

Original Research Article

Hepatoprotective activity of aqueous extract of *Cynodon dactylon* on paracetamol induced hepatotoxic albino rats

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Abstract: *Cynodon dactylon* is commonly known as Bermuda grass. It is a perennial grass and found all over the India. The plant is a rich source of metabolites such as proteins, carbohydrates, minerals, β -sitosterols, flavonoids, alkaloids, glycosides, terpenoids. The plant has also been used traditionally to treat various ailments like cough, convulsion, anasarca, diarrhoea, dysentery, headache, haemorrhage, hypertension, warts and wounds. The hepatoprotective activity of aqueous extract of *Cynodon dactylon* at 200mg/kg body weight and 400mg/kg body weight was carried out against paracetamol induced liver damaged in albino rats. Treatment of the animals with extract caused a significant reduction in the values of SGOT, SGPT, and ALP nearly similar to standard N-acetylcysteine. The hepatoprotective activity was further confirmed by histopathological examination of liver specimens of control and treated groups.

Keywords: *Cynodon dactylon*, hepatoprotective, paracetamol, N-acetylcysteine

INTRODUCTION

Cynodon dactylon is a perennial grass. English name is Bermuda grass and belongs to the family of Poaceae. It is native to East Africa, Asia, Australia and southern Europe. *Cynodon dactylon* is a weed and has been found to possess various potential medicinal properties [1]. It is used as a folk remedy for anasarca, calculus, cancer, carbuncle, convulsion, cough, cramps, cystitis, diarrhoea, dropsy, dysentery, epilepsy, haemorrhoids, leucoderma, headache, haemorrhage, hypertension, hysteria, bronchitis, asthma, tumours, warts and wounds [2]. The paste made of the plant mixed with honey is used in epistaxis. Oral administration of the juice of the plant with honey 2-3 times a day for few days effectively treats menorrhagia. Local application in the form of paste of plant extract upon the lower abdomen reduces severe bleeding in vagina. A decoction of *Cynodon dactylon* mixed with sugar is useful in the problem of urinary retention [2].

Liver diseases have become one of the major causes of morbidity and mortality all over the world. From among this, drug induced liver injury (DILI) is one of the most common causative factor that possess a major clinical and regulatory challenge [3]. The

manifestations of drug-induced hepatotoxicity are highly variable, ranging from asymptomatic elevation of liver enzymes to fulminant hepatic failure. Paracetamol (PCM) also known as Acetaminophen, taken in overdose can cause severe hepatotoxicity and nephrotoxicity. N-acetyl-p-benzo quinoneimine (NAPQI) is a highly reactive arylating minor metabolite of paracetamol which is detoxified by conjugation with glutathione. When a very large dose of paracetamol is taken, glucuronidation capacity is saturated, more of the minor metabolite is formed- hepatic glutathione is depleted and this metabolite binds covalently to proteins in liver cells causing necrosis [4]. The study was conducted to evaluate the hepatoprotective activity of aqueous extract of *Cynodon dactylon* on paracetamol induced hepatotoxic albino rats.

MATERIALS AND METHODS

Drugs and Chemicals

Paracetamol (Para safe 500mg) was purchased from pharmacy. Biochemical estimation kits (Bene SpheraTM) were purchased from Avantor Performance Materials India Ltd, Uttarakhand.

Plant materials

Fresh aerial parts of *Cynodon dactylon* were collected from the Lamphel area, Imphal in the month of August 2014. They were identified and authenticated by Professor P. Kumar Singh of Dept. of Life Sciences, Manipur University, Imphal, Manipur. A voucher sample is kept in the University herbarium under the voucher number [000864].

Preparation of extract

The collected aerial parts of the plant were cleansed and air dried under the shade, powdered by mixer grinder and stored in airtight container for future use. Preparation of aqueous extract of the plant was done by the method described by Verma SCL and Agarwal SL [5]. The powdered aerial parts of *Cynodon dactylon* were extracted using Soxhlet apparatus with distilled water. The greenish brown extract so obtained was filtered, evaporated, shade-dried, weighed and stored in a glazed porcelain jar for use in the experiment. The yield was 15%.

Phytochemical studies

Phytochemical screening of the aqueous extract of *Cynodon dactylon* showed the presence of flavonoids and glycosides.

Acute toxicity studies

Acute toxicity studies for aqueous extract of *Cynodon dactylon* were conducted as per OECD guidelines 423 using mice. Each animal was administered the aqueous extract of the plant by oral route. The animals were observed for any changes continuously for the first 2 h and upto 24 h for mortality. The mice did not show any mortality at 2000mg/kg and hence its 1/10th dose i.e., 200mg/kg and 1/5th dose i.e., 400mg/kg were used as the therapeutic doses in the study [6].

