ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES
Published by JK Welfare & Pharmascope Foundation Journal Home Page: https://ijrps.com

Screening of antioxidant activity of *Mucuna pruriens* by *in vivo* model

Rakam Gopi Krishna[1], Raja Sundararajan*[2]

[1]Department of Pharmaceutical Chemistry, Chaitanya College of Pharmacy Education and Research,Kishanpura, Hanamkonda, Warangal -506001, Telangana, India
[2]GITAM Institute of Pharmacy, GITAM (Deemed to be University), Visakhapatnam- 530045, AndhraPradesh, India

Article History:

Received on: 28.08.2018
Revised on: 01.12.2018
Accepted on: 05.12.2018

Keywords:

Mucuna pruriens,
Antioxidant activity,
FRAP,
Catalase,
TBARS,
GSH

ABSTRACT

The present study was anticipated for reviewing the *in vivo* antioxidant influence of methanolic extract of *Mucuna pruriens*. Methanolic extract of the plant was given to rats separately at dissimilar binary amounts of 250 and 500 mg/kg for 21 days to estimate oxidative stress factors such as thiobarbituric acid reactive substance, ferric reducing ability of plasma & reduced glutathione and to evaluate antioxidant enzyme levels of catalase & superoxide dismutase. The *Mucuna pruriens* extracts of methanol deliberately ($p<0.05$) raised the ferric reducing ability of plasma on seven, fourteen and twenty-one days of treatment. Significant ($p<0.05$) decrease of thiobarbituric acid reactive substance levels beside with an upturn in the superoxide dismutase level in the liver and kidney at two dissimilar doses of the plant was witnessed. Management with a dose of 500 mg/kg b.wt of *Mucuna pruriens* produced a significant progression in catalase levels. However, there was no major effect of thiobarbituric acid reactive substance, superoxide dismutase & catalase in the heart and reduced glutathione level at two different doses of *Mucuna pruriens* was noticed. These outcomes recommend that the leaves of *Mucuna pruriens* have potent antioxidant activity which may be responsible for some of its reported pharmacological actions.

* Corresponding Author

Name: Raja Sundararajan
Phone: +91-9160508261
Email: sraja61@gmail.com

ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v10i1.1875

Production and Hosted by
IJRPS | https://ijrps.com

INTRODUCTION

In the growth of various diseases, free radicals have begun because the chief object is inflicting harm to various organs and tissues. The cytotoxic metabolites are produced by aerobic metabolism inside the tissues that sequentially considerably will raise pathological conditions, resulting in free radical mediate enzymatic deactivation, protein denaturation, base hydroxylation of nucleic acids, strand cutting or cross-linking or maybe death (Aebi, 1984). However, the physiological system has a sequence of defense mechanisms comprising antioxidant enzymes like glutathione peroxidase, catalase, thiobarbituric acid reactive substances; superoxide dismutase, reduced glutathione and other free radical scavengers, curcumin, β-carotene, vitamin C, α-lipoic acid, BHT, rutin, vitamin E, and glutathione to safeguard the cell in contrast to cytotoxic reactive oxygen species (Amin *et al.,* 1996). The antioxidants in the living system can be both enzymatic and non-enzymatic. The enzymatic antioxidants include glutathione, superoxide dismutase and catalase, which catalyze neutralization of many kinds of free radicals (Aqil *et al.,* 2006), whereas the antioxidants which are non-enzymatic involve carotenoids, vitamin C, selenium, polyphenols & vitamin E. Though, when the balance among the antioxidants & oxygen species is changed, a condition of oxidative stress rises, probably leading to endless cellular destruction. There

is confirmation that antioxidants may be valuable in inhibiting the deleterious consequences of oxidative stress, and there is an increasing attention in the defensive biochemical function of natural antioxidants contained in root vegetables, fruits and medicinal herbs (Armstrong *et al.,* 2013). Generally herbs, plants and spices more in phenolic complexes like flavonoids, have been confirmed to have anti-inflammatory, anti-aging, antiallergenic, anticancer & antiviral activities, are attributed to the antioxidant possessions (Arulselvan and Subramanian, 2007). Flavonoids and polyphenolic compounds have received the extreme attention (Bakirel *et al.,* 2008; Bell *et al.,* 1971; Benzie and Strain, 1996).

