In-Vivo Antioxidant Activity of Different Fractions of *Michelia Nilagirica* against Paracetamol Induced Toxicity in Rats

Aminabee SK[1], Lakshmana Rao A[2,*], Chinna Eswaraiah M[2]

[1]Assistant Professor, [2]Professor and Principal, Department of Pharmacology,
V. V. Institute of Pharmaceutical Sciences, Gudlavalleru, A.P., India.
[3]Professor and Principal, Department of Pharmacognosy,
Anurag College of Pharmacy, Kodad, Telangana, India.

[*]**Corresponding Author:**
E-mail: dralrao@gmail.com

ABSTRACT

Objective: *To evaluate the in-vivo antioxidant potential of ethanolic extract of whole plant of Michelia nilagirica against paracetamol induced toxicity in rats.*

Methods: *Animals were treated with ethanolic plant extract for 7 days and the toxicity was induced with a single dose of paracetamol intraperitoneal injection. Pre-treatment with 70 mg/kg p.o of ethanolic extract of whole plant of Michelia nilagirica improved the SOD, catalase, peroxidase and glutathione levels significantly as compared to control group.*

Results: *The present study revealed that Michelia nilagirica has significant in-vivo antioxidant activity and can be used to protect tissue from oxidative stress. The results showed that the activities of SOD, catalase, peroxidase and glutathione in group treated with paracetamol, declined significantly than that of normal group.*

Conclusion: *Ethanolic extract of whole plant of Michelia nilagirica in the dose of 60 mg/kg, p.o. has improved the SOD, catalase, peroxidase and glutathione levels significantly. Based on this study we conclude that ethanolic extract of whole plant of Michelia nilagirica possesses in-vivo antioxidant activity and can be employed in protecting tissue from oxidative stress.*

Keywords: *Michelia nilagirica,* Paracetamol, Sylimarin, Radical scavenging.

INTRODUCTION

Approximately 80% of the world total population depends exclusively on plants for their health and healing. Recently there has been an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing free radical-induced tissue injury[1]. In response to this, increased popularity and greater demand for medicinal and herbal plants, a number of conservation groups and organizations are recommending that wild medicinal plants be brought into cultivation. All plants produce a diverse range of bioactive molecules, making them a good and rich source of different novel types of medicines. A rich heritage of interest and knowledge to preventive and curative medicines was available in ancient scholastic works included in the Atharva veda, Charaka, Sushruta etc[2]. Over 50% of all modern clinical drugs are of natural product origin[3] and natural products play an important role in drug development programs in the pharmaceutical industry[4]. Herbal drugs and products have gained importance in recent years because of their efficacy and cost effectiveness.

Thus, there is an increasing interest in the measurement and use of plant antioxidant for scientific research as well as industrial (dietary, pharmaceutical and cosmetics) purposes. The various antioxidant reactions involve multiple steps including the initiation, propagation, branching, inhibition and termination of free radicals. Free radicals are generated or created when cells use oxygen for various physiological processes. By-products are generally reactive oxygen species (ROS) such as super oxide anion (O_2^-), hypochlorous acid (HClO), hydroxyl radical ($\cdot OH$) and hydrogen peroxide (H_2O_2) that result from the cellular redox process. At low or moderate concentrations, ROS exert beneficial effects on cellular functions and immune response and are crucial for life but at high levels, ROS generate oxidative stress that can damage cell structures and functions, including lipids, proteins, sugars and DNA[5]. Oxidative stress plays a major part in the development of chronic and degenerative ailments such as cancer, altitude sickness, skin diseases, autoimmune disorders, rheumatoid arthritis, osteoarthritis, cataract, aging, coronary heart disease and various neurodegenerative diseases[6]. Antioxidants, which are either naturally produced (in-situ), or externally supplied through diet counteract oxidative stress in human body. These antioxidants capable of scavenging the free radicals act by preventing and repairing damages caused by ROS, and therefore can enhance the immune defense, cellular response and lower the risk of cancer and other degenerative diseases[5].