Experimental protocol

Prior approval from the Institutional Animal ethics committee was obtained for the study (Reg No: 1596/GO/a/12/CPCSEA). 25 wistar healthy albino rats of either sex of (150- 250 g) were taken from Central animal house, RIMS, Imphal, India and were used for the study. Animals were fed with standard pellet diet and water was provided ad libitum. The rats were housed under conditions of controlled temperature (21±2°C) and acclimatized to 12h light: dark cycle.

Experiment was conducted according to guidelines of Institutional Animal Ethics Committee. Animals were divided into five groups of five animals each as Group 1 (normal control), Group 2 (hepatotoxic control), Group 3 (test 1), Group 4 (test 2), and Group 5 (standard). Group 1 received 2% gum acacia dissolved in distilled water at 1, 24, and 48 h per orally (p.o). Group 2 received paracetamol (3g/kg) as single dose on day one followed by distilled water at 1, 24, 48 h p.o. following paracetamol administration. Group 3 received paracetamol (3g/kg) as single dose on day one followed by aqueous extract of *Cynodon dactylon* (200mg/kg) at 1, 24, 48 h p.o. following paracetamol administration. Group 4 received paracetamol (3g/kg) as single dose on day one followed by aqueous extract of *Cynodon dactylon* (400mg/kg) at 1, 24, 48 h p.o. following paracetamol administration. Group 5 received paracetamol (3g/kg) as single dose on day one followed by N-acetylcysteine (100mg/kg) at 1, 24, 48 h p.o. following paracetamol administration [7]. At the end of 72 h, blood samples were collected by retro-orbital puncture method [8, 9]. Collected samples were centrifuged and serum was separated for the estimation of ALP, SGOT and SGPT by using kit method. Animals were sacrificed under high dose of ether; liver specimens were taken for histopathological examination.

Biochemical estimation

Serum ALP was estimated using modified King & King's method [10]. Serum SGOT and SGPT estimation was done by using Reitman and Frankel's method [11].

Histopathological studies of rat kidney

Liver samples were dissected out and washed with normal saline. Initially the materials were fixed in 10% buffered neutral formalin, dehydrated with 100% ethanol solution and embedded in paraffin. It was then processed into 4-5µ thick sections, stained with hematoxylin-eosin and observed under a microscope (magnification power- 40X) [6, 12].

STATISTICAL ANALYSIS

Statistical analysis was done by using SPSS version 16 software. The results were expressed as Mean±S.E.M. Differences between groups were assessed by One-Way ANOVA followed by Dunnett's t test.

RESULTS

Table 1: Biochemical parameters of paracetamol induced hepatotoxicity.

Groups	Drugs given	ALP (IU/L)	SGOT (IU/L)	SGPT (IU/L)
Group 1	2% gum acacia	84.88±200*	37.51±1.76**	37.39±1.41**
Group 2	Paracetamol (3g/kg) + DW	237.74±17.51	130.34±8.25	137.56±5.54
Group 3	Paracetamol (3g/kg) + CDE (200mg/kg)	139.52±4.45*#	82.24±2.45#	80.84±1.35°
Group 4	Paracetamol (3g/kg) + CDE (400mg/kg)	114.45±3.97*ϑ	59.45±1.15**ϑ	72.84±1.98**ϑ
Group 5	Paracetamol (3g/kg) + NAC (100mg/kg)	101.43±3.24*	57.15±3.45**	54.02±1.77**
One-Way ANOVA	F df= (4,20) P<0.05	51.249	70.670	104.847

Values are Mean±S.E.M, n=5, *P<0.05, **P<0.01, when compared with hepatotoxic control.
P<0.01, °P<0.001, when compared with standard. ϑ P<0.01, when compared with test 1.

Serum ALP, SGOT, SGPT of toxic control is 237.74±17.15, 130.34±8.25, 137.56±54 respectively. These values are significantly high with respect to normal control, test 1, test 2 and standard at P values <0.05 and 0.001. ALP and SGOT values of Test 1 are 139.52±4.45 and 82.24±2.25 respectively. These values are highly significant, very highly significant when compared with standard at P values 0.01 and 0.001. ALP, SGOT, SGPT values of Test 2 are 114.45±3.97, 59.45±1.15 and 72.84±1.98 respectively. These values are highly significant when compared with test 1;

suggesting CDE at 400 mg/kg is more effective than CDE at 200 mg/kg.