Mucuna pruriens is a coiling herb generally identified as velvet bean belongs to Fabaceae family. In herbal medicine system, entire plant possesses valuable medicinal properties. Traditionally, the roots are used in the treatment of, asthma, cholera, elephantiasis, as a diuretic and blood purifier. It is also being used to cure in fever, gout, renal stones, cataract and rheumatism (Bhasin *et al.,* 1996). A technical study documented the effectiveness of the seeds in controlling of Parkinson's disease by the quality of their L-DOPA content (Blake *et al.,* 1987). *Mucuna pruriens* revealed to elevate testosterone levels, leading to an affirmation of protein in the muscles and improved muscular power and mass (Brawn and Fridovich 1980; Cao and Prior 1998). Leaves possess numerous plant ingredients like bufotenine, dopamine, choline, genistein, hydroxy genistein, 5-hydroxytryptamine, 6-methoxy tryptamine, N-N-dimethyl, tryptamine (Chandan *et al.,* 2007; Costantino *et al.,* 1992). *Mucuna pruriens* is well-known for the risky itchiness it creates on contact; predominantly with the fresh foliage and the seed pods by the existence of 5- hydroxytryptamine (Ellman 1959; Gitanjali *et al.,* 2016). The main intention of the up-to-date study was to assess the antioxidant effect of methanol extract of *Mucuna pruriens* by *in vivo* model.

MATERIALS AND METHODS

Plant collection & authentication

Aerial parts of the plant were collected from Warangal, Telangana, India. Plant material was dried in the dark away from sunlight and stored suitably. The plant material was taxonomically acknowledged by Dr V.S Raju, Professor, Herbal Systematics Lab, Botany Department, K U, district Warangal, T S, India and a sample was retained in the herbarium against accession number 4612 for upcoming studies.

Chemicals: Analytical grade chemicals were used in the study.

Extraction: About 1000gm of herbal material was extracted in a Soxhlet apparatus with methanol. The extraction was carried out successively till an unadulterated solvent was observed. The extra solvent was dried from methanol extract using a rotary vacuum evaporator and later on concentrated on a water bath. The amount of the extract was assessed. The lastly dried extract was deposited in a desiccator for cardioprotective study.

Safety evaluation study

The safety study was performed out using OECD guidelines No. 423. Three rats belonging to same weight & age group were engaged in unit dose up to the extreme dose of 2000 mg/kg orally of methanol extract of *Mucuna pruriens*. The animals were witnessed for 1 hour constantly and then hourly for 4 hours, and to accomplish after every day up to fifteen days for any disease or gross behavioural variations (Jacob, 1995).

In vivo antioxidant model

Experimental Rats

Wistar albino rats (200-250gm) were acquired from Ghosh enterprises Kolkata, India. Polypropylene cages were used for the accommodation of rats and preserved in a precise environment (28-32°C) with half of day and night cycle. Each day all the rats were fed a normal laboratory diet *ad libitum* and had free access to water. The procedure was sanctioned by the Institutional Animal Ethical Committee established for the purpose. Registration No: - 1287/PO/Re/S/09/CPCSEA. The rats were kept under standard conditions in an animal house as per the guidelines of the committee for the purpose of control and supervision on experiments on animals (CPCSEA).

Experimental protocol

Wistar albino rats were allocated into three groups of six animals each. The first group assisted as control and was given normal saline. The second and third group were given methanol extract of *Mucuna pruriens* orally at 250 and 500 mg/kg body weight, individually. The treatment was given for 21 days, and all the animals were sacrificed by decapitation on the 22nd day of the experiment. Blood was collected through the direct cardiac puncture, and it was used for *in vivo* antioxidant activity. The liver, heart and kidney were separated, washed in cold saline and put in storage in liquid nitrogen for auxiliary biochemical readings. This *in vivo* antioxidant study was analysed by the manner designated by Rajlakshmi et al. (Jadwiga *et al.,* 2000).