Herbal medicines have recently attracted much attention as alternative medicines[7] useful for treating or preventing life style related disorders and relatively very little knowledge is available about their mode of action. There has been a growing

interest in the analysis of plant products which has stimulated intense research on their potential health benefits. In Ayurveda, traditional usages of plants are most commonly in their aqueous extracts form only. Concurrently, some of the papers searched focus for testing these plants in their ethanolic or aqueous extracts and some have also reported activity in petroleum ether, benzene and chloroform extracts[8-10].

Michelia nilagirica, belonging to the genus magnolia (magnoliaceae) is a native to tropical and subtropical South and Southeast Asia, including southern china. It is widely used in both Ayurveda and Homeopathic medicine. Flower buds of *Michelia champaca* Linn. is commonly used by many traditional healers in most of the herbal preparations for diabetes[11] and kidney diseases[12]. Traditionally, it is being used in fever, colic, leprosy, post-partum protection[13] and in eye disorders[14]. It has been reported to possess antipyretic, antiulcer, anti-inflammatory[15], insecticidal[16], antioxidant, antimicrobial[13] and leishmanicidal[17] activities. The active constituents reported in this plant are alkaloids, tannins, saponins, sterols, flavonoids and triterpenoids[9]. Keeping these facts in view, the present study was undertaken to create a scientific base for the use of the extract of *Michelia nilagirica* as a antioxidant agent.

In particular, despite widespread use of this plant as medicines all over the globe, the literature contains few reports of antioxidant activity. In present study, we carried out a systematic record of the free radical scavenging activity of *Michelia nilagirica in-vivo* using different fractions of ethanolic extract.

MATERIALS AND METHODS

Collection of plant material: The whole plant of *Michelia nilagirica* was collected from the deciduous forest of Tirumala Hills in Andhra Pradesh State, India. Samples were authenticated by Dr. K. Madhava chetty, Department of Botany, Sri Venkateswara University, Tirupati, India. The whole plant of *Michelia nilagirica* were sorted, cleaned and air-dried at room temperature for one week. By using the laboratory hammer mill these were ground to powder. Powdered samples were collected and stored in air- and water-proof containers protected from direct sunlight and heat until required for extraction.

Preparation of extracts: The powdered materials of *Michelia nilagirica* (whole plant) were extracted successively each for 18 hrs with petroleum ether, ethyl acetate, chloroform, ethanol and distilled water in soxhlet apparatus. The extracts were concentrated to dryness in rota evaporator till free from the solvents.

Isolation of fractions*:* Thin-layer chromatography method was carried out using silica gel aluminum plate 60F-254, 0.5 mm (TLC plates, Merck). The spots were visualized in UV light and 10% of H_2SO_4 in methanol. The ethanolic extract was subjected to column chromatography (silica gel # 60-100) for further purification. The equilibration of column was carried for one hour with petroleum ether at flow rate 5ml/min. The sample was (2 gm dissolve in acetone) loaded on to the column, 8 fractions were collected using petroleum ether: ethyl acetate (4:1), petroleum ether: ethyl acetate (1:1), petroleum ether: ethyl acetate (2:3), ethyl acetate (100%), chloroform: methanol (9:1), chloroform: methanol (1:1) and chloroform: methanol (2:8).

Above yielded product were pooled into five fractions based on TLC. The yield and appearance of the five fractions was fraction A 50 mg/gm & yellow, fraction B 300 mg/gm & black, fraction C 150 mg/gm & green, fraction D 200 mg/gm & darkish brown and fraction E 150 mg/gm & saffron.

Phytochemical analysis: Phytochemical analysis[18] of fractions was carried out for the presence of alkaloids, tannins, saponins, glycosides, terpenoids, carbohydrates, flavonoids, proteins, amino acids, fixed oils, steroids & sterols by different methods.

Animals: Albino wistar rats weighing 150-200 gm were purchased from National Institute of Nutrition, Hyderabad. The rats were kept in polypropylene cages (3 in each cage) at an ambient temperature of 25±2°C and relative humidity of 55–65%. A 12 hrs light and dark schedule was maintained in the air conditioned animal house. All the rats were fed with common diets for 1 week after arrival and then divided into groups with free access to food and water.

