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Original Research Article

COMPARATIVE EVALUATION OF THE HEPATOPROTECTIVE EFFECT OF COSTUS PICTUS D DON METHANOLIC LEAF EXTRACT AND SILYMARIN ON PARACETAMOL INDUCED LIVER DAMAGE IN ALBINO WISTAR RATS

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ABSTRACT

Background: NSAIDs are the common group of drugs used in self-medication, and this is true for especially Paracetamol (acetaminophen).Although considered safe at therapeutic doses, in overdose, paracetamol causes centrilobular hepatic necrosis which can be fatal. As no data is available on the hepatoprotective effect of Costus pictus D Don, we have made an attempt to investigate the protective effect of Costus pictus D Don leaf extract on paracetamol induced liver damage in rats. The aim of the study is to compare the hepatoprotective effect of methanolic leaf extract of Costus pictus D Don and silymarin on liver damage induced by paracetamol in Wistar rats.

Materials and Methods: 30 Healthy male adult Wistar rats (16 weeks old) weighing > 250g were used for the study. The animals were maintained in a standard cage under controlled temperature (25±2 °C) and light (12:12 light-dark cycle) in MGMC & RI central animal house. The animals were fed with standard rat pellet and hygienic water ad libitum. 30 adult Wistar rats were randomized into 5 groups with 6 rats each as (Normal control -0.5% carboxymethylcellulose (7 days), Toxic control- 0.5% (7 days)+paracetamol 2g/kg(5^{th} day), Test group I-200 mg/kg methanolic leaf extract+paracetamol 2g/kg(5^{th} day) , Test group II-100 mg/kg methanolic leaf extract+paracetamol 2g/kg(5^{th} day) & Standard group - silymarin 25mg/kg (7 days) + Paracetamol 2 g/kg (5^{th} day) The animals were sacrificed on 8^{th} day using sodium pentobarbitone 150mg/kg i.p. serum was sent for biochemical analysis for liver function test. Liver was harvested and a portion was taken for histological examination.

Results: In our study methanolic leaf extract of Costus pictus D Don showed beneficial effect on paracetamol induced liver toxicity which was evident by the significant improvement in liver function test consisting of AST, ALT and ALP in a dose dependent manner which is in consistent with the histological findings.

Conclusions: The study has proved the methanolic leaf extract of Costus pictus D Don posses a significant hepatoprotective activity which was comparable to the standard drug silymarin.

KEY WORDS: Costus pictus D Don, Silymarin, Hepatoprotection.

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INTRODUCTION

In developing countries, it is very common to see drugs dispensed over the counter without medical supervision. Lower socioeconomic status, easy availability of medical products, cost of clinical services are few factors responsible for the increase in incidence of self-medication [1,2].

NSAIDs are the common group of drugs used in self-medication, and this is true for especially Paracetamol (acetaminophen). Prior experience and non-seriousness of illness are the two main reasons for self-medication [3]. Although considered safe at therapeutic doses, in overdose, paracetamol causes centrilobular hepatic necrosis which can be fatal [4]. At therapeutic doses, cytochrome P450 enzymes metabolically activates acetamionophen to a reactive metabolite, N-acetyl-p-benzoquinone (NAPQI) that depletes gluthione(GSH) and covalently bounds to proteins. After toxic dose of acetaminophen, total hepatic GSH is depleted by as much as 90%.As a result, the metabolite covalently binds to cysteine groups on protein forming acetaminophen-protein adducts which subsequently results in loss of function and eventually cell death and lysis [5,6].

Synthetic drugs used for treating liver injury can further cause liver damage. Various plant extracts and polyherbal medications have been clinically approved for their potency and safety in treatment of liver disorder [7].

A novel medicinal plant Costus pictus D. Don commonly known as spiral ginger has diverse biological properties including anti-diabetic [8], anti-oxidant [9], anti-tumor [10], anthelminthic [11], antimicrobial effect [12].

As no data is available on the hepatoprotective effect of Costus pictus D Don, we have made an attempt to investigate the protective effect of Costus pictus D Don leaf extract on paracetamol induced liver damage in rats.

MATERIALS AND METHODS

This prospective cohort study was conducted after receiving the ethical clearance from the Institutional Animal Ethics Committee of Mahatma Gandhi Medical college & Research Institute, Puducherry. Healthy male adult Wistar rats (16 weeks old) weighing > 250g were be used for the study. The animals were procured from Kings Institute, Chennai. The animals were be maintained in a standard cage under controlled temperature (25+2 °C) and light (12:12 light-dark cycle) in MGMC & RI central animal house. The animals were fed with standard rat pellet and hygienic water ad libitum.