Preparation of rat liver, kidney and heart homogenate

Tissue homogenate was organised in a ratio of 1gm of wet tissue to 10 times (w/v) 0.05 mol/L ice-cold phosphate buffer (pH 7.4) and homogenised using a homogeniser (Tissue homogeniser). A 0.2 mL of sample homogenate taken for assessment of thiobarbituric acid reactive substance. The remained part of the homogenate was divided into 2 equal halves. First half was added with 10% trichloroacetic acid (1:1), centrifuged at 5000g (4°C, for 10min) and the supernatant was used for reduced glutathione (GSH) estimation. The other half part of the homogenate was centrifuged at 15000g at 4°C for 1 hour, and the upper layer was taken & utilised for reduced glutathione estimation.

Ferric reducing ability of plasma

The antioxidant ability of plasma was estimated following the ferric reducing ability of plasma method (Benzie and Strain, 1996). The samples of blood were obtained from the rat retro-orbital venous plexus into heparinised glass tubes at treatment for 7, 14 & 21 days. Momentarily, 3mL of newly organized and hot (37°C) FRAP reagent (1mL of 10 mmol/L TPTZ [2,4,6 tripyridyl-s-triazine] solution in 40 mmol/L HCl, 1mL 20 mM/L $FeCl_2.6H_2O$, 10mL of 0.3 mM/L acetate buffer [pH 3.6]) was added with 0.375mL distilled water and 0.025mL of test samples. The absorbance of advanced colour in the organic layer was estimated at 593nm. The temperature was retained at 37°C. The interpretations at three minutes were selected for the design of FRAP values. Ferrous sulphate ($FeSO_4.7H_2O$) was used as a standard for calibration and the data stated as nmol Fe^{2+}/L.

Superoxide dismutase assay

Superoxide dismutase was analysed by the model described by Rai *et al.,* (2006). Test mixture contain 0.1mL of supernatant/serum, 1.2mL of sodium pyrophosphate buffer (pH 8.3; 0.052M), 0.1mL of phenazine methosulfate (186 mM), 0.3mL of nitroblue tetrazolium (300 mM), and 0.2mL of NADH (750 mM). The test has begun by mixing of NADH. After Incubation at 30°C for the 90s, the reaction was terminated by mixing 0.1mL of GAA. The reaction mixture was agitated forcefully with four mL of n-butanol. Color strength of the chromogen in the butanol was measured spectrophotometrically at 560nm, and the concentration of superoxide dismutase was conveyed as units/mg of protein.

Catalase assay

Catalase parameter was analysed by the model of Aebi (1984). A 0.1mL of supernatant/serum was mixed to cuvette containing 1.9mL of 50mM phosphate buffer (pH 7.0). The reaction was initiated by mixing of 1.0mL of freshly prepared 30mM H_2O_2. The extent of decomposition of H_2O_2 was calculated spectrophotometrically at 240 nm. The activity of catalase was conveyed as units/mg of protein.

Estimation of a thiobarbituric acid reactive substance

Lipid peroxidation (LPO) was evaluated by the model of Liu *et al.,* (1990). Acetic acid 1.5mL (20%; pH 3.5), 1.5 of TBA (0.8%), and 0.2mL of sodium dodecyl sulfate (8.1%) was added to 0.1ml of supernatant/serum and heated at 100°C for 61 min. The reaction combination was cooled to room temperature. To this, 5mL of n-butanol: pyridine mixture and 1mL of distilled water was added and shaken dynamically. After centrifugation at 1200g for 10min, the organic layer was removed, and the absorbance was estimated at 532nm using a spectrophotometer. Malonyldialdehyde (MDA) was the final product of lipid peroxidation, and it reacts with TBA to form pink chromogen–TBA responsive constituent. It was evaluated using a molar extinction coefficient of 1.56 X 105M-1 cm-1, and it was shown as nM/g wet wt.

Estimate of reduced glutathione

Glutathione was estimated by Ellman (1959) method. An equivalent amount of homogenate was mixed with 10% trichloroacetic acid, and it was centrifuged to distinct the proteins. To 0.01mL of this supernatant, 2mL of phosphate buffer (pH 8.4), 0.5mL of 5'5-dithio, bis (2-nitrobenzoic acid) and 0.4mL double distilled water was mixed. The mixture was shaken, and the absorbance was recorded at 412nm within 15 min. The concentration of reduced glutathione was indicated as µg/g tissue.

