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Hepatoprotective activity of *Cynodon dactylon* leaf extract against rifampicin- induced liver damage in albino rats

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ABSTRACT

Liver plays an important role in maintaining the biological equilibrium of vertebrates. Liver diseases are a major worldwide health problem with high endemicity in developing countries. They are mainly caused by chemicals and some drugs when taken in very high doses. Despite advances in modern medicine, there is no effective drug available that stimulates liver function, offer protection to the liver from damage or help to regenerate hepatic cells. There is urgent need, therefore, for effective drugs to replace/supplement those in current use. The plant kingdom is undoubtedly valuable as a source of new medicinal agents. The main aim of any medication in the treatment of liver disorders is to prevent degeneration of hepatocytes and associated metabolic abnormalities and promote regeneration of hepatic cells. In present study the hepatoprotective activity of Cynodon dactylon extracts was evaluated in rifampicin induced liver toxicity by biochemical parameters like SGPT, SGOT, ALP, BIT and by histopathological study. Acute administration of rifampicin produced marked elevation of the serum levels of the above parameters compared to that of the control group. Treatment with ethanolic and aqueous extracts of *Cynodon dactylon* leaves at doses of 200 and 400 mg/kg produces significant prevention in rifampicin induced rise of the above parameters. *Silymarin* at 100 mg/kg body weight significantly prevented such rise in study. The effect of *Cynodon dactylon* leaves extracts was found possess promising hepatoprotective activity. Further studies in other species and on other parameter would throw more light on this plant.

Keywords: Plant extract, Hepatoprotective activity, *Cynodon dactylon*, Rifampicin- induced liver damage.

INTRODUCTION

Ayurvedic system of medicine is one of the oldest systems in India. The World health Organization has estimated that 80% of the world's population continues to use traditional therapies such as herbs and herbal products, which has incredibly wide use throughout time and place. It has been providing real health benefits with maintaining safety profile [1]. In India, Herbal formulations have widely used as therapeutic agents include antidiabetics, nootropics, hepatoprotective and lipid lowering agents [2].

Cellular necrosis, reduction of GSH levels in tissue and increases the lipid peroxidation in tissue is associated with liver damage. In addition, serum levels of many biochemical markers like ALT, AST, ALP, Direct and Total Bilirubin, Total Cholesterol, HDL Cholesterol, are elevated. In spite of phenomenal growth of modern medicine, there are few synthetic drugs available for the treatment of hepatic disorders such as Silymarin, is a popular remedy extracted from the *Silybium marainum* (milk thistle) for hepatic disorder [3].

Presently only a few natural sourced hepatoprotective drugs are available for the treatment of liver diseases [4].

Literature survey indicates *Cynodon dactylon* (Family: *Poaceae*), commonly known as Bermuda grass [5]. It is abundantly available along the roadsides and lawns. The grass grows faster in uncultivated area [6]. *Cynodon dactylon* is used as a folk remedy for bronchitis, anasarca, calculus, dropsy, hemorrhage, urogenital disorders, cough, headache, sores, cancer, carbuncles, convulsions, cramps, cystitis, dysentery, epilepsy, hemorrhoids, leukoderma, hypertension, hysteria, asthma, tumors, measles, rubella, snakebite, stones, warts, wounds, eye disorders week vision and dandruff, fever. It is also useful against the algesia, inflammations, toothache in children.

Various parts of the herb *Cynodon dactylon* have been studied for pharmacological action and fresh leaves extract was tested for its hepatoprotective activity on mice liver [7]. Keeping this in view it was thought

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that the plant *Cynodon dactylon* which is abundantly available and have a protective role in organ toxicities induced by different chemicals. Therefore, in the present study it was planned to evaluate the organ protective property of *Cynodon dactylon* plant.

MATERIALS AND METHODS

Collection of plant material

Leaves of *Cynodon dactylon* (Darbgrass) were purchased from the local market of Gulbarga, Karnataka and authenticated at Pharmacognosy department of HKES's MTR Institute of Pharmaceutical Sciences, Kalaburagi. A specimen of the plant is stored in the herbarium with specimen no. HKE cog col – 07.

a) Preparation of petroleum ether and ethanolic extract:

The extraction process of powder material of *Cynodon dactylon* leaves was done by Soxhlet extraction with the increasing order of solvents polarity i.e., petroleum ether (60-80 °C), methanol and distilled water. First it was extracted with petroleum ether to defeat the powder, then it was subjected to successive solvent extraction with methanol (95%) for about 36 hours. The temperature was maintained with thermostat control. The Appearance of colorless solvent in the siphon tube was taken as the termination of extraction. The extractions were concentrated by distilling the solvent and were air dried at room temperature. The extracts were weighed and percentage yield was calculated and the results (percentage yield) are tabulated in table I. The color and consistency of the extracts were noted.

