International Standard Serial Number (ISSN): 2249-6807

International Journal of Institutional Pharmacy and Life Sciences 1(3): November-December 2011

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Life Sciences **Research Article……!!!**

Received: 06-12-2011; Accepted: 13-12-2011

HEPATOPROTECTIVE ACTIVITY OF *VANILLA PLANIFOLIA* AGAINST PARACETAMOL INDUCED HEPATOTOXICITY IN ALBINO RATS

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Keywords:
Vanilla Planifolia, Paracetamol, hepatoprotective, hepatotoxicity

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ABSTRACT

The present study was conducted to evaluate the hepatoprotective activity of ethanolic extract of *Vanilla planifolia* against paracetamol induced liver damage in rats. The ethanolic extract of *Vanilla Planifolia* (100mg/kg) was administered orally to the animals with hepatotoxicity induced by paracetamol (500mg/kg). Silymarin (100mg/kg) was given as reference standard. All the test drugs were administered orally by suspending in 0.5% Carboxy methyl cellulose solution. The plant extract was effective in protecting the liver against the injury induced by paracetamol in rats. This was evident from significant reduction in serum enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin. It was concluded from the result that the ethanolic extract of *Vanilla planifolia* possesses hepatoprotective activity against paracetamol induced hepatotoxicity in rats.

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[1]. Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects[2]. In the absence of a reliable liver protective drug in modern medicine there are a number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders[3]. In view of severe undesirable side effects of synthetic agents, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity. 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 disease[4]. Antioxidant[5], Antimicrobial[6], Anticancer[7] activity of *Vanilla planifolia* has been reported. The study was conducted to establish the traditional use of *Vanilla planifolia* as hepatoprotective against paracetamol induced hepatotoxicity in rats.

MATERIALS AND METHODS

Animals

Male albino rats weighing 150-200g were used for this study. These animals were treated with robust health by providing pellet diet and water *ad libitum* in the animal house, which is well ventilated and lighted. A total of 30 healthy male albino rats selected were acclimatized to the lab conditions for 15 days and then randomly divided into five groups of six each. All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (IAEC) and were in accordance with the guidelines of the IAEC.

Plant Material

The healthy plant of *Vanilla planifolia* were collected from the Idukki, Kerala, India and used for study. The plants were identified and the voucher specimens were deposited in the herbarium cabinet facility sponsored by St. Joseph's College, Tiruchirappalli. After authentification, the plants were cleaned and shade dried and milled into coarse powder by a mechanical grinder.

Preparation of Extract

The beans of the plant was cut into small pieces, shade dried and made into powder. 100g of plant powder was suspended in 300ml of ethanol and the mixture was filtered and air dried by low pressure using soxhlet apparatus. The residue was collected and dissolved in water in a fixed dose and used for the treatment.

Hepatoprotective Activity

A total of 30 animals were equally divided into 5 groups of six each. Group -1 served as control and had free access to food and water. Group- 2 received 0.5 ml of saline for 5 days. On day 5th, Paracetamol (500mg/kg) was given orally. Group -3 treated orally with ethanolic extract of *Vanilla planifolia* (100mg/kg) once in a day for 7 days. Group -4 pretreated with ethanolic extract of *Vanilla planifolia* (100mg/kg) for 5 days as single dose on 5th day, Paracetamol (500mg/kg) was given orally. Group- 5 pretreated with silymarin (100mg/kg) for 5 days as single dose on day 5th, Paracetamol (500mg/kg body weight) was given orally. All the test drugs and paracetamol were administered orally by suspending in 0.5% CMC solution. After 48h of paracetamol feeding, the blood was collected by direct cardiac puncture under light ether anesthesia and serum was separated for the estimations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP)[8,9] and bilirubin[10].

Statistical Analysis

The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnet's 't' - test. P values <0.05 were considered significant.

RESULTS

The results of hepatoprotective activity of ethanolic extract of *Vanilla planifolia* on Paracetamol treated rats are shown in Table 1. The hepatic enzymes ALT, AST, ALP and bilirubin in serum was significantly (P <0.01) increased in paracetamol treated animals when compared to control. The ethanolic extract of *Vanilla planifolia* treatments significantly (P < 0.01) reversed the levels of AST, ALP and bilirubin and ALT (P<0.01) when compared to paracetamol alone treated rats. Silymarin (100 mg/kg) treated animals also showed significant decrease in AST, ALT, ALP and bilirubin (P<0.01) levels when compared to paracetamol alone treated rats.

Table 1. Effect of ethanol extract of *Vanilla planifolia* on paracetamol-induced hepatotoxicity in rats

Treatment	Dose mg/kg	ALP(U/L)	SGOT (U/L)	SGPT (U/L)	Total Bilirubin mg/dl
Control		252.68 ± 54.00	42.88 ± 13.40	24.44 ± 10.10	0.57 ± 0.25
Paracetamol induced	500	475.12 ± 31.59	89.84 ± 20.00	57.52 ± 13.72	2.36 ± 0.48
Vanilla planifolia extract alone	100	388.50 ± 48.80*	79.64 ± 2.87*	22.12 ± 1.12*	1.55 ± 0.25*
Paracetamol + *Vanilla planifolia* extract	100	322.98 ± 18.89*	61.38 ± 9.77*	32.08 ± 9.23*	1.00 ± 0.18*
Paracetamol + silymarin	100	329.98 ± 28.88*	58.62 ± 9.63*	38.88 ± 5.25*	0.60 ± 0.08*

Data are expressed as Mean ± SEM, n = 6 in each group. *P<0.01 compared to control group.

DISCUSSION

Paracetamol is a well known antipyretic and analgesic, which produces hepatic necrosis in high doses and of the most commonly, used hepatotoxins in the experimental study of liver disease. Paracetamol damages liver by covalent binding of its toxic metabolite N-acetyl-p-benzoquinone imine to sulphydrl groups of proteins resulting in cell necrosis and lipid peroxidation induced by decrease in glutathione in the liver[11]. This is evidenced by an elevation in the serum marker enzymes namely AST, ALT, ALP, and total bilirubin. 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[12].

The inhibitory effect of the ethanolic extract of *Vanilla planifolia* on hepatotoxicity was compared to that of positive control group. The significant protection in the biochemical parameters like AST, ALT and ALP against Paracetamol induced elevations in pretreatment of the animals with the 100 mg/kg of the ethanolic extract of leaves of *Vanilla planifolia*. The administration of hepatoprotective drugs may induce the hepatocytes to resist the toxic effect of Paracetamol. The results indicated that the ethanolic extract of *Vanilla planifolia* has significant hepatoprotective activity. The obtained results indicated a high degree of protection against the hepatotoxic effect of Paracetamol. The serum enzyme levels were significantly ($P<0.01$) declined in Paracetamol treated group.

Bilirubin is one of the most useful clinical clues to the severity of necrosis and its accumulation is a measure of binding, conjugation and excretory capacity of hepatocyte. Decrease in serum bilirubin after treatment with the extract in liver damage induced by paracetamol, indicated the effectiveness of the extract in normal functional status of the liver.

CONCLUSION

The results of the present investigation indicate that the ethanol extract of *Vanilla planifolia* possess good hepatoprotective activity. Further investigations are required to characterize the active hepatoprotective principle and its mechanism of action.

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