Statistical analysis

All investigational reports were conveyed as the mean ± standard error of the mean (SEM). This Statistical examination was performed out using one-way analysis of variance (ANOVA) followed by Dunnet-t-test with the SPSS statistical software for judgment to the control group. $P < 0.05$ was measured as statistically significant.

RESULTS

Safety evaluation study

Rats, when nourished with methanol extract of *Mucuna, pruriens* up to 2000 mg/kg, p.o. Shown no death or any symptom of gross behavioural variations when witnessed initially for three days, and last up to fifteen days.

Ferric reducing ability of plasma

The ferric reducing ability of plasma (FRAP) of rats later administration of methanol extracts of *Mucuna pruriens* done for 21 days was presented

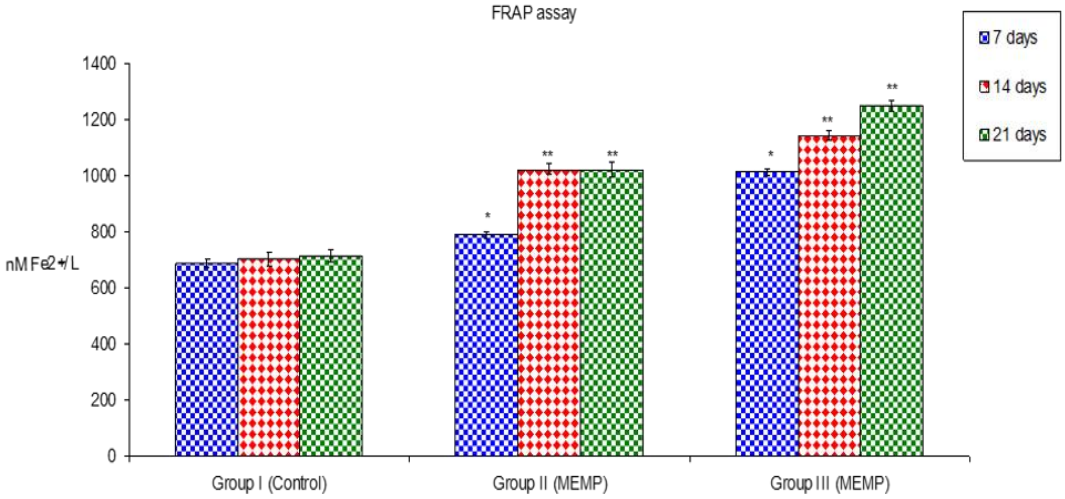

Figure 1: Variations of the antioxidant capability of ferric reducing ability of plasma in rats evaluated by Fe^{2+} equivalent after giving *Mucuna pruriens* methanol extract. Mean values were found to be ± s.d (n=6). Group II and III (plant extracts treated rats) compared to rats of group I. p < 0.05* and p < 0.01**

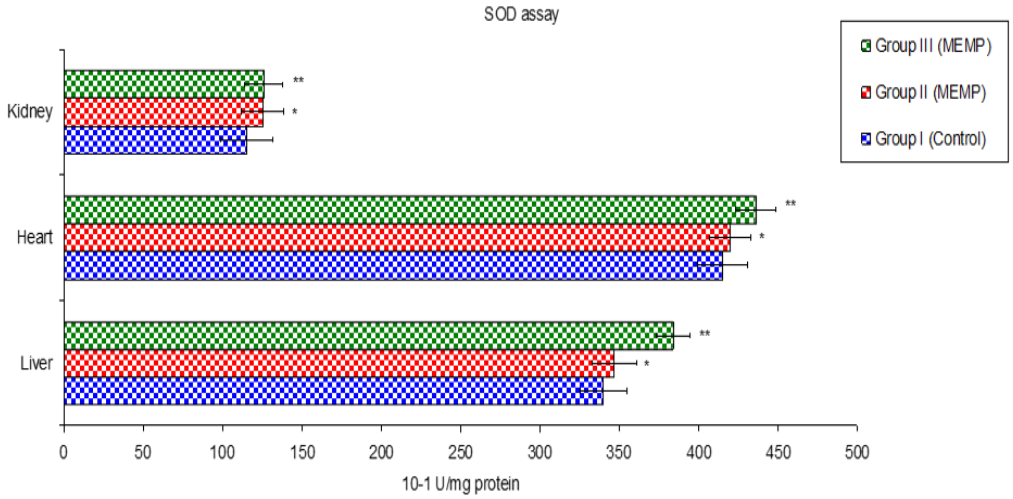

Figure 2: Deviations of superoxide dismutase (SOD; 10^{-1} U/ mg protein) resulting in oral management of methanol extract of *Mucuna pruriens* in rat liver, kidney and heart. Mean values are mean ± s.d. (n=6). IInd and IIIrd Groups (plant extracts treated rats) were compared with Ist Group (control rats). **P< 0.01, *P< 0.05