Preparation of the methanolic leaf extract:

Costus pictus D Don leaves was collected from domestic gardens of Pondicherry and confirmed by a botanist. The leaves were washed thoroughly with water, air dried in shade for 5 days and powdered. The powdered leaf material was be soxahaleted with methanol. The methanol was then be distilled, allowed to evaporate and the final content is air dried and stored in air tight brown capped bottle in a refrigerator [13].

Experimental design: 30 adult Wistar rats were randomized into 5 groups with 6 rats each.

Group 1: Normal control – 0.5% carboxymethylcellulose (CMC) 1ml/kg X 7 days

Group 2: Toxic control – 0.5 % CMC for 7days + Paracetamol (2 g/kg) p.o on 5th day

Group 3: Test group I - 200 mg/kg methanolic leaf extract of Costus pictus D Don for 7 days + Paracetamol (2 g/kg) p.o on 5th day

Group 4: Test group II - 100 mg/kg methanolic leaf extract of Costus pictus D Don for 7 days + Paracetamol (2 g/kg) p.o on 5th day

Group 5: Standard group - silymarin 25mg/kg for 7 days + Paracetamol (2 g/kg) p.o on 5th day

For 7 days, the animals in test group I and II was pretreated with methanolic leaf extract of Costus pictus D Don in doses of 200 mg/kg BW and 100 mg/kg BW respectively. Standard group animals will be pre-treated with silymarin 25mg/kg for 7 days. Hepatotoxocity will be induced by oral administration of paracetamol at a dose of 2 g/kg BW on 5th day.

On 8th day, the animal was sacrificed using sodium pentobarbitone 150mg/kg i.p. Blood samples was collected by intra cardiac puncture for biochemical analysis to estimate hepatocellular enzymes: Aspartate transaminase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) using standard clinical autoanalyser. Liver was washed in saline and a

small portion of these organs were quickly fixed in 10% formalin and slides were prepared using standard histological technicque and stained with Haematoxylin and Eosin stain. The stained slides were analysed under microscope and photographed.

Statistical Analysis: The collected data was analysed using SPSS version 17.0. the values were expressed as mean± Standard Error of mean (SEM). The statistical analysis was carried out using one way ANOVA followed by post hoc Tukey's test. P value < 0.05 were considered be statistically significant at 95% confidence interval.

RESULTS

The results are discussed under 1) biochemical parameters and 2) histological examination

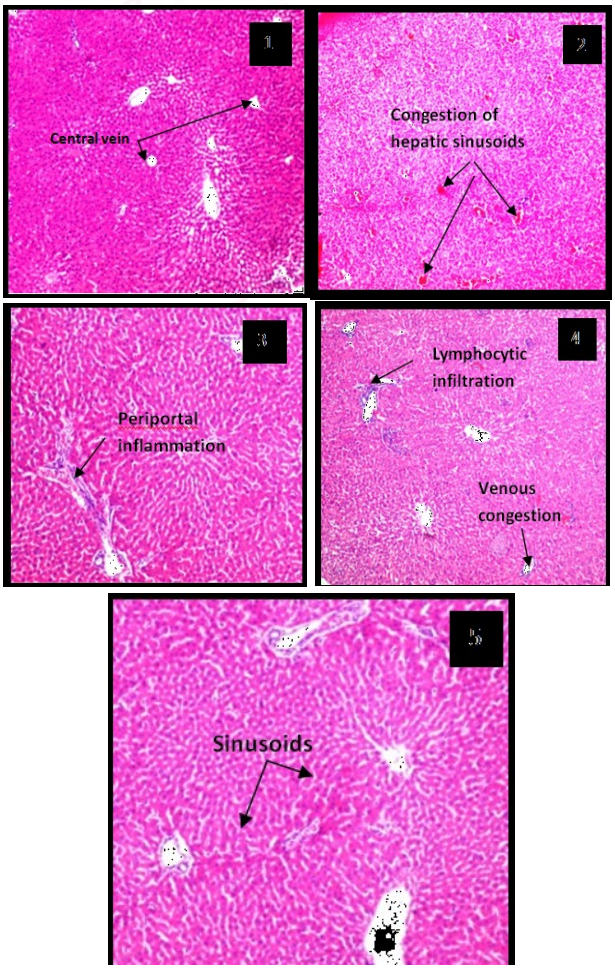
Biochemical parameters: The values of Liver Function Test such as AST, ALT and ALP were compared between each study groups are shown in Table 1. There was significant increase in ALT,AST & ALP levels in paracetamol toxic control group. Silymarin administered rats (standard control) showed significant reduction ($p<0.001$) of liver enzymes. Rats in Group no.4 (Test group II) demonstrated significant difference ($p<0.05$) in the liver parameters when compared to that of standard group. Group no.3 (Test group I) showed alterations of liver parameters which were not significant ($p>0.05$) when compared to standard control group. This implies that the alterations in liver parameters demonstrated By test group 1 was comparable to that of standard control.

