International Standard Serial Number (ISSN): 2249-6807

International Journal of Institutional Pharmacy and Life Sciences 2(4): July-August 2012

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Life Sciences **Original Article……!!!**

Received: 05-08-2012; Revised; Accepted: 18-08-2012

COMPARATIVE STUDY ON ANTI-MICROBIAL, HEPATOPROTECTIVE ACTIVITY OF *TERMINALIA CHEBULA* DIFFERENT FRACTION

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Keywords:

Terminalia chebula; Antimicrobial, Hepatotoxicity, Zone of inhibition

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ABSTRACT

Terminalia chebula fractions were measured for hepatoprotective and antimicrobial activities. The water, ethanol, methanol, acetone, hexane and butanol solvent were used for fractionalization. Antimicrobial susceptibility test showed that different fraction inhibited the growth of *Streptococcus pneumoniae* in disk diffusion method. In this all fractions ethanol and methanol solvent have produce higher zone inhibition. Hence they again treat for antimicrobial activity at different concentrations. The higher zone inhibition activity of 10:10 and 2:18 of ethanol mingled methanol fractions were selected for hepatoprotective study. Treatment with these two fractions were decrease alkaline phosphatase (ALP), amino transferase (AST), alanine amino transferase (ALT), total bilirubin and gamma glutamate transpeptidase (GGTP) as well as increase (GPx) and glutathione S-transferase (GST) superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase against CCl4-induced hepatotoxicity. Among the two fractions ethanol mingled methanol 2:18 concentration were significant activity than 10:10, which was comparable to that of standard drug Silymarin.

INTRODUCTION

Traditional medicine from plant is still the backbone of about 75-80% of the entire population for primary health care for better compatibility with the human body because of their lower side effects. It use was highly increased in the developed world in the last few years (1). However in Indian sub-continent and the tribal communities most people in particular, rely on traditional medicine for treatment of their ailments from ancient ages (2). *Terminalia chebula* Retz. an important medicinal plant is distributed in the sub Himalayan tracks, and the eastern, western and southern parts of India. It is a medium to large deciduous tree, attaining a height of up to 30 m, with widely spreading branches and a broad roundish crown. Its wood is hard and bulky (3). *T. Chebula* possesses a wide variety of activities like Cytoprotective (4), spasmogenic (5), NF-κB inhibition in human lymphoblastic T cells (7), antioxidant activity, and neuroprotective (8), antinociceptive (9), antiulcerogenic (10), antiplasmodial activity and cytotoxicity (11), hepato and nephrotoxicity (12), anti-arthritic (13), anti-aging (14), antgi-hyaluronidase effect (15) and anticaries (16) activity. Recent study suggested that fractions from plant active extract prove highly treat disease than crude extract. Therefore, we aimed to identify the active fraction of *Terminalia chebula* against multiple resistance microbes and to study the hepatoprotective activity of *Terminalia chebula* selective fraction in CCL4 induced hepatotoxic rat.

MATERIALS AND METHODS

Plant Materials and Extraction

Leaves of *Terminalia chebula* were collected in University campus, Vels University. The shaded dried leaves were weighted and ground in a sterile mortar. Extract Preparation plant powder (75g) was extracted in Soxhlet apparatus with of 95% methanol at controlled temperature. The solvent were removed by using rotary evaporator. The extract was then subjected to qualitative phytochemical investigation for the identification of phytoconstituents.

Fractionation

Soxhlet extract was impregnated with 10 g of silica gel and loaded on to a silica gel column. The column was packed silica with hexane and benzene successively. Fractions were eluted with water, ethanol, methanol, acetone, hexane and butanol solvent for mobile phase, respectively.