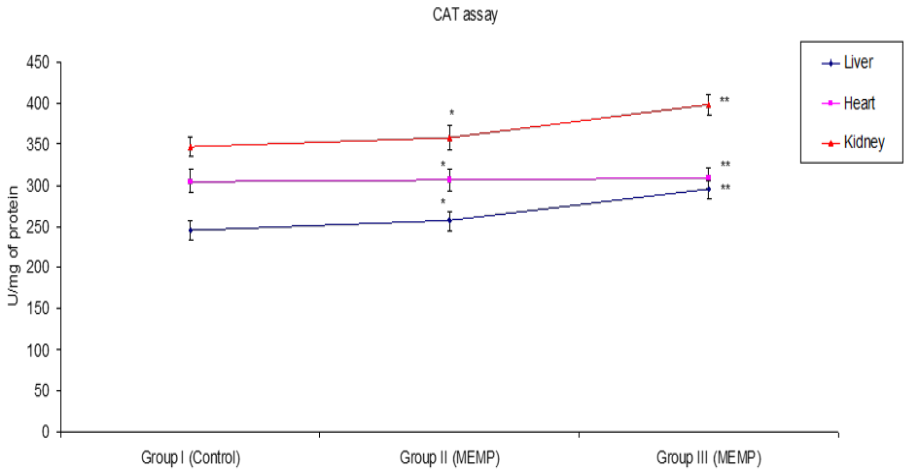

Figure 3: Changes of catalase (CAT; units/mg protein) following oral administration of methanol extract of *Mucuna pruriens* in rat liver, kidney and heart. Mean values are ± s.d. (n=6). Groups II and III (plant extracts treated rats) compared with group I (control rats). **P< 0.01, *P< 0.05

Figure 4: Changes of thiobarbituric acid reactive substance (nmol/g wet weight [wt] following oral administration of methanol extract of *Mucuna pruriens* in rat liver, kidney and heart. Values are mean ± s.d. (n=6). Groups II and III (plant extracts treated rats) compared with group I (control rats). **P< 0.01, *P< 0.05

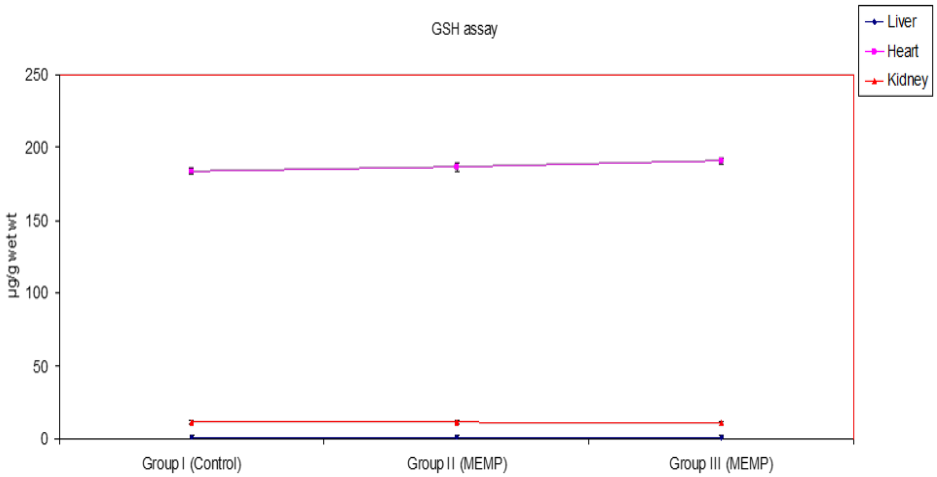

Figure 5: Changes of reduced glutathione (GSH; µg/g wet wt) following oral administration of an extract of methanol of *Mucuna pruriens* in rat liver, kidney and heart. Values are mean ± s.d. (n=6). IInd and IIIrd Groups (plant extracts treated rats) compared with group I rats. **P< 0.01, *P< 0.05