Table I: Data Showing the Extractive Values of Leaves of *Cynodon dactylon*

Plant name	Part used	Method of extraction	Colour			Yield in percentage		
			Petroleum Ether extract	Ethanolic extract	Aqueous extract	Petroleum Ether extract	ethanolic extract	Aqueous extract
Cynodon Dactylon	Leaves	Soxhlet extraction of powdered leaves	Dark green	Dark green	Dark green	1.8 %	5.61 %	14.29 %

b) Preparation of aqueous extract

100 g of powder was accurately weighed and taken in a round bottom flask (2000 ml) and macerated with 500 ml of distilled water. 10 ml of chloroform was added as preservative. The closed vessel was kept for 24 hr. After 24 hr. the marc was removed by filtering the extract, and it was concentrated on a water bath at 50 °C for semi solid mass.

The aqueous and ethanolic extracts were stored in an airtight container in a refrigerator below 10 °C. The two extracts were examined for their color change. The percentage yield was calculated with reference to air-dried sample.

Animals

The male and female Wistar albino rats weighing between 150-250 g were procured from central animal house, M.R. Medical College, Kalaburagi. The animals were kept in pathogen free environment in a standard well aerated condition at temperature (25 ± 2 °C) with relative humidity (30-70%) for one week. The animals were fed with standard pellet supplied by Hindustan lever. Co. Mumbai with water *ad libitum* throughout the study. During the starting phase, the animals were fasted for 18 hr.

Determination of Acute Toxicity (Ld_{50})

Acute oral toxicity study was performed as per OECD-423 guidelines (Ecobichon. 1997). The ethanolic and aqueous extract of *Cynodon dactylon was* administered orally in doses of 5, 50, 300, 2000, 5000 mg/kg bodyweight to groups of rats (n=3) and the percentage mortality was recorded over a period of 24 h. During the first 1 h of drug administration, rats were observed for gross behavioral changes as described by Irwin *et al. (*1968) [8]. The extracts were found to be safe and fall under GSH- 5 ranges (2000-5000mg/kg).

Evaluation of Hepatoprotective Activity

The rats were selected and divided into 7 groups each containing six animals. Silymarin and herbal extracts were suspended in 2% gum acacia suspension. The present study was planned to study the effect of herbal extract in preventive aspect of rifampicin induced hepatotoxicity [9]. The dose of rifampicin to induce hepatic damage was selected as 1g/kg body weight for ten days. The dose of Silymarin used was 25 mg/kg body weight. The ethanolic and aqueous extracts dose of *Cynodon dactylon* was selected 200 mg/kg and 400 mg/kg body weight. The treatment protocol is summarized and given below.

Group 1- **Control**: 2% w/v gum acacia (5mg/kg) p.o.

Group 2- **Standard Drug**: Silymarin (25 mg/kg) p.o. + after 30 min Rifampicin (1g/kg) p.o. for every 72 hours for 10 days.

Group 3- **Toxicant**: Rifampicin (1g/kg) p.o. once for every 72 hours for 10 days.

Group 4- *Cynodon dactylon* aqueous extract (low dose, 200 mg/kg) p.o. + after 30 min Rifampicin (1g/kg) p.o. for every 72 hours for 10 days.

Group 5- *Cynodon dactylon* aqueous extract (High dose, 400 mg/kg) p.o. + after 30 min Rifampicin (1g/kg) p.o. for every 72 hours for 10 days.

Group 6- Cynodon *dactylon* ethanolic extract (low dose, 200 mg/kg) p.o. + after 30 min Rifampicin (1g/kg) p.o. for every 72 hours for 10 days.

Group 7- *Cynodon dactylon* ethanolic extract (High dose, 400 mg/kg) p.o. + after 30 min Rifampicin (1g/kg) p.o. for every 72 hours for 10 days.

On 0 and 4th day blood specimen was collected from all animals by retro orbital puncture method. Serum samples were separated by centrifugation (3000 rpm for 15 min) and subjected for estimation of biochemical parameters like SGPT, SGOT, ALP and BIT. The rats were sacrificed under ether anesthesia and the liver was dissected, blotted off blood, washed with saline and stored in 10% formalin for histopathology study to evaluate the details of hepatic architecture in each group microscopically [10].