Acute toxicity studies: Acute toxicity studies were performed according to organization for economic co-operation and development (OECD) guidelines[19]. Animals were divided in groups (n=5). The animals were fasted for 4 hrs with free access to water only. The extracts were administered orally in doses of 100, 300, 1500, 3000 mg/kg to different groups of mice and observed over 14 days for mortality and physical/behavioral changes.

EXPERIMENTAL

Group-I animals served as normal control, treated with vehicle (gum acacia 3% solution). Group-II animals served as toxic control, treated with Paracetamol in a single dose of 2 gm /kg, oral to produce acute toxicity. Group-III served as standard group and was administered Sylimarin (100 mg/kg), Group-IV treated with fraction A 70 mg/kg, Group-V treated with fraction B 70 mg/kg, Group-VI treated with fraction C 70 mg/kg, Group-VII treated with fraction D 70 mg/kg and Group-VIII treated with fraction E 70 mg/kg. The animals of groups III to VIII were given single dose of Paracetamol 2 gm /kg, orally, 6 hr after the last treatment. On day 8 the rats were sacrificed by carotid bleeding and liver was

rapidly excised, rinsed in ice-cold saline, and a 10% w/v homogenate was prepared using 0.15 M KCI, centrifuged at 800 rpm for 10 min at 4^0C. The supernatant obtained was used for the estimation of catalase and peroxidase and other enzymes. Further, the homogenate was centrifuged at 1000 rpm for 20 min at 4^0C and the supernatant was used for biochemical estimation.

BIOCHEMICAL ESTIMATION

Estimation of SOD: The assay of SOD was based on the reduction of nitro blue tetrazolium (NBT) to water insoluble blue formazan[20]. Liver homogenate (0.5 ml) was taken and 1 ml of 50 mM sodium carbonate, 0.4 ml of 24 mM NBT and 0.2 ml of 0.1 mM EDTA were added. The reaction was initiated by adding 0.4 ml of 1 mM hydroxylamine hydrochloride. Zero time absorbance was taken at 560 nm followed by recording the absorbance after 5 min at 25°C. The control was simultaneously run without liver homogenate. Units of SOD activity were expressed as the amount of enzyme required to inhibit the reduction of NBT by 50%. The specific activity was expressed in terms of units per milligram of proteins.

Estimation of catalase: Catalase[21] activity was measured in which Supernatant (0.1 ml) was added to cuvette containing 1.9 ml of 50 mM phosphate buffer (pH 7.0). Reaction was started by the addition of 1.0 ml of freshly prepared 30 mM H_2O_2. The rate of decomposition of H_2O_2 was measured spectrophotometrically from changes in absorbance at 240 nm. Activity of catalase was expressed as units/mg protein.

Estimation of peroxidase: The peroxidase[22] assay was carried out in which the iver homogenate (0.5 ml) was taken, and to this were added 1 ml of 10 mM KI solution and 1 ml of 40 mM sodium acetate solution. The absorbance of potassium periodide was read at 353 nm, which indicates the amount of peroxidase. Twenty microliters of hydrogen peroxide (15 mM) was added and the change in the absorbance in 5 min was recorded. Units of peroxidase activity were expressed as the amount of enzyme required to change the OD by 1 unit per minute**.** The specific activity was expressed in terms of units per milligram of proteins.

Estimation of glutathione: The procedure to estimate the reduced glutathione (GSH) level followed to the method as described by Ellman[23]. The homogenate (in 0.1 M phosphate buffer, pH 7.4) was added with equal volume of 20 % trichloroacetic acid (TBA) containing 1 mM EDTA to precipitate the tissue proteins. The mixture was allowed to stand for 5 min prior to centrifugation for 10 min at 200 rpm. The supernatant (200 µl) was then transferred to a new set of test tubes and added 1.8 ml of the Ellman's reagent (5, 5'-dithio *bis*2- nitrobenzoic acid) (0.1 mM) was prepared in 0.3 M phosphate buffer with 1% of sodium citrate solution). Then all the test tubes make up to the volume of 2ml. After completion of the total reaction, solutions were measured at 412 nm against blank. Absorbance values were compared with a standard curve generated from standard curve from known GSH. The glutathione level in liver was calculated as micromole/g liver.

