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Original Article

HEPATOPROTECTIVE ACTIVITY OF *PHYLLANTHUSNIRURI* IN THIOACETAMIDE INDUCED HEPATOTOXICITY IN MALE WISTAR RATS

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ABSTRACT

Objective: To assess the hepatoprotective and hepatoregenerative activity of *Phyllanthusniruri* in liver damage caused by long term administration of high doses of thioacetamide.

Methods: Sixty, albino wistar male rats weighing 150 – 200g were divided into six groups of ten rats each. The study was conducted in two phases of twelve weeks each. Group I served as control. In group II hepatotoxicity was induced with thioacetamide during phase 1 and no active treatment was given in phase 2. In group III hepatotoxicity was induced during phase 1 and treatment with extract of *Phyllanthusniruri* was given in phase 2. In group IV hepatotoxicity was induced during both the phases. In group V *Phyllanthusniruri* was given in phase 1 and hepatotoxicity was induced during phase 2. Group VI received *Phyllanthusniruri*during both the phases. Daily fluid intake and weekly body weight was measured. Histopathology and superoxide dismutase (SOD) activity of the liver were studied.Statistical analysis used: Data was analysed using SPSS software version 11.5. One way ANOVA followed by post hoc Tukey test was used. Data has been expressed as mean ± Standard deviation (SD), p value < 0.05 was considered significant.

Results: There were four deaths in group II, two in group III and IV. All the rats in groups II, III and IV and 50% of the rats in group V developed cirrhosis; 50% of the rats in group also developed dysplasia. There was an increase in SOD activity in groups II and III and a decrease in the same in group IV.

Conclusion: *Phyllanthusniruri* is an effective prophylactic against thioacetamide induced liver cirrhosis.

Keywords: *Phyllanthusniruri,* thioacetamide, hepatotoxicity

INTRODUCTION

Drug induced liver injury still remains a challenge not only to patients and health care professionals but also to the pharmaceutical industry and drug regulatory agencies. Hepatotoxicity has become one of the principle limitations of some important commonly used drugs. Because of its strategic placement in the body, toxins gain access first to the liver and make it one of the most vulnerable organs. Long term exposure of the liver to various toxic insults may cause irreversible injury to the hepatic parenchyma and consequently chronic inflammatory changes. Thioacetamide is a proven hepatotoxin causing centrilobular hepatic necrosis [1]. The toxic effects of thioacetamide are postulated to be due to the formation of free radicals and inhibition of enzymatic activity of hepatocytes [2].

Phyllanthusniruri (Bhumyamalaki in Sanskrit) is a common herb grown in India. The roots, leaves and also the whole plant have been traditionally used in Indian folklore medicinefor the treatment of jaundice, dysentery, dyspepsia, colic, cough and diabetes [3].A compound herbal product with a mixture of six herbs including *Phyllanthusniruri*has been shown to prevent liver cell necrosis in acute and chronic liver damage induced by carbon tetrachloride in rats [4]. Two active principles, phyllanthin and hypophyllanthin isolated from a hexane extract of *Phyllanthusniruri*protected against carbon tetrachloride and galactosamine induced cytotoxicity in primary cultured rat hepatocytes [5]. Despite the tremendous advances in modern medicine, no effective hepatoprotective drug is available. Not many studies have investigated the hepatoprotective effects of *Phyllanthusniruri* in thioacetamide induced liver injury.Hence this study was undertaken to assess the hepatoprotective and hepatoregenerative activity of *Phyllanthusniruri*in liver damage caused by long term administration of high doses of thioacetamide.

MATERIALS AND METHODS

Plant material

The whole plant of *Phyllanthusniruri* L. was collected locally in the month of September, crushed and the juice was obtained. After addition of honey, it was stored in a closed earthen vessel for a month. It was later filtered and used.

Animals

A total of 60 healthy,in-bred, albino wistar adult male rats aged approximately 2 months, weighing 150 – 200g were obtained from the institutional central animal house after approval from the Institutional Animal Ethics Committee. The animals were individually housed under standard laboratory conditions in clean polypropylene cages, in a controlled environment (22-24^{0}C) with a 12 hour light and dark cycle and fed standard laboratory pellet diet and water *ad libitum*. The animals were divided into six groups of ten rats each. The study was conducted in two phases as shown in table 1.

Assessment of hepatoprotective activity

Daily fluid intake and weekly body weight was measured. At the end of the study, rats from all the groups were anaesthetized using ether. The abdomen was opened by a midline incision and the liver was dissected out. The weight of the liver was noted and a portion of the liver was put in formalin and sent for histopathological examination, which was done blindly by a pathologist. A portion of the liver was perfused with cold saline to remove as much blood as possible, was blotted with blotting paper and refrigerated until the assays of superoxide dismutase (SOD) in the liver homogenate were performed. Liver homogenate was prepared using 1g liver tissue and 10ml 0.005M potassium phosphate buffer (pH 7.8). Unbroken cell and cell debris were removed by centrifugation at 700g for 10 minutes. The supernatant was used for assay of SOD activity. The

procedure adapted for SOD enzyme assay was that of Beauchamp and Fridovich[6].

Statistical analysis

Data was analysed using SPSS software version 11.5. One way ANOVA followed by post hoc Tukey test was used. Data has been expressed as mean ± Standard deviation (SD), p value < 0.05 was considered significant.

RESULTS

Mortality

There were four deaths in group II, two in group III and IV. No deaths were recorded in the other groups.

Body Weight

As shown in Table 2, the control group showed an increase in the mean body weight gain during the study period.

The increase was significantly higher at the end of phase 1 as compared to the end of phase 2.