Histopathological examination

The processed histological slides were examined for any histopathological changes. The liver histological parameters of hepatocyte necrosis, binocularity of hepatocytes, congestion and portal tract infiltration were examined and scoring was done as none (-), mild (+), moderate (++) , severe (+++).

Table 2: Histopathological scoring of paracetamol induced hepatotoxicity.

Histological parameters	Normal control	Hepatotoxic control	Test 1	Test 2	Standard
Hepatocyte necrosis	-	+++	-	-	-
Binocularity of hepatocytes	-	++	-	-	-
Congestion	-	+++	++	-	+
Portal tract infiltration	-	+++	+	-	-

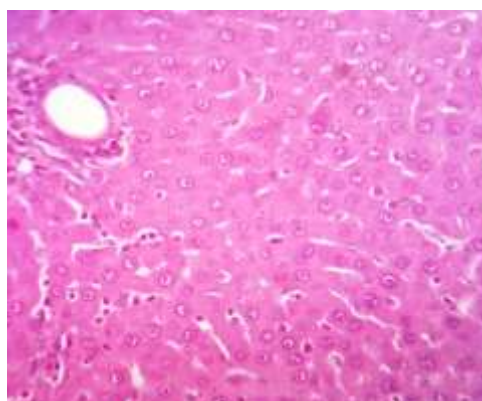


Fig 1: Normal control

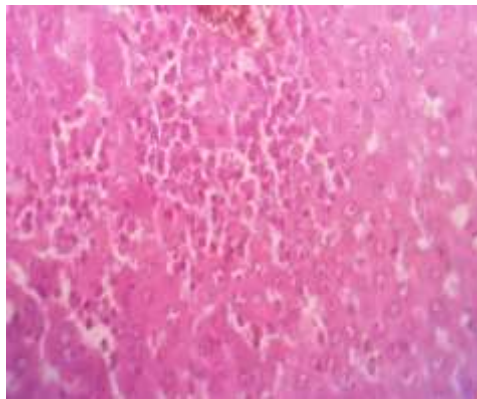


Fig 2: Hepatotoxic control

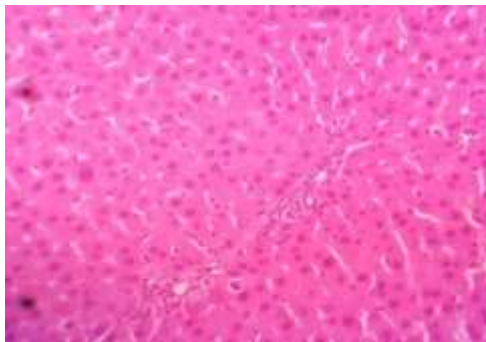


Fig 3: Test 1

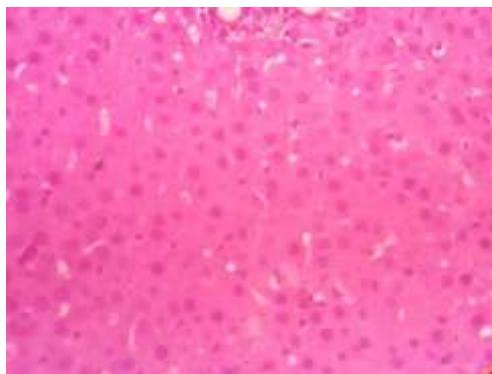


Fig 4: Test 2

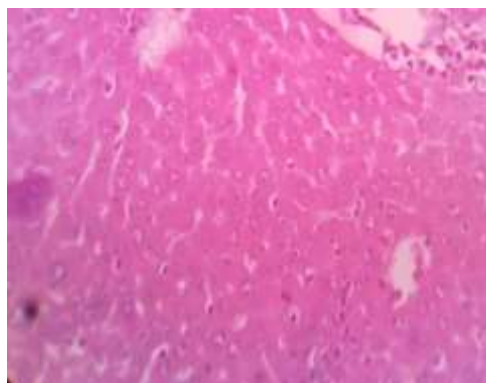


Fig 5: Standard

DISCUSSION

Liver dysfunction due to various causes in our life is increasing worldwide. Liver diseases are mainly caused by toxic chemicals (CCL₄, certain antibiotics, chemotherapeutic agents, aflatoxins, chlorinated hydrocarbons), excessive consumption of alcohol, infection and by autoimmune disorder [13]. Experimentally induced hepatotoxic models provide essential models for the study of physiological and biological reflections of hepatic diseases. Almost all the known lesions of liver can be induced by various hepatotoxic agents like paracetamol, alcohol, and rifampicin.

