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## EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF *PHYLA NODIFLORA L.* AGAINST CCl<sub>4</sub> INDUCED HEPATOTOXICITY

\*Narendra Naik. D<sup>1</sup>, Bhupesh Choudary Naadella\*<sup>2</sup>, Rameswara Reddy. G<sup>2</sup>, Bastipati Suresh<sup>3</sup>

<sup>1</sup>Department of Biotechnology, Jawaharlal Nehru Technological University Kakinada, Vizianagaram, Andhra Pradesh-535003.

<sup>2</sup>School of Biochemistry, SRM University, Chennai, Tamil Nadu-603 203.

<sup>3</sup>Centre for Biotechnology, Jawaharlal Nehru Technological University Hyderabad, Hyderabad-500 082.

### ABSTRACT

*Phyla nodiflora* Linn. (Verbenaceae) is commonly called as Bhujokra in Hindi, Ratoliya in Gujarati and Jalpippali in Sanskrit. It is found throughout warmer parts of India ascending up to 900m in the hills. It is common in wet places, along irrigation channels, canal edges and river banks. The whole plant of *Phyla nodiflora L.* is used for ulcer, jaundice, asthma and bronchitis. To investigate the hepatoprotective activity and acute oral toxicity of extract of whole plant of *Phyla nodiflora L.* (MPN) in male Wistar albino rats by using CCl<sub>4</sub> induced hepatotoxicity. The MPN at doses of 200 and 400mg/kg, p.o and the standard drug Silymarin (100mg/kg, p.o) were administered three times at 12h intervals and then CCl<sub>4</sub> (1ml/kg) was administered to all the groups except normal control for 2 days. The hepatoprotective activity was assessed by using various biochemical parameters like SGOT, SGPT, ALP,  $\gamma$ -GT, TP and total bilirubin along with histopathological studies were observed after 36h of CCl<sub>4</sub> treatment. The MPN at the doses of 200 and 400mg/kg inhibited CCl<sub>4</sub> induced liver toxicity in Wistar albino rats as assessed by the biochemical changes and histopathological studies. The methanol extract of whole plant of *Phyla nodiflora L.* afforded significant protection against CCl<sub>4</sub> induced hepatocellular injury.

**Key words:** *Phyla nodiflora L.*, Hepatoprotective, CCl<sub>4</sub>, Silymarin, Hepatotoxicity.

### INTRODUCTION

The liver is the largest organ in the body weighing 1200-1500g. It is a key organ in regulating homeostasis within the body. It regulates several important functions including protein synthesis, storage and metabolism of fats and carbohydrates, detoxification of drugs and other toxins, metabolism of hormones and excretion of bilirubin. Liver diseases are associated with distortion of these metabolic functions [1,2]. Although viruses are the main cause of liver diseases, the liver lesions arising from xenobiotics, excessive drug therapy, environmental pollution and alcoholic intoxication are not uncommon [3]. Every year about 20,000 deaths are found due to liver disorders [4]. Thus to maintain a healthy liver is a crucial factor for overall health and well beings [5]. Thus, liver diseases remain one of the serious health problems and its disorders are numerous with no effective remedies [6-8]. There is no rational therapy

available for treating liver disorders and management of liver diseases is still a challenge to the modern medicine [9-11]. In the absence of reliable liver protective drugs in allopathic medical practices, herbs play a role in the management of various liver disorders [6]. The use of natural remedies for the treatment of various hepatic diseases has a long history and medicinal plants and their derivatives are still used all over the world [4].

*Phyla nodiflora* Linn. (Verbenaceae) is commonly called as Bhujokra in Hindi, Ratoliya in Gujarati and Jalpippali in Sanskrit. It is found throughout warmer parts of India ascending up to 900m in the hills. It is common in wet places, along irrigation channels, canal edges and river banks. It is a creeping, prostrate, much branched perennial herb with branches spreading profusely and rooting at the nodes. The stem is woody at the base, light violet if exposed

to the sun on drying; it remains straight, pale creamy or white brown in color, shriveled, glabrate forming furrows with vertical channels. The leaves are simple, small, obtuseobovate, deeply and sharply serrate towards the apex; both surfaces are shiny, hairy with medifixed white strigose hairs. The roots of this plant are tough, knotty with fibrous fracture and whitish wood. In literature review it was found that the aerial parts are used as anodyne, antibacterial, diuretic, emmenagogue, parasiticide, refrigerant, febrifuge and cooling. According to traditional uses and Unani system of medicine the plant is acrid, hot and dry; diuretic, maturant, useful in fevers and cold, astringent to bowels, stomachic, used in lack of bowel movements, pain in knee joints and in lithiasis. *Phyla nodiflora* contains flavonoids, sugars, sterol, an essential oil, resin, nonglucosidal bitter substance, tannin, large amount of potassium nitrate and other constituents. Several workers have reported many pharmacological properties including antispasmodic, hair affections, anti-inflammatory, analgesic and antipyretic, antibacterial, anti *Helicobacter pylori* activity, hypotensive activity, antinociceptive and antifungal. The whole plant of *Phyla nodiflora L.* is used for ulcer, jaundice, asthma and bronchitis [12]. Therefore we attempt to investigate the hepatoprotective activity of this plant against  $\text{CCl}_4$ -induced liver damage in rats to support the claim. Hence, the present study was designed to verify the claims of the native practitioners.