Determination of antimicrobial activity

In disc diffusion method various fraction of *Terminalia chebula* were tested against hazardous and multiple resistance *Streptococcus pneumoniae* in different petri dish. Strains were swabbed

on the surface of the sabouraud agar plates and discs (Whatman No.1 filter paper with 9 mm diameter) impregnated with the 50 µl of each plant sample was place on the surface individually. To compare the anti-bacterial activities, Nystatin (20 µg/disc) used as standard antibiotic and negative control, a blank disc impregnated with solvent followed by drying was used. The plates (triplicates) were incubated 28°C for 72 h. The antimicrobial potency of the test samples was measured by determining the diameter of the zones of inhibition in millimeter.

Animal

Adult male healthy Albino rats weighing 200–250 gm were selected for this study. They were kept in polypropylene cages at 25 ± 3°C temperature, 50–55% humidity, and a 12 h light-dark cycle for at least a week before the experiment. They were maintained at standard housing conditions and free access to standard diet (Chakan Oil Mills, Sangli, India) and water ad libitum during the experiment.

Hepatoprotective study

Healthy and mature male Wistar albino rats weighing 150-200 g were equalized with respect to body weight and randomly divided following groups of six animals each.

Group I: Rats received normal saline and served as control.

Group II: Rats were intraperitoneally applied CCl4 as a 50% solution in olive oil (1ml/kg) twice a week for six weeks.

Group III: Rats administered Fraction (10:10) daily and CCl_4: olive oil (1:1 v/v) (1 ml, ip) on alternate days for seven days.

Group IV: Rats administered Fraction (2:18) daily and CCl_4: olive oil (1:1 v/v) (1 ml, ip) on alternate days for seven days.

Group V: Rats administered silymarin (50 mg/kg po) daily and CCl_4: olive oil (1:1 v/v) (1 ml, ip) on alternate days for seven days.

The animals were sacrificed under light ether anaesthesia 24 h after the last treatment of CCl_4. On the 8^{th} day, blood was collected by cardiac puncture into marked sample bottles and allowed to clot for 45 min at room temperature. The serum was obtained by centrifugation at 2500 rpm at 30°C (5 min) and liver tissue was excised for biochemical estimation.

Statistical analysis

The data were statistically analyzed and all values were expressed as mean ± S.E.M. The data were also analyzed by one way ANOVA followed by Dunnet's t-test. $P<0.05$ was considered significant.

RESULT AND DISCUSSION

CCL4 is the one of the most commonly used hepatotoxins for induce liver injury in experimental research rats (17). Because of CCL4 generates free radical formation to defects antioxidants result in formation liver injury. Plant phytochemical compound have antioxidants which act free radical scavenging activity and antilipoperoxidants leading to hepatoprotection (18). The active fraction from the effective extract is important in the development of novel drugs (19).

Table 1: Antimicrobial activity of *Terminalia chebula* individual fraction tested against *Streptococcus pneumoniae* by disk diffusion method.

Plant sample / Solvent	Zone of inhibition (mm)					
Streptococcus pneumoniae	**Water**	**Ethanol**	**Methanol**	**Acetone**	**Hexane**	**Butanol**
	0.6	3	5	1	3	0.5

Table 2: Antimicrobial activity of ethanol and methanol combined fractions of the *Terminalia chebula* tested against *Streptococcus pneumoniae* by disk diffusion method.

Plant sample / Solvent	Zone of inhibition (mm)								
Streptococcus pneumoniae	**18:2 (E/M)**	**16:4 (E/M)**	**14:6 (E/M)**	**12:8 (E/M)**	**10:10 (E/M)**	**8:12 (E/M)**	**6:14 (E/M)**	**4:16 (E/M)**	**2:18 (E/M)**
	0.5	2	1	3	4	0.7	2	1	7

Table 3: Effect of selective fractions of *Terminalia chebula* on biochemical parameters in CCl4 induced hepatotoxicity in rats.