in the figure. 1. In the control group, there was no significant change in the ferric reducing ability of plasma (FRAP) value on days 7 (685 nM Fe^{2+}/L) 14 (702 nM Fe^{2+}/L) and 21(714 nM Fe^{2+}/L) respectively. But, in group II and III on days 7 (789 and 1012 nM Fe^{2+}/L, respectively), 14 (1022 and 1144 nM Fe^{2+}/L, respectively) and 21 (1021 and1249 nM Fe^{2+}/L, respectively) there was a noteworthy ($p < 0.05$, p and $p < 0.01$) increase in ferric reducing ability of plasma (FRAP) value. The maximum enhancement of ferric reducing ability of plasma (FRAP) level was found in group III, which resemble animals administered with 500 mg/kg body mass of the methanol extract of *Mucuna pruriens*.

Approximation of Superoxide Dismutase

The treatment of methanol extract of *Mucuna pruriens* caused minor significant ($p < 0.05$, and $p < 0.001$) increase at 250 mg/kg (420 units/mg of protein) and 500 mg/kg (436 units/mg of protein) b.wt in superoxide dismutase in the heart when matched with the control group (415 units/mg of protein). Conversely, the value of superoxide dismutase (SOD) in the kidney and liver of the plant treated rats was dose correlated and was found to be significantly increased ($p < 0.05$ and $p < 0.01$) at the two different doses of 250 mg/kg (125 and 347 units/mg of protein, respectively) and 500 mg/kg (126 and 384 units/mg of protein, respectively) equated with the control (Group-I) (115 and 340units/mg of protein, respectively). The result was exposed in the figure. 2.

Assessment of Catalase

The management of methanolic extract of *Mucuna pruriens* to rats for 21 days induced a dose-dependent elevation in catalase level in liver and kidney and minor changes in the heart (figure. 3). Methanol extract of plant was significantly augmented at 500 mg/kg body weight, dose of the treatment ($p < 0.001$) for liver (295 units/mg of

protein) and kidney (398 units/mg of protein) associated with the control group (245 & 347 units/mg of protein, respectively). Similarly, at a dose of 250 mg/kg a significant increase ($p < 0.01$) in the catalase (CAT) levels were observed for liver (257 units/mg of protein) and kidney (358 units/mg of protein) equated with the control group. But, there was a little adjustment in the endogenous antioxidant values [305 units/mg of protein (control), 307 units/mg of protein (250 mg/kg) and 309 units/mg of protein (500 mg/kg)] in heart tissue.

Assessment of thiobarbituric acid reactive substance (TBARS)

The outcome of two different doses of methanol extract of *Mucuna pruriens* on the lipid peroxidation and endogenous antioxidants of liver, heart and kidney of rats was shown in figure 4. A significant ($p< 0.05$ and $p< 0.01$) decline in TBARS concentration was observed in liver (267 nM/g and 264 nM/g wet wt tissues) and kidney (257 nM/g and 245 nM/g wet wt tissue tissues) for group II and group III when compared to control group (307 nM/g and 285 nM/g wet wt tissue). There were no changes of TBARS levels of heart tissue in the methanol extract of *Mucuna pruriens* treated groups II (296 nM/g) and III (297 nM/g) respectively, when compared to the control group (295 nM/g wet wt tissue).

Evaluation of reduced glutathione

The results indicated no change of reduced glutathione level in liver (1.129 and 1.102 mg/g wet weight tissue), heart (187 & 191 µg/g wet weight tissue) and kidney (11.5 &11.0 µg/g wet weight tissue) respectively for groups II and III, when paralleled to control group of liver, heart and kidney (1.136, 184 and 11.4 µg/g wet wt tissue, respectively). The result was indicated in the figure. 5.

DISCUSSION

Excessive production of reactive oxygen species (ROS) plays a key role within the pathologic process and progression of many diseases together with completely different organs (Maxwell, 1995). The principle for the consumption of antioxidants is well recognised in interfering and therapy of long-lasting diseases where aerobic stress shows a serious aetiopathological role. Varied people studies support that intake of natural sources of fruits and vegetables, high in antioxidants, are related to a reduced frequency of stress evoked diseases (Meister and Anderson, 1983).

