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Protective Effect of Methanolic Extract of Sida Veronicaefolia against Paracetamol Induce Hepatotoxicity in Experimental Animals

Arti Pandey*[1], Vidhi Jain [1], Manmeet Singh Saluja[2]

[1]Research Scholars, Department of Pharmacy, SunRise University, Alwar, Rajasthan.
[2]Professor, Department of Pharmacy, SunRise University, Alwar, Rajasthan.

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Corresponding author: Arti Pandey

ABSTRACT

The present study was intended to evaluate the hepatoprotective activity of Methanolic Extract of the leaves of Sida veronicaefolia (MESV). The Methanolic Extract of the leaves of Sida veronicaefolia (MESV) at dose 250 & 500 mg/kg were administered orally to the animals with hepatotoxicity induced by paracetamol. Silymarin (25mg/kg) was given as reference standard. The hepatoprotective activity was also supported by histopathological studies of liver tissue. An effective significant alteration in all biochemical parameters and histopathological sections was observed. Since results of biochemical studies of blood samples of paracetamol treated rats showed significant increase in the levels of serum enzyme activities, reflecting the liver injury caused by paracetamol and blood samples from the animals treated with the oral administration of Methanolic Extract of the leaves of Sida veronicaefolia (MESV), produced a significant reduction in serum enzymes alanine aminotransferase (ALT), aspartate aminotransferase, (AST), alkaline phosphatase (ALP) to the acute hepatotoxic induced rats. They exhibited a significant inhibition of hepatic toxicity by using various marker enzymes and the histopathological analysis. From these results, concluded that the Methanolic Extract of the leaves of Sida veronicaefolia (MESV) was effective in protecting the liver against the injury induced by paracetamol in rats at the dose of 250 & 500 mg/kg/body weight. These results suggest that leaves of methanolic extract of Sida veronicaefolia (MESV) may supports the hepatic cells protection.

Introduction

Liver diseases have become one of the major causes of morbidity and mortality all over world. From among, drug induced liver injury is one of the most common causative factor that poses a major clinical and regulatory challenge **(Russmann at el., 2009)**. Liver diseases are some of the fatal disease in the world today. They pose a serious challenge to international public health. Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for their treatment of liver disorders **(Karan at el., 1999, Chaterrjee at el., 2000)**. Herbal medicines remain a popular alternative throughout the India. The phytochemical components of medicinal plants often act individually, additively or synergistically in improvement of health. Clinical research in this century has confirmed the efficacy of several plants in the treatment of liver disease4. After having analyzed the various chemical components present in leaves Sida veronicaefolia it is imperative that focus shifts to the medicinal applications of the plant. **(Karan at el., 1999, Chaterrjee at el., 2000)**

Aceta-aminophen (N-acetyl-p-aminophenol, Paracetamol) is widely used as analgesic and antipyretic drug is known to cause hepatotoxicity in experimental animals and humans at high doses. It is established that following an oral therapeutic dose, a fraction of acetaminophen is converted via the cytochrome

P-450 pathway to a highly toxic metabolite, N-acetyl-p-benzoquinone- imine (NAPQI) which is normally conjugated with glutathione and excreted in the urine as conjugates. Overdoses of aceta- aminophen deplete glutathione stores leading to accumulation of NAPQI, mitochondrial dysfunction, the development of acute hepatic necrosis. Also depletion of glutathione enhances the expression of tumour necrosis factor alpha (TNFα). TNFQ primes phagocytic NADPH oxidase to the enhanced production of oxygen free radicals and contributes to the liver damage. Acetaminophen induced hepatotoxicity in rodents is a widely used animal model to assess hepatoprotective activity of new compounds. It is a powerful inducer of cytochrome P-450 and produces a highly reactive quinineimine, which combines with sulphahydral groups of proteins and cause rapid depletion of intracellular GSH. Normally GSH contributes significantly to the intracellular antioxidant defensive system as it is a powerful consumer of superoxide singlet oxygen, and hydroxyl radicals. The breakdown of the GSH- dependent antioxidant defensive system increases the intracellular flux of oxygen apotopsis. The rise in serum levels of AST, ALT and ALP has been attributed to the damaged structural integrity of the liver because these are cytoplasmic in location and are released into circulation after cellular damage **(Mukherjee, 2008)**. The present study was conducted to evaluate hepatoprotective activity of methanolic extract against paracetamol-induced toxicity in rats.