RESULTS

Preliminary phytochemical screening: Phytochemical screening revealed the presence of flavonoids & amino acids in fraction A, terpenoids & proteins in fraction B, terpenoids in fraction D and alkaloids, tannins, carbohydrates & flavonoids in fraction E (Table 1).

Acute toxicity studies: Acute toxicity studies were carried by up-down regulation method. It was found that the extract at a limit dose from 1500 to 3000 mg/kg is safe and does not show any mortality.

In-vivo antioxidant activity: Phytochemical screening of the plant showed the presence of flavonoids in fraction A. The present study was taken to assess the *in-vivo* antioxidant effect of different fractions of ehanolic extract of *Michelia nilagirica* whole plant on paracetamol induced toxicity in rats. The result showed that the levels of SOD, catalase, peroxidase and glutathione levels in groups treated with paracetamol declined significantly than that of normal group. Co-administration of fraction A of *Michelia nilagirica* at a dose of 70 mg/kg markedly prevented the paracetamol induced alteration and maintained enzymes level near to normal values (Table 3). Standard treated group also significantly increased the levels of SOD, catalase, peroxidase and glutathione (Fig. 2-5).

Statistical analysis: All analyses were run in triplicates. Data were analysed by an analysis of variance (ANOVA). Statistical analysis was performed by the Student's t-test and by ANOVA.

Table 1: Preliminary phytochemical screening of different fractions of ethanolic extract of *Michelia nilagirica*

S. No.	Phytochemicals	Fraction A	Fraction B	Fraction C	Fraction D	Fraction E
1	Alkaloids	----	----	----	----	+
2	Tanins	----	----	----	----	+
3	Saponins	----	----	----	----	----
4	Glycosides	----	----	----	----	----
5	Terpinoids	----	+	----	+	----
6	Carbohydrates	----	----	----	----	+
7	Flavonoids	+	----	----	----	+
8	Proteins	----	+	----	----	----
9	Aminoacids	+	----	----	----	----
10	Fixed oils	----	----	----	----	----
11	Steroids & Sterols	----	----	----	----	----

Table 2: Glutathione Standard curve

S. No.	Conc. (µM)	OD
1	5	0.001
2	10	0.032
3	20	0.068
4	40	0.120
5	60	0.180
6	80	0.230
7	100	0.290

Table 3: Radical scavenging activity of different fractions of ethanolic extract of *Michelia nilagirica*

S. No.	Treatment group	SOD (U/mg protein)	Catalase (U/mg protein)	Peroxidase (U/ml)	Glutathione (µM)
1	Normal control (Vehicle treated)	***68.5±0.12	***13.31±0.98	**0.989±0.12	***95.2±1.41
2	Hepatotoxic control (Paracetamol treated)	32.8±0.08	5.2±0.21	0.129±0.14	45.4±5.65
3	Standard (Sylimarin 100 mg/kg)	***64.5± 4.6	***11.2±0.74	**0.931±0.23	90.2±1.4
4	Fraction A (70 mg/kg)	***53.8±0.09	***10.1±0.12	***0.884±0.13	***82.6±0.7
5	Fraction B (70 mg/kg)	**41.3±0.1	**5.83±0.13	***0.549±0.21	***76.5±0.7
6	Fraction C (70 mg/kg)	***40.5± 0.08	**6.1±0.24	**0.433±0.21	***75.2±0.53
7	Fraction D (70 mg/kg)	32.1±0.09	**6.19±0.25	0.359±0.14	***60.2±2.8
8	Fraction E (70 mg/kg)	**38.3±0.16	***6.28±0.21	**0.421±0.23	***65.4±2.12

*Value are Mean±SD, n=6 for each group: ** P< 0.05, ***P<0.001 compared with their corresponding value in Paracetamol treated toxic group.*

