [26] We also observed significant decrease ($p<0.05$) in the level of total protein and albumin in serum, which was increased by the administration of the plant extract at 400mg/kg and 700mg/kg for 21 days. *Sesamum indicum* appears to be effective in reducing the injurious effect of Paracetamol observed in the study. This was an indication of stabilization of plasma membrane, as well as repair of hepatic tissue damage, caused by Paracetamol. Similar studies have proved the hepatoprotective effect of *Withania somnifera*, [27] *Ocimum sanctum*, [28] *Ricinus communis* and *Phyllanthus niruri*, [29] *Eclipta alba*, [30] *Phyllanthus embelica* [31] and *Spilanthes ciliate.* [32]

Hepatoprotective effect of *Sesamum indicum* was further confirmed by histopathological studies of the liver, which basically supported the results from serum assays. The results are in agreement with the commonly accepted view that serum level of Transaminase returns to normal with healing of hepatic parenchyma and the regeneration of hepatocytes. [33] Further, the stimulation of hepatic regeneration was known to make the liver more resistant to damage toxins. [34] *Sesamum indicum* administration resulted in bringing about an almost normal histological architecture of the liver.

CONCLUSION

The present study has lead to conclusion that extract of *Sesamum indicum* has the potential to protect liver from toxic substances. Furthermore profound studies can expect herbal drug to act as lead compound for development of economical, effective, and nontoxic hepatoprotective agents therefore doses of these extract are useful when patient having Paracetamol (a well known NSAID) toxicity after self medication or wrong dose calculation by prescribers.

In most of the developed countries, the incidence of viral hepatitis is more so, the investigation for an efficient hepatoprotective drug from the natural resources is an urgent necessity.

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