In the current investigation, the ferric reducing ability of plasma test evaluated total antioxidant ability, and it was determined through non-enzymatic antioxidants. Numerous approaches have been established to evaluate the total antioxidant capacity of plasma or serum because of the trouble in measuring each antioxidant factor distinctly in the serum or plasma (Mergort *et al.,* 1996). Some of these are the ferric reducing ability of plasma, which evaluates the reduction of Fe^{3+} to Fe^{2+} in the company of water-soluble exogenous antioxidants. The significant increase in the ferric reducing ability of plasma level after oral administration of methanol extract of *Mucuna pruriens* shows the existence of bio-available antioxidants in the plants. Responses with the cell membrane elements lead to lipid peroxidation (Mukherjee, 2002). Augmented LPO harms membrane function by reducing membrane fluidity and altering the action of membrane-bound enzymes and receptor (Niedernhofer *et al.,* 2003). Thiobarbituric acid reactive substance (TBARS) level was specified as an indicator of lipid peroxidation and malondialdehyde generation. Malondialdehyde is an endogenous genotoxic formation of enzymatic and ROS-induced lipid peroxidation whose adducts are recognised to occur in DNA isolated from healthy adult (Orrenius and Moldeus, 1984). In the present study, the level of a thiobarbituric acid reactive substance in the groups treated with extracts declined in a dose-dependent way when matched to control. The existing study exhibited the depletion in the lipid peroxidation as observed by a decline in the thiobarbituric acid reactive substance level of the kidney and liver in the plant extracts treated groups, but there was no change of thiobarbituric acid reactive substance level in the heart as compared to control.

The superoxide dismutase catalyses the dismutation of superoxide to hydrogen peroxide and oxygen, thereby reducing the probability of superoxide anion responding with nitric oxide to form reactive peroxynitrite (Verma *et al.,* 2014). It is an actual defense of the cell against an endogenous and exogenous generation of superoxide (Rai *et al.,* 2006). Catalase is an enzyme that catalyses the decay of H_2O_2, a reactive oxygen species, and it is a toxic product of aerobic metabolism as well as pathogenic ROS production (Rajlakshmi *et al.,* 2003). The ROS scavenging activity of superoxide dismutase is effective only if it is followed by the action of catalase and glutathione peroxidase since the dismutase activity of superoxide dismutase produces H_2O_2 from the superoxide ion, which is highly toxic than oxygen-derived free radicals and vital to be scavenged further by catalase and glutathione peroxidase (Davies, 1995). The management of methanolic extract of *Mucuna pruriens* at 500 mg/kg body weight significantly increased the level of superoxide dismutase and catalase in kidney & liver. The existing study illustrates the anti-

oxidant affinity of the plant extract. Normally, outcomes for the kidney have revealed slight changes in antioxidant activity related to the liver (Sai karthik *et al.,* 2017). However, no changes in result of superoxide dismutase and catalase were observed in the heart, which could define the present opinion. Reduced glutathione is a defensive molecule against chemical prompted cytotoxicity (Vayalil, 2002). Glutathione is involved in several imperative cellular events, extending from the regulation of physicochemical belongings of cellular proteins and peptides to the detoxification of free radicals (Visioli *et al.,* 2000). Though, long period administration of *Mucuna pruriens* methanol extract had not shown important outcomes in reduced glutathione levels of liver, heart and kidney indicating a protective antioxidant effect.

CONCLUSION

From the above study, It was determined that the methanol extract of *Mucuna pruriens* had vital *in vivo* antioxidant activity. The methanol extract was also efficient in decreasing lipid peroxidation in tissues of liver, heart and kidney. The antioxidant action of methanol extract of the plant may be accredited to the presence of known bioactive compounds (flavonoids), which brings maximum conjugation with radical species, thus reducing the number of free radicals available as well as oxidative stress-related ailments (diabetes, heart related problems, Alzheimer's disease, atherosclerosis, arthritis, cancer) of major organs such as, heart, liver and kidney. Further studies on the active ingredient in the extracts are required to assess the antioxidant effect of the plant extract.