## MATERIALS AND METHODS

### Plant material

The whole plant of *Phyla nodiflora L.* was collected from Tirumala hills, Tirupati, Andhra Pradesh, India. It was identified and authenticated by Prof. Madhava Chetty, K., Taxonomist, S.V. University, Tirupati, Andhra Pradesh, India. A voucher specimen has been kept in our laboratory for future reference.

### Preparation of plant extract

The collected whole plant was dried at room temperature, pulverized by a mechanical grinder, sieved through 40mesh. About 100g of powdered materials were extracted with Methanol (90%) using soxhlet apparatus. The extraction was carried out until the extractive becomes colourless. The extracts is then concentrated and dried under reduced pressure. The solvent free semisolid mass thus obtained is dissolved in tween 80 and used for the experiment. The percentage yield of prepared extract was around 10.5% w/w.

### Animals Used

Albino rats (180–200 g) of either sex were maintained in a 12 h light/dark cycle at a constant temperature 25 °C with free access to feed (Sai durga feeds and foods, Bangalore) and water. All animals were fasted prior to all assays and were allocated to different experimental groups each of 6 rats. Moreover the animals

were kept in specially constructed cages to prevent coprophagia during the experiment. All experiments were carried out according to the guidelines for care and use of experimental animals and approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA.

### Acute toxicity study

The procedure was followed according to the OECD guidelines 423 (Acute toxic class method). The acute toxic class method is a step wise procedure with 3 animals of single sex per group. Depending on the mortality and or moribund status of the animals, on an average 2-4 steps may be necessary to allow judgment on the acute toxicity of the testing substance. According to this procedure minimum number of animals were to be used for acceptable data and scientific conclusion. The method uses defined doses (5, 50, 300, 2000 mg/kg body weight) and the results allow a substance to be ranked and classified according to the globally harmonized system (GHS) for the classification of chemical which causes acute toxicity.

Adult female wistar rats were used for this study. The starting dose of whole plant of *Phyla nodiflora L.* extract was 2000 mg/kg body weight, as most of the crude extracts possess  $\text{LD}_{50}$  value more than 2000 mg/kg body weight. The dose was administered to overnight fasted rats and food was withheld for a further 3-4 hours after administration of the drug and observed for signs of toxicity.

Body weight of the rats before and after treatment were noted and any changes in skin, eye, and mucous membranes, salivation, nasal discharge, urination and behavioral (sedation, depression), neuromuscular (tremors, convulsions), cardiovascular, lethargy, sleep and coma were noted. The onset of toxicity was also noted. The animals were kept under observation for 14 days.

The acute toxicity of Methanol extract of *Phyla nodiflora L.* whole plant was determined as per the OECD guideline no. 423 (Acute Toxic Class Method). It was observed that the test extract was not lethal to the rats even at 2000mg/kg dose. Hence,  $1/10^{\text{th}}$  (200mg/kg) and  $1/5^{\text{th}}$  (400mg/kg) of this dose were selected for further study [13].

### Carbon tetrachloride induced hepatotoxicity in rats

The liver protective effect was evaluated using the carbon tetrachloride ( $\text{CCl}_4$ ) model described by Rao and Mishra [14]. Wistar albino rats (150-200g) were divided into five groups and were subjected to the following treatments; group-I served as normal control; received vehicle only. Group-II served as untreated group; received only  $\text{CCl}_4$ , to assist assessing the severity of toxicity

produced by carbon tetrachloride administration. Groups III-V served as treated groups; received MPN at the dose of 200 and 400mg/kg, p.o. and standard drug Silymarin at a dose of 100mg/kg, p.o. were administered orally to rats of the respective groups three times at 12h intervals. Carbon tetrachloride diluted with liquid paraffin (1:1) was administered in dose of 1ml/kg, p.o. for 2 days to all animal groups except for normal control. After 36h of carbon tetrachloride treatment, blood was collected from all groups of rats by puncturing the retro-orbital sinus. Serum was separated by centrifugation at 2500rpm at 37°C for 15min and analyzed for various biochemical parameters.

#### Biochemical estimation

The separated serum was subjected to estimate SGOT and SGPT by *Reitman and Frankel* method [15], alkaline phosphatase (ALP) by *Kind and King* method [16], and bilirubin by *Malloy and Evelyn* method [17].