Treatment	AST U/L	ALT U/L	ALP U/L	Total bilirubin mg%	Total Protein mg%	GGTP U/L
Control	47.52 ± 2.13	98.25 ± 1.8	128.17 ± 1.77	0.99 ± 0.11	9.41 ±1.18	49.18±1.22
CCl4	296.56 ± 1.38	481.21 ± 1.71	251.52 ± 1.79	3.22 ± 0.14	6.08± 0.33	72.1 ±2.21
Silymarin	85.75 ± 2.21**	129.73 ±1.55**	139.8 ± 2.88**	1.24 ± 0.15**	8.68± 0.23 **	46.3±1.45**
Terminalia chebula (10:10)	126.21 ± 2.19*	241.91 ± 1.05*	175.8 ± 2.15*	2.11 ± 0.01*	7.63 ±0.17*	61.4±1.56*
Terminalia chebula (2:18)	91.1 ± 0.15**	157.5 ±3.07**	150.83 ± 1.53**	1.44 ± 0.41**	8.12 ±0.67**	49.18±3.77**

Values are mean ± S.E.M., n = 6 in each group, data were analyzed by one way ANOVA followed by Dunnet's test. *P<0.05,**P<0.01 when compared with hyperglycemic hyperlipidemic control.

Table 4: Effect of selective fractions of *Terminalia chebula* on antioxidant activity in CCl4 induced hepatotoxicity in rats.

Treatment	SOD	Catalase	GPx	GST
Control	37.12 ± 2.12	58.14±1.55	48.65 ± 2.67	0.45 ± 0.09
CCl4	20.14± 1.44	37.29 ± 1.51	21.27 ± 2.34	0.12 ± 0.08
Silymarin	30.38 ± 1.51**	50.10 ± 1.86**	38.14± 1.55**	0.36 ± 0.06**
Terminalia chebula (10:10)	24.44±1.44*	36.19 ± 2.66*	29.84 ± 2.45*	0.27±0.08*
Terminalia chebula (2:18)	28.18±1.34**	45.29 ± 2.44**	35.27± 1.88**	0.33 ± 0.20**

Values are mean ± S.E.M., n = 6 in each group, data were analyzed by one way ANOVA followed by Dunnet's test. *P<0.05,**P<0.01 when compared with hyperglycemic hyperlipidemic control.

In our study, *Terminalia chebula* water, ethanol, methanol, acetone, hexane and butanol solvents fractions were exhibit against tested bacterial *Streptococcus pneumoniae* strains showed different patterns of inhibition in disc diffusion method (0.6, 3, 5, 1, 3, 0.5) (Table 1). In this activity methanol and ethanol fraction have highly killed microbes hence they once again treated with novel *Streptococcus pneumoniae* strain at different concentration. In this study the ethanol mingled methanol fraction 10:10 and 2:18 concentrations has produce higher Zone of inhibition (Table 2). Ahmad et al (20) also reported that isolated compound from alcoholic solvent exhibited greater activity than other polar and non-polar solvent compound against microbes, with no cellular toxicity. Hence these two fractions were taken for hepatoprotective research.

In hepatoprotective activity, CCL4 increased the levels of serum markers AST, ALT, ALP, γ-glutamate transpeptidase (GGTP), total bilirubin, total protein and lipid peroxidation (LPO), indicating liver damage. Because liver is considered to be highly sensitive to toxic agent in all over part of the body. However, treatment of *Terminalia chebula* ethanol mingled methanol fraction 10:10 and 2:18 remarkably prevented CCl4-induced hepatotoxicity, which was compared to that of standard drug Silymarin (Table 3).

The enzymic antioxidant defense system is the nature protector against lipid peroxidation for important scavengers of superoxide ion and hydrogen peroxide (21). In Table 4, the levels of superoxide dismutase (SOD), Catalase, glutathione peroxidase (GPx), and glutathione S-transferase (GST) were decreased by induction of CCL4. Treatment with *Terminalia chebula*

significant fraction such as 10:10 and 2:18 were recover the decreased antioxidant levels when compared with CCL4 treated rats. Silymarin treated animals also showed a significant increase in antioxidant enzymes levels compared to CCL4 treated rats. In conclusion, *Terminalia chebula* fractions possess hepatoprotective activity against CCL4 intoxication rats suggested that disk diffusion method is novel way to identify new antioxidant drug for treat hepatic trouble.

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