Methods and Materials

Procurement and Authentication of the Plant

The leaves of Sida veronicaefolia were gathered from the surrounding region of Alwar, Rajasthan, and then sent to Sunrise University, Alwar, Rajasthan, where they were certified by Dept. of Botany, Sunrise University, Alwar, Rajasthan.

Preparation of extracts of Sida veronicaefolia

In a Soxhlet apparatus, 500g of powdered leaves were extracted with solvent in order to increasing polarity. The materials were concentrated by evaporation **(Farnsworth et al., 1966)**.

Animals

Wistar albino rats (150-200 g) were procured from Central Drug Research Institute, Lucknow, India. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water ad libitum. All the animals were acclimatized for a week before use. The experimental protocols were approved by Institutional Animal ethics Committee after scrutinization. Animals were received the drug by oral gavages tube. All the animals were care of under ethical consideration as per the CPCSEA guidelines (CPCSEA, 2003) with regular inspections of rats. The laboratory conditions duly undertaken by registered veterinary practitioner.

Chemicals

All the chemicals and solvents were of analytical grade. Silymarin was obtained as gift sample from Micro Lbs, Goa, India. Standard kits for SGOT, SGPT and ALP etc. were obtained from Span Diagnostics Ltd., India.

Experimental design

The rats, of both sexes, were split up into 6 groups of six each. (n = 6) **(Balakrishnan et al., 2011; Haque et al., 2011)**

- **Group I (Control)**: administered water (5 mililitre/kilogram, p.o.) o.d. for nine days.
- **Group II (-ve control):** administered water (5 mililitre/kilogram, p.o.) o.d. for nine days, meanwhile on the seventh day, paracetamol (1 g/kg p.o.) was administered.
- **Group III (+ve control):** administered the normal medicine silymarin (25 mg/kg, p.o.) o.d. for nine days, meanwhile on the seventh day, paracetamol (1 g/kg p.o.) was administered.
- **Group IV and V (Test Sample)** administered Methanolic Extract of the leaves of Sida veronicaefolia (**MESV**) (250 and 500 mg/kg) o.d. for nine days, meanwhile on the seventh day, paracetamol (1 g/kg p.o.) was administered.

Assessment of hepatoprotective activity

On last day, blood was obtained from animals by puncturing retro orbital plexus. Blood

samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 30°C for 15 min and utilized for the estimation of various biochemical parameters including **SGOT & SGPT (Reitman et al., 1957), ALP (Kind et al., 1954), serum bilirubin (Amour et al., 1965) and serum protein (Lowry et al., 1951)** After collection of blood samples, the animals were sacrificed under deep ether anesthesia.

Morphological parameters like weight of animals, weight of liver have also been used to evaluate the protective effect of the drug. Hepatoprotective chemical causes loss in liver weight/100 gm body weight of rats **(Avadhoot et al., 1991; Bhanwra et al., 2000)**.

Histopathology studies

A portion of liver tissue of all the animal groups was excised and then washed with normal saline. The liver tissues were fixed in 10% buffered neutral formalin for 48 hrs and then with bovine solution for 6 hrs and were then processed for paraffin embedding. By using a microtome, sections of 5 mm thickness were taken and stained with hematoxylin and eosin. These sections were examined under light microscope using a magnification of 100X **(Mankani et al., 2005)**.

Statistical Significance

The results of the study were expressed as mean ± SEM, n=6. ANOVA **(Gennaro et al., 1995)** was used to analyze and compare the data, followed by Dunnet's **(Dunnet et al., 1964)** test for multiple comparisons.