#### Statistical analysis

The data were expressed as mean  $\pm$  standard error mean (S.E.M). The Significance of differences among the group was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Tukey-Kramer multiple comparison tests, the p values less than 0.05 were considered as significance.

## RESULTS

#### Acute toxicity study

The body weight of the rats before and after administrations were noted that there is slightly increased the body weight. But there are no changes in skin and fur,

eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous system and motor activity and behavior pattern were observed and also no sign of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma were noted. The onset of toxicity and signs of toxicity also not there. In this study there was no toxicity/ death were observed at the dose of 2000mg/kg b.w. The acute toxicity study in rats showed that at 2000 mg/kg dose, the plant is safe for consumption and for medicinal uses (Table 1). In the acute toxicity study, the animals treated with the MPN at a higher dose of 2000 mg/kg did not manifest any significant abnormal signs, behavioral changes, body weight changes, or macroscopic findings at any time of observation. There was no mortality in the above-mentioned dose at the end of the 14 days of observation.

#### Effect of MPN on CCl<sub>4</sub> – induced hepatotoxicity

The results of MPN on Carbon tetrachloride-induced hepatotoxicity were represented in Table 2. The animals treated only with CCl<sub>4</sub> exhibited a significant increase ( $P<0.001$ ) the levels of SGOT, SGPT, ALP,  $\gamma$ -GT and total bilirubin as well as decrease in the levels of TP when compared to the normal control group after 36h of CCl<sub>4</sub> treatment, indicating hepatocellular damage. The MPN at tested doses (group-III & IV) produced a significant reduction ( $P<0.001$ ) in the CCl<sub>4</sub> induced elevated levels of SGOT, SGPT, ALP,  $\gamma$ -GT and total bilirubin as well as increases the TP when compared to the animals treated only with CCl<sub>4</sub> (group-II) after 36h of CCl<sub>4</sub> treatment. Overall, MPN at tested doses significantly reduced the levels of hepatic enzymes and total bilirubin.

**Table 1. Acute toxicity study of methanol extract of *Phyla nodiflora* L. Linn. (MPN) in rats**

S.No	Groups	Dose/kg b.w, p.o	Weight of animals		Signs of Toxicity	Onset of Toxicity	Duration of study
			Before Test	After Test			
1	MPN	2000 mg	185 g	190 g	No signs of Toxicity	Nil	14days
2	MPN	2000 mg	200 g	205 g	No signs of Toxicity	Nil	14days
3	MPN	2000 mg	195g	200 g	No signs of Toxicity	Nil	14days
4	MPN	2000 mg	200 g	205 g	No signs of Toxicity	Nil	14days
5	MPN	2000 mg	210 g	215 g	No signs of Toxicity	Nil	14days
6	MPN	2000 mg	195 g	200 g	No signs of Toxicity	Nil	14days

**Table 2. Effects of MPN on alternation of hepatic enzyme and serum bilirubin in rat after 36h. of CCl<sub>4</sub> treatment**

Groups (n=6)	Biochemical Parameters					
	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	γ-GT (IU/L)	TP (gm/dl)	Total Bilurubin (mg/dl)
Group-I (Normal Control)	36.66 ± 0.17***	25.44 ± 0.14***	191.45 ± 0.18***	53.42 ± 1.12***	9.52 ± 0.18***	0.85 ± 0.04***
Group-II (CCl <sub>4</sub> : 1ml/kg)	68.41 ± 1.29	42.23 ± 0.34	416.22 ± 0.29	104.45 ± 0.41	2.42 ± 0.12	3.72 ± 0.04
Group-III (MPN: 200mg/kg)	49.19 ± 0.21***	35.52 ± 0.46***	244.39 ± 0.12***	64.84 ± 0.35***	3.37 ± 0.27***	1.25 ± 0.04***
Group-IV (MPN: 400mg/kg)	41.5 ± 0.22***	24.43 ± 0.22***	226.52 ± 0.48***	59.36 ± 0.22***	5.48 ± 0.44***	0.85 ± 0.02***
Group-V (Silymarin: 100mg/kg)	34.12 ± 0.27***	22.65 ± 0.45***	191.72 ± 0.68***	54.47 ± 0.13***	7.64 ± 0.52***	0.74 ± 0.07***

Values are expressed as mean ± SEM of 6 rats in each group. \*\*\*  $p < 0.001$ , as compared to CCl<sub>4</sub>-treated group. SGOT = Serum glutamate oxaloacetate transaminase, SGPT = Serum glutamate pyruvate transaminase, ALP = Alkaline phosphatase, γ-GT = Gamma glutamyl transpeptidase, TP = Total proteins.