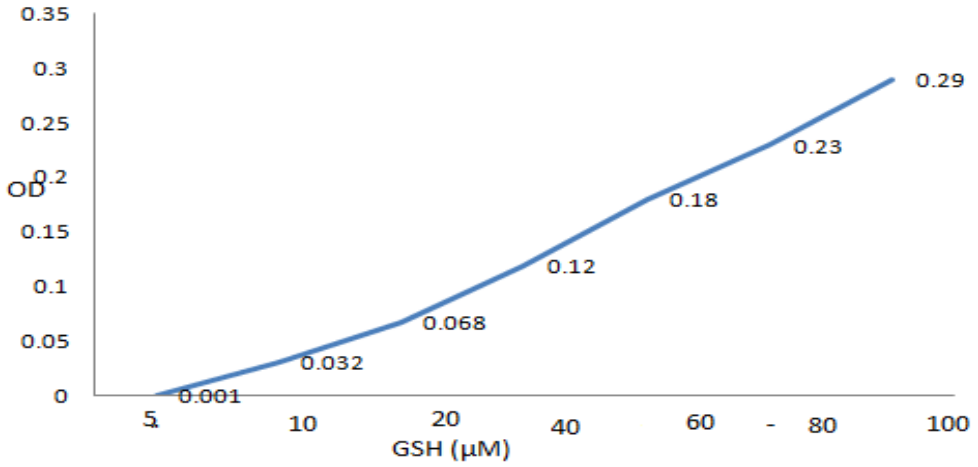

Fig. 1: Glutathione Standard curve

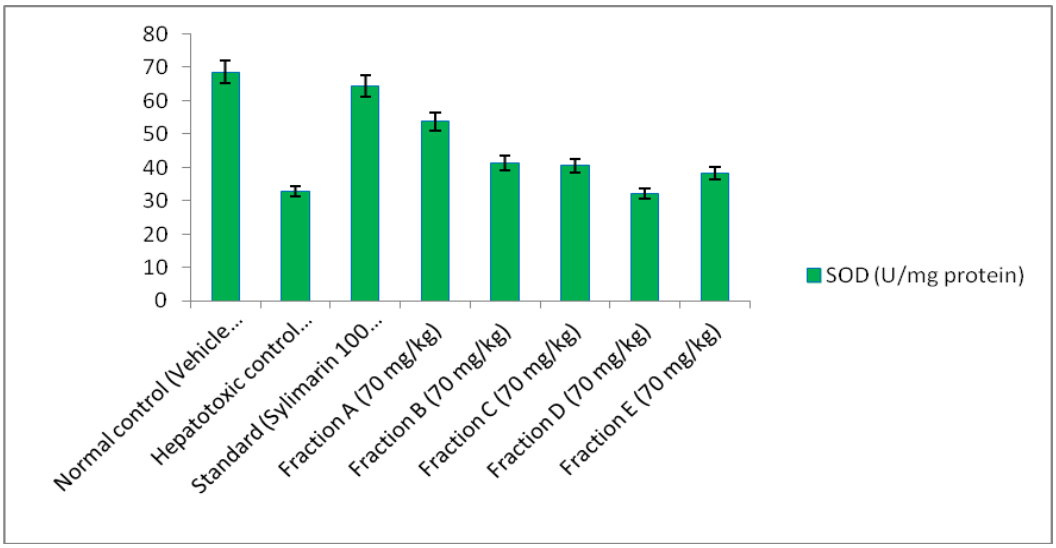

Fig. 2: Comparison of SOD values of different fractions of ethanolic extract of *Michelia nilagirica*