Histological study: The sections of liver from normal control group shows normal liver architecture with cords of hepatocytes radiating from centre to periphery (fig 1:1). Sections studied from liver with paracetamol treated group shows venous congestion with periportal chronic lymphocytic infiltration. There is also congestion of hepatic sinusoids with mild distortion in the hepatocyte architecture (fig1:2). Sections studied from liver treated with 200 mg/kg methanolic leaf extract of Costus pictus D Don shows reduced periportal inflammation with mild hepatic venous congestion (fig 1:3). Section studied from liver of test group 4 treated with 100 mg/kg methanolic leaf extract of Costus pictus D Don shows central venous congestion with mild periportal chronic lymphocytic infiltration (fig 1:4). Section studied from liver with treated with silymarin shows absence of periportal inflammation. Mild congestion in very few central veins seen (fig 1:5)

Table 1: Effect of Methanolic leaf extract of Costus pictus D Don on liver function test values expressed in Mean±SEM.

SL.NO	GROUPS	ALT(IU/L)	AST(IU/L)	ALP(IU/L)
1	Normal control	34.53+3.26	48.55+2.87	96.33+3.43
2	Toxic control	250.12+6.67	157.43+1.5	217.78+4.64
3	Test group I	140.5+4.27	102.6+4.31	144.5+4.99
4	Test group II	182.1+3.21	129.56+2.21	176.5+4.56
5	Standard group	141.5+2.36	93.76+2.34	141.25+4.75

Fig. 1: Photomicrograph showing liver section of control, Toxic control, Test group I, Test group II & Standard control as 1,2,3,4 & 5 in Haemotoxlin & Eosin stain under 10X magnification.



DISCUSSION

Blood serum markers like ALT, AST and ALP are commonly used in assessing the liver disease. Increase in levels of both transaminases ALT and AST is highly indicative of hepatotoxicity[14]. ALP is a highly sensitive marker of hepatobiliary

injury. Increased levels are associated with congestion or obstruction of bile ducts indicating cholestasis [15].

In our study, the Toxic control (group 2) which received only paracetamol showed an abnormal increase in liver enzymes namely ALT,AST & ALP. Paracetamol was shown to cause hepatotoxicity by forming a highly reactive alkylating metabolite which binds to cell constituents covalently to cause cell damage and necrosis. Under therapeutic dose the metabolite are removed rapidly by conjugation but in toxic dose the excess metabolites produced will produce more hepatocyte damage [4].

In the test group I & II the rats pretreated with Methanolic leaf extract of Costus pictus D Don with doses of 200mg/kg and 100mg/kg tapered the increase in liver enzymes value caused by paracetamol toxicity. More studies evaluating the effect of leaf extract of Costus pictus D Don on liver function test is lacking in scientific literature. The improvement in the liver function tests clearly implicates the ability of Costus pictus D to maintain the liver function during acute paracetamol toxicity.

The group 5 was pre treated with silymarin and served as the standard control. There was marked reduction in liver enzymes when compared to that of paracetamol toxic control group.these findings are comparable to the result obtained by Kazemifera et al.[16] Silymarin possess membrane stabilizing activity which prevents hepatocellular damage thus maintaining the normal functioning of the liver [17].

The congestion of central vein and hepatic sinusoids are typical features of paracetamol as per Zhang et al. [18], are consistent with our observation in Toxic control group.

The test group I which was treated with 200 mg/kg methanolic leaf extract of Costus pictus D Don shows reduced periportal inflammation with mild hepatic venous congestion. The test group II which was treated with 100 mg/kg methanolic leaf extract of Costus pictus D Don shows central venous congestion with mild periportal chronic lymphocytic infiltration.Studies focusing on histopathological studies of hepatoprotective effects of Costus pictus D Don are lacking.

The standard group which was treated with silymarin showed the absence of periportal inflammation. Mild congestion was observed in very few central veins. These findings are in accordance with Girish et al., and further confirm the hepatoprotective effects of Silymarin [19].

CONCLUSION

Naturally occurring antioxidant phytochemicals shows a promising potential agent capable of prevention and protection against liver damage caused by oxidative stress. In this study Methanolic leaf extract of Costus pictus D Don is proved to have protective effect against paracetamol induced liver toxicity which is similar to silymarin treated liver. Hence, the result of the study will be of great therapeutic importance in treating liver injury with naturally available plant.

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Conflicts of Interests: None

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