Statistical analysis

The statistical analysis of results was expressed as mean ± SEM and assessed by one-way analysis of variance (ANOVA) followed by a comparison between different groups using "Turkey - Kramer" test. A value of $p < 0.05$ was considered to be statistically significant. The toxicant group was compared with all other treatment groups and with normal control group.

Histopathology

Histopathological studies of livers of model were performed in histopathology lab by consultant histopathologist Dr. Zeenath Begum, K.B.N Medical College, Gulbarga.

RESULTS AND DISCUSSION

Successive Solvent Extraction

The *Cynodon dactylon* leaves powder was subjected to batch extraction in Soxhlet apparatus. The percentage yield; colour, consistency and solubility in water of different solvents are noted in table I.

Acute Toxicity Study

As per OECD 423 guidelines, LD_{50} studies were conducted in albino mice for *Cynodon dactylon* extracts. It was found that the extract even at 5000 mg/kg dose had not shown any mortality confirming its nontoxic nature and falls in GHS category 5.

Liver weight of animals after 10 days treatment against rifampicin induced hepatotoxicity in rats

1 g/kg body weight dose of rifampicin, induced a significant increase in liver weight with an increase of 75.69% (6.29/100g) compared to normal control (3.58/100g). Rifampicin induced rise of liver weight was protected by 25mg/kg body weight dose of Silymarin and 200 and 400mg/kg body weight doses of *Cynodon dactylon* aqueous and ethanolic extracts. The rise was only 18.71% (4.25/100 g) in silymarin, and 47.76 % (5.29/100 g) and 26.53% (4.53/100 g) in 200 mg/kg and 400 mg/kg aqueous extract and 22.9% (4.4/100 g) and 21.22% (4.34/100 g) in200 mg/kg and 400 mg/kg ethanolic extract respectively after three days treatment. The results are given in table II.

Table II: Liver weight of animals after 10 days treatment against rifampicin induced hepatotoxicity.

Groups	Treatment	Dose (mg/kg)	Liver wt in (g)	Percentage increase
1	Gum acacia	5	3.58 ± 0.07	_
2	RIF	1000	6.29 ± 0.21 ***	75.69
3	SIL + RIF	25 + 1000	4.25 ±0.07 ***	18.71
4	AECD + RIF	200+ 1000	5.29 ± 0.15 ***	47.76
5	AECD + RIF	400+ 1000	4.53 ± 0.11 ***	26.53
6	EECD +RIF	200+ 1000	4.4 ± 0.15 ***	22.90
7	EECD + RIF	400+ 1000	4.34 ± 0.11 ***	21.22

*** P < 0.001 significant when compared group -2 with group -1 and group – 3, 4, 5, 6 & 7 with group -2. RIF – Rifampicin, SIL – Silymarin, AECD – Aqueous extract of *Cynodon dactylon,* EECD – Ethanolic extract of *Cynodon dactylon.*

The various extracts prepared were subjected to preliminary phytochemical studies and the outcome of these tests reveal that ethanolic extract contained only alkaloids, flavonoids, glycosides, tannins, saponins, coumarin, terpenoids and phenols. Flavonol aglycone and their glycosides as main phenolic content are potent antioxidants which are believed to prevent degenerative disease including liver diseases [11].

For acute toxicity studies, Ethanolic and aqueous extract of *Cynodon dactylon* was selected at a dose of 2000 mg/kg. There was no mortality observed at 2000 mg/kg. So, its 1/10th (200 mg/kg) and 1/5th (400 mg/kg) doses were selected for hepatoprotective activity.

Rifampicin induced hepatotoxicity was used by several researchers as a model for screening of hepatoprotective agents. The dose used for induction of hepatotoxicity by different workers was found to vary. In the present study 1 g/kg body weight rifampicin was used as toxicant for 10 days in albino rats of Wistar strain.[10] Rifampicin (rifampin) is a safe, effective and widely used antitubercular drug however an overdose can induce severe hepatotoxicity in experimental animals and humans. Excessive administration of rifampicin can induce enzymes CYP3A4 and CYP3A7 m RNAs in adult's human hepatocytes in culture [13]. Rifampicin strongly induced cytochrome P-450 3A-dependent enzyme and UDP-glucosyltransferase activities in rat liver microsomes [12]. Acute administration of rifampicin produced physical changes such as enlarged heavy liver, dark brown coloration. Biochemical changes i.e. marked elevation of serum marker enzymes *viz...* SGPT, SGOT, ALP and BIT levels. Histological changes such as extensive central vein dilation, extensive portal inflammation was observed in toxicant (Rifampicin Group II) compared to that of control group (Group I). The rise in SGPT, SGOT, ALP. BIT indicate the damage of liver and other tissues as depicted in Table III.