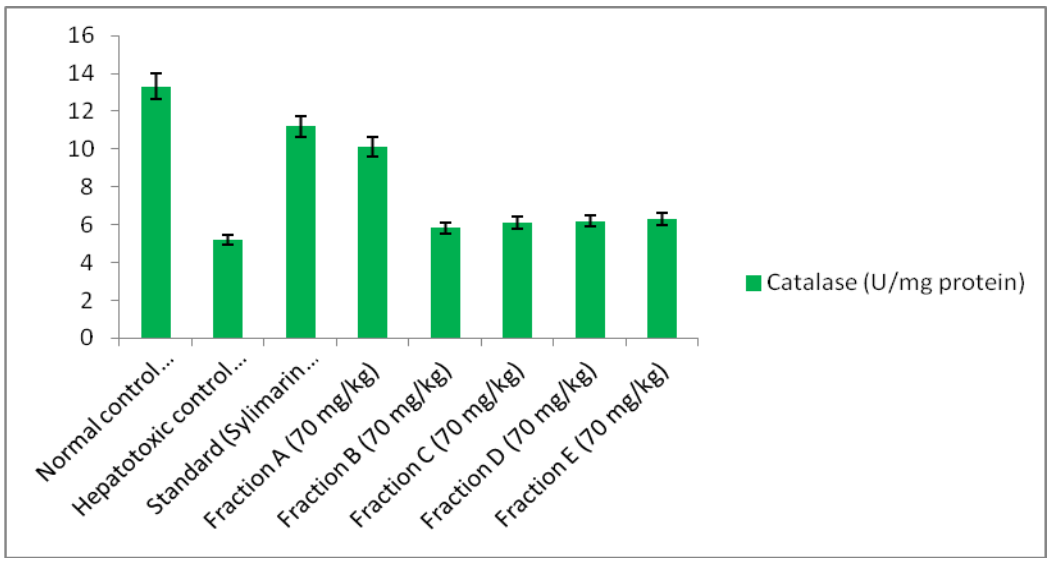

Fig. 3: Comparison of catalase values of different fractions of ethanolic extract of *Michelia nilagirica*

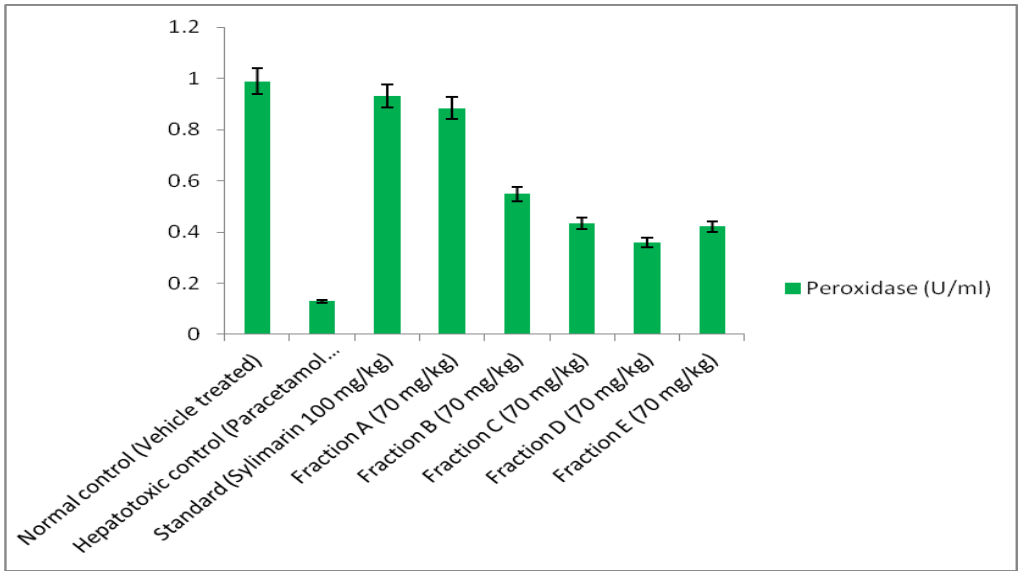

Fig. 4: Comparison of peroxidase values of different fractions of ethanolic extract of *Michelia nilagirica*