## DISCUSSION AND CONCLUSION

Liver is the vital organ of metabolism and excretion. It produces and secretes bile; it also produces fibrinogen, prothrombin, heparin and sulfuric acid ester. The liver converts sugar into glycogen [18]. Any changes in anatomy or functions of liver are characterized by cirrhosis, jaundice, tumors, liver cell necrosis and hepatitis, metabolic and degenerative lesion etc. The management of hepatic diseases is still a challenge to the modern medicines [10,19]. Herbal medicines play a major role in the treatment of liver disorders. A number of medicinal plants and their formulations are widely used for the treatment of these disorders [20,21]. However, there were not enough scientific investigations on the hepatoprotective activities conferred to these plants. One of the plants from Indian flora is *Phyla nodiflora* L. The present studies were performed to investigate the hepatoprotective activity of methanol extract of whole plant *Phyla nodiflora* L. in rats against carbon tetrachloride as hepatotoxin to prove its claims in folklore practice against liver diseases.

Carbon tetrachloride (CCl<sub>4</sub>) is one of the most commonly used hepatotoxins in the experimental study of liver diseases [22]. CCl<sub>4</sub> is potent hepatotoxin producing centrilobular hepatic necrosis. It is accumulated in hepatic parenchyma cells and metabolized to trichloromethyl free radicals (CCl<sub>3</sub>) by liver cytochrome P-450 dependent monooxygenases. This CCl<sub>3</sub> free radical combined with cellular lipids and proteins in the presence of oxygen to produce lipid peroxides [23]. Thus, antioxidant or free

radical generation inhibition is important in protection against CCl<sub>4</sub> induced liver lesion [24]. The flavonoids constituents possess free radical scavenging properties [25].

In general, the extent of liver damage is assessed by histopathological evaluation and levels of hepatic enzymes such as ALP, SGOT, SGPT and also Bilurubin release in circulation [26,27]. The estimation of gamma glutamyl transpeptidase (γ-GT) is an important screening test with a high negative predictive value for hepatic disease [28].

Administration of hepatotoxins CCl<sub>4</sub> elevated the serum levels of SGOT, SGPT, ALP, γ-GT and bilurubin as well as decreases total serum proteins (TP) significantly [29,30]. The rise in serum enzymes level and bilurubin has been attributed to the damaged structural integrity of the liver, because they are cytoplasmic in location and released into circulation after cellular damages [31].

In our investigation, the biochemical changes were observed after 36h. of CCl<sub>4</sub> treatment. Thereby, it was found that the animal groups which are pretreated with MPN at the dose of 200 and 400mg/kg (groups-III and IV) as well as silymarin at the dose of 100mg/kg (group-V) for three times at 12h. intervals, resulted in significantly decreases the hepatic enzymes such as SGOT, SGPT, ALP and γ-GT and also total bilurubin; as well as increases the total serum proteins (TP) as compared to animals treated only with CCl<sub>4</sub> (group-II). These results give us the suggestion that, the

animals which are pretreated with MPN as well as silymarin, showed a protection against the injurious effects of CCl<sub>4</sub> that may results from the interference with cytochrome P-450. These biochemical restoration may be due to the inhibitory effects on cytochrome P-450 or/and promotion of its glucuronidation [32,33]. Silymarin is a known hepatoprotective drug. It is reported to have a protective effect on the plasma membrane of hepatocytes [34].

In histopathological assessment, it was found that the normal liver architecture was disturbed by CCl<sub>4</sub> intoxication. In the liver section of rats treated with MPN showed the ability of MPN to prevent hepatocellular necrosis, thereby further confirming the significant hepatoprotective effect of whole plant of *Phyla nodiflora L.*

It is well documented that the phytoconstituents comes under the category of flavonoids, alkaloids, glycosides, carotenoids, phenols, coumarins, lignans, essential oil, lipids, monoterpenes, xanthenes and organic acids are reported to have hepatoprotective activity [35]. Literature review revealed that various chemical

investigations were carried out with this plant. *William Carey Mamidipalli et al.*, have been reported the preliminary phytochemical screening of the methanol extract of *Phyla nodiflora L.* revealed that presence of steroids, flavonoids, tannins, alkaloids and glycosides [36]. *Mulabagal vanisree et al.*, have been reported that the purification of the methanol extract yielded n-hentriacontane, ferulic acid, 4-hydroxycinnamic acid, quercetin-3-rhamnoside and kaempferol-3-glucoside; along with beta-sitosterol, beta-sitosterol-glucoside and d-manitol [37]. The hepatoprotective activity of *Phyla nodiflora L.* may be attributed due to presence of these constituents. This study supports the traditional claims and the MPN could be added in traditional preparations for the various liver diseases.

It is concluded from the data, that the methanol extract of whole plant of *Phyla nodiflora L.* possesses significant hepatoprotective activity and may prove to be effective for the treatment of liver disorders. However, longer duration studies on chronic models are necessary to elucidate the exact mechanism of action so as to develop it as a potent hepatoprotective drug.

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