Groups II, III and IV showed a significant reduction in mean body weight gain as compared to group I at the end of phase 1.Groups V and VI that received *Phyllanthusniruri* showed a very highly significant increase in mean body weight gain as compared to the control in phase 1.

SOD activity and liver histopathology

Groups II and III in phase 1 and group V in phase 2 received thioacetamide in a dose of 5.1, 5.2 and 5.4 mg per rat per day respectively and group IV received the toxin in a dose of 4.5 and 4.8mg per rat per day in phase 1 and 2 respectively. As shown in Table 3,initial exposure to thioacetamide increased SOD activity. When *Phyllanthusniruri*was given as a corrective (group III) it enhanced the already increased activity of the enzyme following initial exposure of thioacetamide. This increase in activity of SOD was still associated with cirrhotic changes in all rats of group III, but two rats showed only early cirrhosis as compared to group II where all ten rats showed cirrhosis. This favourable picture in group III is further associated with a mean liver weight comparable to that of the control group. Among the four groups that received thioacetamide, group IV registered the lowest level of SOD activity, and all the rats of this group not only showed cirrhosis but five of them also showed early dysplasia. Group VI which received *Phyllanthusniruri*throughout the study registered the lowest level of SOD activity in the study, lower than the control group (Table 3). Though groups III and V received thioacetamide and the extract of *Phyllanthusniruri*for a similar period of time in the two phases of the study at different intervals, group V registered significantly less SOD activity than group III. Equally significant was the finding that in group V histopathology of five livers were normal and five showed cirrhosis of which three were early changes of cirrhosis; while all the rats in group III showed cirrhosis. Moreover, there were no deaths registered in group V whereas group III registered two deaths.

Table 1: Plan of the study

Groups	Treatment received during Phase 1 (initial 12 weeks)	Treatment received during Phase 2 (latter 12 weeks)
I (Control)	Standard pellet diet and water	Standard pellet diet and water
II	Standard pellet diet and water containing thioacetamide 0.0004M	Standard pellet diet and water
III	Standard pellet diet and water containing thioacetamide 0.0004M	Standard pellet diet and water containing *Phyllanthusniruri* 10ml/L
IV	Standard pellet diet and water containing thioacetamide 0.0004M	Standard pellet diet and water containing thioacetamide 0.0004M
V	Standard pellet diet and water containing *Phyllanthusniruri*10ml/L	Standard pellet diet and water containing thioacetamide 0.0004M
VI	Standard pellet diet and water containing *Phyllanthusniruri* 10ml/L	Standard pellet diet and water containing *Phyllanthusniruri*10ml/L

Table 2: Mean body weight gain in Phase 1 and 2

Groups	Mean body weight gain ± SD (g)	
	Phase 1	**Phase 2**
I	60.20±11.86	33.56±17.49
II	14.00±26.85*	74.50±22.88
III	28.09±19.43*	74.13±28.04
IV	31.27±13.35*	28.33±9.54
V	86.91±22.77#	17.30±17.13
VI	127.27±13.89*	29.45±8.42

*p<0.00001, #p<0.01 vs Group I

Table 3: SOD activity, liver weight and liver histopathology

Groups	Mean SOD ± SD(units/g)	Mean liver weight ± SD (g)	Liver histopathology	
			Cirrhosis(n=10)	**Cirrhosis with dysplasia (n=10)**
I	912.56±122.89	12.62±1.59	0	0
II	1266±201.25	14.03±3.36	10	0
III	2145.5±203.72$	12.27±1.73	10	0
IV	786.33±86.74	17.62±2.23	10	5
V	994.5±104.07#	18.38±3.65	5	0
VI	748.82±116.99*	12.05±1.24	0	0

*p<0.05 vs Group II, III and V; #p<0.05 vs Group II, III; $p<0.05 vs Group I, IV, V and VI

DISCUSSION

The significant increase in body weight in the control group in phase 1 may be representative of the normal pattern of growth in rodents. The reduction in mean body weight gain in groups II, III and IV was anticipated as they all received thioacetamide. The increase in mean body weight gain in groups V and VI as compared to the control in phase 1shows that *Phyllanthusniruri*may possess anabolic activity. This spurt in anabolic activity where index of activity was the measurement of body weight reached its ceiling at the end of phase 1 and tapered off to a plateau in phase 2.

Initial exposure to thioacetamide increased SOD activity which could be due to a protective response against injury. However, if the protective mechanisms against thioacetamide only was of importance, all four groups which received thioacetamide should have shown comparable results with reference to SOD activity which was not so. The rats which were exposed to thioacetamide for the longest period (24 weeks) displayed low SOD activity and carcinogenic changes in the liver histopathology.This finding corroborates the findings of other studies[7,8].

The normal rats which received *Phyllanthusniruri*(Group VI) had SOD activity lower than the control rats, this indicates that this product may exert an active protective role against routinely found oxidants to which groups I and VI were uniformly exposed. This protection was independent of SOD activity.

Pre-treatment with *Phyllanthusniruri*protected the rats from hepatotoxicity induced by thioacetamide but hepatoregenerative activity was not found,as has been depicted by the inability of the plant extract to correct the cirrhotic changes and deaths that occurred in group 3, though SOD activity was very high. Hence the authors are convinced that *Phyllanthusniruri* extract is more effective as a prophylactic (as given in group V) than as a corrective (as given in group III). However, group V was associated with SOD activity that was lower than in group III, indicating that *Phyllanthusniruri* extract exerts a protective activity by a mechanism that is not entirely dependent on SOD activity.

Conclusion: An extract of the whole plant of*Phyllanthusniruri* is an effective prophylactic against thioacetamide induced liver cirrhosis, by an unknown mechanism other than the action through SOD. However, when used after the injury has already occurred, it increases the inherent SOD activity.

CONFLICTING INTEREST: None

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