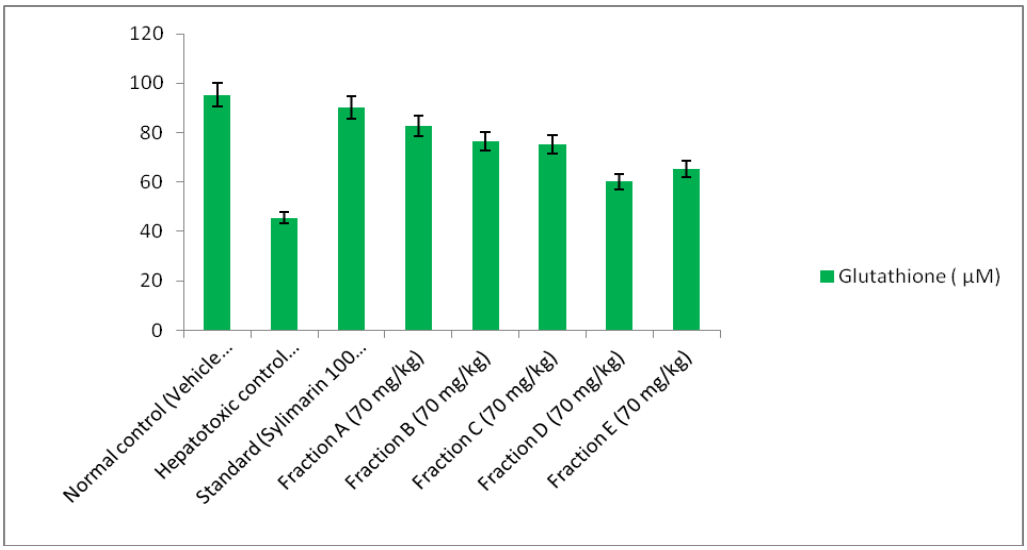

Fig. 5: Comparison of Glutathione values of different fractions of ethanolic extract of *Michelia nilagirica*

DISCUSSION

In paracetamol induced toxicity, fraction A of ethanolic extract of *Michelia nilagirica* treatment increased the depleted levels of cellular GSH significantly in paracetamol induced toxicity in rats. The fraction A of ethanolic extract of *Michelia nilagirica* also restored the levels of antioxidant enzymes such as SOD and catalase almost back to the normal levels. SOD plays an vital role in the depletion and elimination of reactive oxygen species (ROS) and protects cells against the deleterious effects of super oxide anion derived from the peroxidative process in liver and kidney tissues[24] and the observed increase in SOD activity suggests that the fraction A of ethanolic extract of *Michelia nilagirica* has an efficient protective mechanism in response to ROS. Catalase, considered as most important H_2O_2 removing enzyme and also a key component of antioxidative defense system[25]. Here catalase activity was increased and then restored to normal levels on administration of fraction A of ethanolic extract of *Michelia nilagirica*. Peroxidase is an enzyme that catalyzes the reduction of hydroperoxides, including hydrogen peroxides (H_2O_2), and functions to protect the cell from peroxidative damage[26]. We propose that the additive and synergistic antioxidant activity of phytochemicals such as flavonoids, present in *Michelia nilagirica* are responsible for its potent antioxidant activity.

CONCLUSION

Phytochemical screening of the fractions showed the presence of flavonoids in fraction A. In our investigation on *Michelia nilagirica*, the enzymatic oxidants such as glutathione, SOD,

catalase, and peroxidase were improved in drug treated group as compared to control. Based on this we conclude that fraction A of ethanolic extract of *Michelia nilagirica* possess *in-vivo* antioxidant activity and may be employed in protecting tissues from oxidative stress.

ACKNOWLEDGEMENTS

Authors are sincerely thankful to Dr. K. Madhava Chetty, Plant Taxonomist, Department of Botany, Sri Venkateswara University, Tirupati, India for authentification of plant materials. Authors are thankful to V. V. Institute of Pharmaceutical Sciences, Gudlavalleru, India for providing necessary facilities of research work.