Table III: *Cynodon dactylon* extracts report on selected serum biochemical parameters

Group	Treatment	SGPT (IU/L)		SGOT (IU/L)		ALP (IU/L)		BIT (mg/dL)	
		0 day	11th day	0 day	11th day	0 day	11th day	0 day	11th day
1.	Gum acacia (5mg/kg)	41.06 ± 0.35	41.5 ± 0.41	46.4 ± 0.9	45.3 ± 0.99	124.23± 2.04	121.4± 0.96	0.53± 0.6	0.64 ± 0.10
2.	RIF (1 g/kg)+ SIL(25mg/kg)	42.5 ± 0.7	60.9±2.3***	45.9 ± 1.4	57.9± 1.04***	121.8 ± 0.7	162.9±0.8***	0.48 ± 0.76	0.41 ± 0.06***
3.	RIF(1 g/kg)	47.8 ± 0.8	106.45±1.5***	51.4 ± 0.97	115.9± 1.53***	132.6 ± 1.97	254.8±4.4***	0.47 ± 0.04	2.6±0.1***
4.	RIF (1/kg) + *C.dactylon* aqueous Ext (200mg/kg)	41 ± 0.4	66.9 ± 2.4**	43.5 ± 0.59	72.0± 1.96***	122.7 ± 1.2	177.8±2.2***	0.36 ± 0.6	0.32±0.05***
5.	RIF (1/kg) + *C.dactylon* aqueous Ext.(400mg/kg)	38.9 ± 0.8	58.2±2.2***	45.4 ± 1.4	63.7± 1.6***	123.0 ± 0.95	159.3±2.91***	0.37 ± 0.07	0.29±0.07***
6.	RIF (1/kg) + *C.dactylon* ethanolic Ext (200mg/kg)	43.5 ± 1.13	60.7±0.70***	47.7 ± 1.4	72.5± 1.10***	125.6 ± 1.34	183.4± 1.22***	0.7 ± 0.11	0.58±0.06***
7.	RIF (1/kg) + *C.dactylon* ethanolic Ext (400mg/kg)	41.2 ± 1.2	53.7±0.99***	50.55 ± 1.35	59 ± 2.3***	125 ± 1.3	166.3±1.7***	0.42 ± 0.09	0.39±0.14***

Values indicate mean ± SEM for 6 animals in each group.

***P < 0.001 significant when compared to group-1 with group-2

***P<0.1, ***P < 0.001 significant when compared group-2 with group-3, 4, 5, 6 and 7

*P < 0.1, **P< 0.05 significant when compared group-3 with group 4, 5, 6 and 7

RIF- Rifampicin, SIL-Silymarin, Ext- Extract, *C, dactylon –Cynodon Dactylon*

Treatment with *Cynodon dactylon* ethanolic and aqueous extract at doses of 200 and 400 mg/kg produce significant prevention in paracetamol induced rise in the above biochemical parameters. Silymarin at dose of 100 mg/kg body weight significantly prevented such rise in study. The effect of Silymarin was found to be in between the effect of selected doses of *Cynodon dactylon* ethanolic and aqueous extract.

Histopathological studies of rat liver in toxicant group the fatty changes showed severe central vein dilation, and intense inflammation. Ethanolic extract of the leaves of *Cynodon dactylon* showed no fatty acid changes, and also there was no intense inflammation. It helps to retain the cytoarchitecture of the liver as compared with control animals with less severe degenerative changes. In 400 mg/kg of ethanolic extract, hepatocytes were normal and also the inflammation was very mild. Therefore, ethanolic extract of the leaves of *Cynodon dactylon* shows significant hepatoprotective activity.

Reported data indicate that, plant materials containing chemical moieties such as phenols, flavonoids and saponins exhibit organ protective activities, by virtue of their ability to scavenge free radicals generated during such diseases. Indeed, the plant extract under study also contains the above mentioned phytoconstituents and may also be responsible for the observed hepatoprotective effect.

Table IV: Average percentage change of serum biochemical parameters in rats in Rifampicin induced hepatotoxicity.