Results

Acute toxicity study

There was no mortality found amongst the graded dose groups of animals and they did not show any toxicity or behavioral changes at a dose level of 5000 mg/kg. This finding suggests that the Methanolic Extract of the leaves of Sida veronicaefolia (**MESV**) was safe or non-toxic to rats and hence doses of 250 & 500 mg/kg, p.o. were selected for the study.

Effect of Methanolic Extract of the leaves of Sida veronicaefolia (MESV) on serum marker enzyme levels

There was a significant elevation in the levels of serum marker enzymes like SGOT, SGPT and ALP content of hepatotoxic treated groups. In contrast, pretreatment with Methanolic Extract of the leaves of Sida veronicaefolia (**MESV**) (250 & 500 mg/kg, p.o) and silymarin (25 mg/kg, p.o) exhibited an ability to counteract the hepatotoxicity by decreasing serum marker enzymes. The results were showed in table no 1.

Effect of Methanolic Extract of the leaves of Sida veronicaefolia on biochemical parameters

In hepatotoxic treated groups, there was a significant increase in total bilurubin and significant reduction in total protein content. Whereas, pretreatment with Methanolic Extract of the leaves of Sida veronicaefolia (**MESV**) (250 & 500 mg/kg, p.o) caused significant reduction in total bilirubin and significant increase in total protein. The results were showed in table no. 1.

Table No 1: Effect of leaves of Sida veronicaefolia (MESV) on serum enzyme parameter in Paracetamol induced hepatotoxic rats.

Normal	64.0 ± 3.74	169.05 ± 2.70	188.0 ± 8.02	0.49 ± 0.07	9.75 ± 0.23
Induced (RIF+INH)	188.31 ± 7.43*	369.69 ± 8.44*	346.43 ± 7.55*	6.68 ± 7.04*	5.59 ± 0.16*
Standard (Silymarin)	74.50 ± 3.41***	175.81 ± 4.56***	200.28 ± 8.36***	0.52 ± 2.57***	9.65 ± 4.70***
MESV (2500mg/kg)	138.02 ± 3.65***	267.01 ± 4.63***	233.14 ± 6.41***	0.67 ± 4.33***	7.85 ± 4.05**
MESV (500mg/kg)	120.21 ± 4.76***	198.0 ± 9.46***	200.22 ± 8.66***	0.62 ± 0.58***	8.18 ± 1.48***

Effect of Methanolic Extract of the leaves of Sida veronicaefolia (MESV) on liver weight

Paracetamol intoxicated group of animals, weight of the liver & Vol. of liver were

significantly increased, but it was normalized in Methanolic Extract of the leaves of Sida veronicaefolia (**MESV**) (250 & 500 mg/kg, p.o) treated groups of animals. A significant reduction in liver supports this finding. The results were showed in table no 2.

Table No 2: Effect of Methanolic extract of Sida veronicaefolia (MESV) on liver weight in Paracetamol induced hepatotoxic rats.

S.No.	Treatment/ Dose	Liver weight (wt/100gm bw)	Liver Volume (ml)
1	Normal	6.93 ± 0.07	6.97 ± 0.05
2	Induced (CCl_4)	8.88 ± 0.66*	9.12 ± 0.49*
3	Standard (Silymarin)	7.08 ± 0.45***	7.09 ± 0.49***
4	MESV (250mg/kg)	8.08 ± 0.24***	8.39 ± 1.28**
5	MESV (500mg/kg)	7.60 ±0.80***	7.80 ± 0.90***

Values are mean ± SEM, n = 6. (One way ANOVA Followed by Dunnette multiple comparisons test). Statistically significance of ** P<0.01, *** P<0.001, when compared with Paracetamol induced group and * P<0.05, when compared with normal group.

Histopathology

Histopathological studies of liver also provided a supportive evidence for biochemical analysis. Histological changes such as steatosis (fatty changes in hepatocytes) and perivenular fibrosis were observed in paracetamol treated (toxic) control group. Both the plant extracts has prevented these histological changes. The results were showed in fig. no. 1.