REFERENCES

1. Pourmorad F, Hosseinimehr SJ, Shahabimajd N. Antioxidant activity, phenols, flavanoid contents of selected Iranian medicinal plants. South African Journal of Biotechnolology. 2006; 5: 1142-1145.
2. Ripa FA, Haque M, Haque MIU. *In-vitro* antimicrobial, cytotoxic and antioxidant activity of flower extract of *Saccharum spontaneum* linn. European Journal of Scientific Research. 2009; 30(3): 478-483.
3. Stuffness M, Douros J. Current status of the NCI plant and animal product Program. Journal of Natural Products. 1982; 45: 1-14.
4. Baker JT, Borris RP, Carte B. Natural product drug discovery and development: New perspective on international collaboration. Journal of Natural products. 1995; 58: 1325-1357.
5. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. International Journal of Biomedical Science. 2008; 4(2): 89-96.
6. Willcox JK, Ash SL, Catignani GL. Antioxidants and prevention of chronic disease. Food Science and Nutrition. 2004; 44; 275-295.
7. Van Der Weyden MB, Armstrong RM, Gregory AT. The 2005 Nobel Prize in physiology or medicine. Medical Journal of Australia. 2005; 183(11-12): 612-614.
8. Nagarajan NS, Murugesh N, Thirupathy KP, Radha N, Murali A. Antidiabetic and antihyperlipidemic effects of *Cleome feline*. Fitoterapia. 2005; 76: 310-315.
9. Nalamolu KR, Srinivasu N. Antidiabetic and renoprotecticve effects of chloroform extract of *Terminalia chebula* seeds in streptozotocin induced diabetic rats. BMC Complementary and Alternative Medicine. 2006; 58(1): 128-135.
10. Phuong ML, Ali BA, Aziz E, Abdellatif S, Yahia C, Pierre SH. The petroleum ether extract of *Nigella sativa* exerts lipid lowering and insulin-sensitizing action in the rats. Journal of Ethnopharmacology. 2004; 94: 251-259.
11. Srinivasan K. Plant foods in the management of diabetes mellitus. Spices as beneficial antidiabetic food adjuncts. International Journal of Food Sciences and Nutrition. 2005; 56: 399-414.
12. Soltan ME, Sirry SM. Usefulness of some plant flowers as natural acid-base indicators. Journal of Chinese Chemical Society. 2002; 49: 63-68.
13. Khan MR, Kihara M, Omoloso AD. Antimicrobial activity of *Michelia champaca*. Fitoterapia. 2002; 73: 744-748.
14. Sobhagini N, Soumit KB, Malaya KM. Ethno-medico-botanical survey of kalahandi district of Orissa. Indian Journal of Traditional Knowledge. 2004; 3: 72-79.
15. Vimala R, Nagarajan S, Alam M, Susan T, Joy S. Anti-inflammatory and antipyretic activity of *Michelia champaca* Linn., (white variety), *Ixora brachiata* Roxb. and *Rhynchosia cana* (Willd.) D.C. flower extract. Indian Journal of Experimental Biology. 1997; 35: 1310-1314.
16. Ulla J, Vijaya K, Shantini S. Sesquiterpene lactones from *Michelia champaca*. Phytochemistry. 1995; 39: 839-843.
17. Takahashi M, Fuchino H, Satake M, Agatsuma Y, Sekita S. *In-vitro* screening of leishmanicidal activity in myanmar timber extracts. Biological and Pharmaceutical Bulletin. 2004; 27: 921-925.
18. Khandelwal KR. Practical Pharmacognosy Techniques and Experiments. 2000; 149-56.
19. OECD Guidelines for testing of chemical, revised draft guidelines. Acute Oral Toxicity-Up-and-Down Procedure: 2001; 425: 30.
20. Beauchamp C, Fridovich I. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry Review. 1971; 44: 276-287.
21. Aebi H. Catalase *in vitro*. Methods in Enzymology. 1984; 105: 121-126.
22. Nicholos MA. A spectrophotometric assay for iodide oxidation by thyroid peroxidase. Analytical Biochemistry. 1962; 4: 311-345.
23. Ellman GL. Tissue sulphydryl groups. Achieves of Biochemistry and Biophysics. 1959; 82: 70-77.
24. Fridovich I. Superoxide dismutases. Annual Review of Biochemistry. 1975; 44: 147-159.
25. Luck H. Methods of enzymatic analysis. Academic Press, New York. 1971; 3:885-894.
26. Sandhir R, Gill KD. Hepatoprotective effects of Liv-52 on ethanol induced liver damage in rats. Indian Journal of Experimental Biology. 1999; 37: 762-766.