Group	Treatment	SGPT	SGOT	ALP	BIT
1.	Control (Gum acacia) (5mg/kg)	1.07	2.37	2.28	2.07
2.	RIF (1/kg) + SIL(25mg/kg)	43.29***	26.14***	33.74***	14.58***
3.	RIF (1/kg)	122.69***	125.49***	92.6***	453.19***
4.	RIF (1 g/kg + *Cynodon dactylon* aqueous Ext.(200mg/kg)	63.17***	65.5***	44.90***	11.1***
5.	RIF (1 g/kg + *Cynodon dactylon* aqueous Ext.(400mg/kg)	49.61***	40.3***	29.51***	21.62***
6.	RIF (1g/kg + *Cynodon Dactylon* ethanolic Ext.(200mg/kg)	39.54***	51.9***	46.02***	18.28***
7.	RIF (1 g/kg + *Cynodon Dactylon* ethanolic Ext.(400mg/kg)	30.34***	16.72***	33.04***	7.14***

***P < 0.001 significant when compared to group-1 with group-2

***P < 0.001 significant when compared group-2 with group-3, 4, 5, 6 and 7.

*P< 0.1 **P< 0.05 significant when compared group-3 with group 4, 5, 6 and 7.

RIF – Rifampicin, SIL-Silymarin, Ext- Extract.

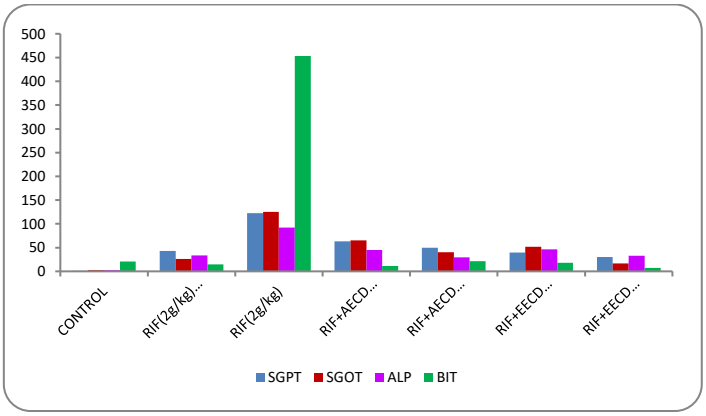

Figure 1: Average percentage change in selected serum biochemical parameters in rifampicin induced hepatotoxicity in rats

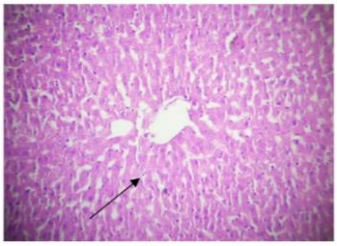

Figure R1: control-Normal Histopathology of rat liver

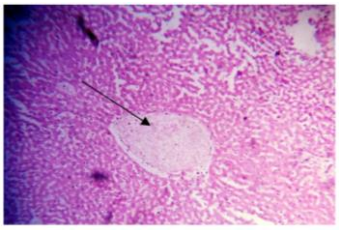

Figure R2: Microphotograph of rat liver with standard group (SIL +RIF) shows mild central vein dilation.

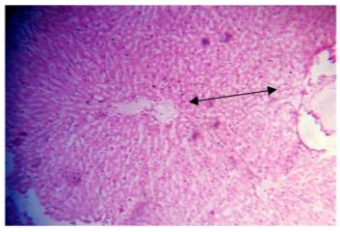

Figure R3: Microphotograph of rat liver with RIF induced toxicity shows extensive central vein dilation.Areas of heamorahages, focal areas shows ballooning of hepatocytes.

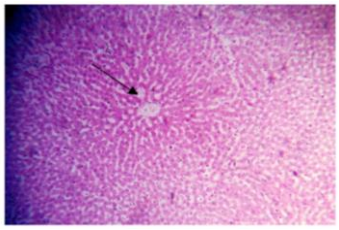

Figure R4: Microphotograph of rat liver treated with RIF + AECD (200mg/kg) shows very mild central vein dilation.

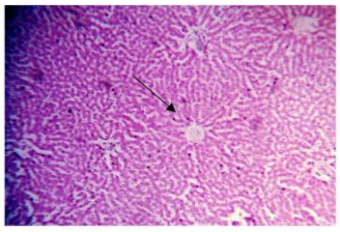

Figure R5: Microphotograph of rat liver treated with RIF + AECD (400mg/kg) shows normal hepatocytes, with no evidence of hepatic damage.