The aqueous extract of aerial parts of *Cynodon dactylon* was studied for hepatoprotective activity. In this study, paracetamol was chosen for inducing hepatotoxicity as it is extensively used in medical sciences. This can lead to severe exposure to the chemical to mankind, resulting in acute liver diseases. Moreover it is extensively studied hepatotoxic drugs. Paracetamol is a potent hepatotoxic drug. N-acetyl-p-benzo quinoneimine (NAPQI) is a highly reactive arylating minor metabolite of paracetamol which is detoxified by conjugation with glutathione. When a large dose of paracetamol is taken, glucuronidation capacity is saturated, more of minor metabolite is formed, hepatic glutathione is depleted and these metabolites bind covalently to protein in liver cells [4]. The hepatocellular necrosis eventually leads to marked changes in biochemical parameters and histopathological features. Serum enzyme levels and histopathological features changes are the standard approach for the measurement of hepatic injury. N-acetylcysteine (NAC) replenishes the glutathione stores of liver and prevents binding of the toxic metabolite to other cellular constituents. The drug is recommended in the management of paracetamol toxicity [4]. It can be administered orally or intravenously. For this study, 100mg/kg was chosen and administered orally.

The serum transaminase levels are exclusively used for assessing hepatic injury due to ease of measurement and high degree of sensitivity. In all mammalian species SGOT is a sensitive measure of acute hepatic necrosis but in rats the SGPT is almost as sensitive as SGOT. In this study, albino rats were chosen were as experimental model and hepatotoxicity was induced by paracetamol orally. SGOT, SGPT and ALP were measured after 72 hour of paracetamol administration. Blood was collected from retro orbital sinus of the rats and then sacrificed under high dose ether.

The hepatoprotective effect of *Cynodon dactylon* on paracetamol induced hepatotoxicity was studied on following parameters.

1. Hepatic biochemical parameters, serum ALP, SGOT, SGPT were assessed by mod. Kind & King's method and Reitman & Frankel's method respectively [10, 11].
2. Histopathology of hepatic tissues was assessed on following parameters of hepatocyte necrosis, binocularity of hepatocytes, congestion and portal tract infiltration.

The findings of the present study shows that aqueous extract of *Cynodon dactylon* produced a significant reduction in serum alkaline phosphate, SGOT, SGPT in dose dependent manner. The serum ALP values in test 1 and test 2 are (139.52±4.45, 114.45± 3.97) respectively, which is significant at (P<0.05) when compared with toxic control (237.74±17.15) [14, 15]. SGOT values in test 1 and test 2 are (82.24±2.45, 59.54±1.15) respectively, which is significant at P<0.01, when compared with hepatotoxic group (130.34±8.25). SGPT levels in test 1 and test 2 are (80.84±1.35, 72.84±1.98), which is significant at P<0.01, when compared with hepatotoxic group (137.56±5.54) suggesting paracetamol is a potent hepatotoxic drug [16]. The values of alkaline phosphatase, SGOT, SGPT in test 2 are (114±45, 59.45±1.15, 72.84±1.98) respectively, which is highly significant at P<0.01, when compared with those values of test 1 (139.52±4.45, 82.24±2.45, 80.84±1.35). However, alkaline phosphatase, SGOT, SGPT values in test 1 (139.52±4.45, 82.24± 2.45, 80.84±1.35) respectively, which is highly significant at P<0.01, P<0.001, when compared with standard values (101.43±3.24, 57.15±3.45, 54.02±1.77), suggesting *Cynodon dactylon* is effective in dose dependant manner [7]. The histopathological evaluation of hepatic tissues shows severe hepatic necrosis, moderate binocularity of hepatocytes, severe congestion, severe portal tract infiltration in hepatotoxic control group, shows severe hepatotoxic potential of paracetamol in higher doses. However, mild congestion, mild hepatic necrosis and portal tract infiltration in test, near normal in test 2 and standard group's shows aqueous extract of *Cynodon dactylon* possesses hepatoprotective activity.

CONCLUSION

Paracetamol produce significant injury to the hepatic parenchyma. The elevation of liver enzymes SGOT, SGPT and ALP are considered as indicator for the disruption of liver parenchyma. Administration of aqueous extract of *Cynodon dactylon* prevents the paracetamol induced elevation of liver enzymes in dose dependant manner. Biochemical and histopathological

studies confirmed the protective effect of aqueous extract of *Cynodon dactylon*. Thus, the present study shows that the aqueous extract of *Cynodon dactylon* has promising ameliorative effects on hepatotoxicity caused by paracetamol in albino rats.

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