Discussion

There are many factors which are responsible for the liver damage or injuries such as chemicals and drugs. In the present study, paracetamol was used to induce hepatotoxicity, since it is clinically relevant. Elevated levels of serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) are indications of hepatocellular injury **(Yue et al., 2006)**.

Paracetamol is a common analgesic and antipyretic. Several studies have demonstrated the induction of hepatocellular damage by acetaminophen higher doses in experimental animals and humans. For screening of Hepatoprotective agents, paracetamol-induced hepatotoxicity has been used as a reliable method. Paracetamol is metabolizedprimarily in the liver and eliminated by conjugation with sulfate and glucuronide, and then excreted by the kidney. Moreover, paracetamol hepatotoxicity has been attributed to the formation of toxic metabolites, when a part of paracetamol is activated by hepatic cytochrome P-450 to a highly reactive metabolite N acetyl-p- benzoquinoneimine(NAPQI). Toxic metabolites (N-acetyl-p-benzoquineimine) can alkylate and oxidise intracellular GSH, which results in liver GSH depletion subsequently leads to increased lipid peroxidation by abstracting hydrogen from a polyunsaturated fatty acid and ultimately, liver damage due to higher doses of paracetamol **(Mitchell 1973, Savides, 1983)**. Reactive metabolites can exert initial cell stress through a wide range of mechanisms including depletion of glutathione (GSH) or binding to enzymes, lipids, nucleic acids and other cell structures **(Pauli-Magnus at el., 2005)**. Normally, AST and ALP are present in high concentration in liver. Due to hepatocyte necrosis or abnormal membrane permeability, these enzymes are released from the cells and their levels in the blood increases. ALT is a sensitive indicator of acute liver damage and elevation of this enzyme in non-hepatic diseases is unusual. ALT is more selectively a liver paranchymal enzyme than AST **(Nkosi at el., 2005, Mahalakshmi at el., 2010)**.

Administration of 1 gm/kg body weight of Paracetamol to experimental animals for 7 day produced statistically significant rise in the enzymes levels, namely SGOT, SGPT, ACP, ALP indicating the chemical induced hepatocellular toxicity. The inhibitory effect of the Methanolic Extract of the leaves of Sida veronicaefolia (**MESV**) on hepatotoxicity was compared to that of positive control group. The significant protection in the biochemical

parameters like SGOT, SGPT, ACP and ALP against Paracetamol induced elevations in pretreatment of the animals with the Methanolic Extract of the leaves of Sida veronicaefolia (**MESV**). Further there was increase in weight of the liver treated with the Paracetamol is seen as compared to the normal. The treatment with the Methanolic Extract of the leaves of Sida veronicaefolia (**MESV**) retains the liver weight near to the normal. Liver section of control rat showing a normal hepatic architecture wall brought about from the central vein. The liver samples of Paracetamol treated rats showed gross necrosis of the centrilobular hepatocytes characterized by gross necrosis, degeneration, karyolysis and eosinophilic infiltration which are significantly prevented by treatment with the Methanolic Extract of the leaves of Sida veronicaefolia (**MESV**) that showed the hepatoprotective activity. The histopathological pattern of the livers of the rats treated with Paracetamol plus extract showed minimal necrosis in centrilobular and regeneration of hepatocytes. A number of scientific reports indicated that certain flavonoids, triterpenoids and steroids have protective effect on liver due to its antioxidant properties. Administration of Methanolic Extract of the leaves of Sida veronicaefolia (**MESV**) that showed significant hepatoprotective activity; while qualitative phytochemical investigations on the Methanolic Extract of the leaves of Sida veronicaefolia (**MESV**) also showed test positive for flavonoids by chemical tests. Further, it has been reported that the flavonoid constituents of the plant possess antioxidant properties and was found to be useful in the treatment of liver damage (**Mahalakshmi at el., 2010)**.

The administration of hepatoprotective drugs may induce the hepatocytes to resist the toxic effect of Paracetamol. The results indicated that the Methanolic Extract of the leaves of Sida veronicaefolia (**MESV**) has significant hepatoprotective activity. The obtained results indicated a high degree of protection against the hepatotoxic effect of Paracetamol.

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