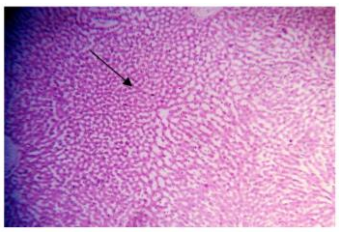

Figure R6: Microphotograph of rat liver treated with EECD (200 mg/kg + rifampicin) showing mild degeneration and inflammation.

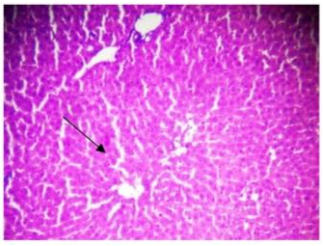

Figure R7: Microphotograph of rat liver treated with RIF + EECD (400mg/kg) shows normal hepatocytes, with no evidence of hepatic damage.

Figure 2: Effect of leaves extracts of *Cynodon dactylon*on histopathological examination of rat liver in rifampicin induced hepatotoxicity

CONCLUSION

Ethanolic and aqueous extract of the leaves of *Cynodon dactylon* at higher dose (400mg/kg) possesses both statistically and clinically significant hepatoprotective activity against various toxicants. However, further evaluation of the same against other different toxicants in animals and in human subjects may yield rewarding results. The results obtained from the studies suggest that the hepatoprotective activity of *Cynodon dactylon* mediated was through the stabilization of plasma membrane, repair of hepatic tissue damage, reversal of elevated biochemical levels to normal values and regeneration of hepatocytes.

Comparative histopathological studies of liver exhibit almost normal architecture as compared to control group, and also there was an improvement in the architecture of liver due to the treatment with ethanolic and aqueous extracts of *Cynodon dactylon* against rifampicin induced liver damage.

The present findings suggest that ethanolic and aqueous extracts of *Cynodon dactylon* possess significant hepatoprotective activity in Albino rats.

Conflict of Interest

None declared.

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Nil.

REFERENCE

1. Akerele O. Summary of WHO Guidelines for the assessment of herbal medicines. *Herbal Gram*. 1993; 28
2. Kurvilla A. Herbal formulations as pharmacotherapeutic agents. *Indian Journal of Experimental Biology*. 2002; 40(1): 7-11.
3. Ramchandra SS, Absar AQ, Viswanath Swamy AH, Tushar Patil PT, Prabhu K, Veeran Gouda A. Hepatoprotective activity of *Calotropis Procera* flowers against Paracetamol-induced hepatic injury in rats. *Fitotrapia* 2007; 8:451-54.
4. Gujrati V *et.al,* Hepatoprotective activity of alcoholic and aqueous extracts of leaves of *Tylophora indica* (Linn.) in rats. *Indian journal of pharmacology*, 2007; 39(1):43-47.
5. Oudhia P. Traditional Medicinal knowledge about useful herb Doobi (*C.dactylon*) in chhattisgarh, India. *http://www.botanical.com/ site/colni/111_doobi.html* 2003.
6. Huxley A. The New Royal Horticulture Society *Dictionary of Gardening. Macmillan Press, London*. 1992; 474-94.
7. Prabha DC, Annapoorani S. Hepatoprotective effect of *Cynodon Dactylon* on ccl_4 induced experimental mice. *Journal of Bio-science*.2009;17(1):7-34.
8. Irwin. The organisation of screening. In: Robert A Turner. *Screening methods in Pharmacology. India: Elsevier*; 2009.p.22- 40.
9. Shyamladevi CS and Devipriya S. Liver diseases are a large public healthy problem in the world*. Ind. J. Pharmacol.*1999; 3:422-426.
10. Agarwal SK, Samanta KC and Chipa RC. Hepatopratective activity of alcoholic and aqueous leaves of *Anisochilus carnosus, International Journal of Pharmacy and Life Sciences.* 2010; 1(2):99-104.
11. Ninad VS, Shailendrs SG. *Cynodon Dactylon*: A systemic review of pharmacognosy, phytochemistry and pharmacology. *International J. Pharmacy and Pharmaceutical sciences*. 2014; 6(8):7-12.
12. Rubin EI (1995). '*The liver and biliary system; In essential Pathology', Lippincott Williams & Wikins, Wolters Kluwer Company, U.S.A*.Vol.14 No.3 pp. 393-440.
13. Oesch F. Inducing properties of rifampicin and rifabutin for selected enzyme Activities of the cytochrome P-450 and UDP-glucuronosyltransferase Superfamilies in female rat liver. *Journal of antimicrobial Chemotherapy*.1996; 37: 1111.

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