Acknowledgements

The authors are thankful to the management of GITAM University, Visakhapatnam, Andhra Pradesh, India, for providing the required facilities to carry out the research work. Further, authors are also thankful to Chaitanya College of Pharmacy Education & Research, Kishanpura, Hanamkonda, Warangal, Telangana, India for supporting the research work.

REFERENCES

Aebi, H, 1984. Catalase *In vitro*. *Methods in Enzymology*, 10, 121-126.

Amin, YMN, Rehman, ZS, Khan, NA, 1996. Sexual functions. improving effect of *M. pruriens* in sexually normal male rats. *Fitoterapia*, 67, 53–58.

Aqil, F, Ahmad, I, and Mehmood, Z, 2006. Antioxidant and Free Radical Scavenging Properties of Twelve Traditionally used Indian Medicinal Plants. *Turkish Journal of Biology*, 30, 177–183.

Armstrong, JA, Cash, N, Soares, PM, Souza, MH, Sutton, R, Criddle, DN, 2013. Oxidative stress in acute pancreatitis: lost in translation? *Free Radical Research*, 47(11), 917-933.

Arulselvan, P and Subramanian, SP, 2007. Beneficial effects of *Murraya koenigii* leaves on antioxidant defense system and ultrastructural changes of pancreatic ß-cells in experimental diabetes in rats. *Chemico Biological Interactions*, 165, 155–164.

Bakirel, T, Bakirel, U, Keles, OU, Ulgen, SG, and Yardibi H, 2008. *In vivo* Assessment of Antidiabetic and Antioxidant Activities of Rosemary (*Rosmarinus officinalis*) in alloxan-diabetic rabbits. *Journal of Ethnopharmacology*, 116, 64–73.

Bell, EA, Nulu, JR, Cone, C, 1971. L-DOPA and L-3-carboxy-6,7-dihydroxy-1,2,3,4- tetrahydroisoquinoline, a new imino acid, from seeds of *Mucuna mutisiana*. Phytochemistry, 10(9), 2191-2194.

Benzie, IFF and Strain, JJ, 1996. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power the FRAP assay. *Analytical Biochemistry*, 23, 70-76.

Bhasin, S, Storer, TW, Berman, N, Callegari, C, Clevenger, B, Phillips, J, Bunnell, TJ, Tricker, R, Shirazi, A, Casaburi, R, 1996. The effects of supraphysiologic doses of testosterone on muscle size and strength in normal men. *New England Journal of Medicine*, 335(1), 1-7.

Blake, DR, Allen, RE and Lunee, J, 1987. Free Radicals in Biological Systems, A Review Oriented to the Inflammatory Process. *British Medical Bulletin*, 4, 371-385.

Brawn, K and Fridovich, I, 1980. Superoxide Radical and Superoxide Dismutase Threat and Defense. *Acta Physiologica Scandinavica Supplementum*, 49, 9-18.

Cao, G and Prior, RL, 1998. Comparison of Different Analytical Methods for Assessing Total Antioxidant Capacity of Human Serum. *Clinical Chemistry*, 4, 1309-1315.

Chandan, BK, Saxena, AK, Shukla, S, Sharma, N, Gupta, DK, Suri, KA, Suri, J, Bhadauria, M, Singh, B, 2007. Hepatoprotective potential of *Aloe barbadensis* mill against carbon tetrachloride induced hepatotoxicity. *Journal of Ethnopharmacology*, 111, 560–566.

Costantino, L, Albasini, A, Rastelli, G, Benvenuti, S, 1992. Activity of Polyphenolic Crude Extracts as Scavengers of Superoxide Radicals and Inhibitors of Xanthine Oxidase. *Planta Medica*, 5, 342-344.

Davies, KJA, 1995. Oxidative stress: The paradox of aerobic life. In: Rice-Evans, C., Halliwell, B., Lunt, G.G., Eds., Free Radicals and Oxidative Stress: Environment, Drugs, and Food Additives, Biochemical Society Symposium 61, Portland Press, London, 1-32.

Ellman, GL, 1959. Tissue Sulfhydryl Groups. *Archives of Biochemistry and Biophysics*, 82(1), 70-77.

Gitanjali, D, Harshada, K, Sanjay, K, 2016. Phytochemistry and pharmacological activity of *Mucuna pruriens*: a review. *Pharmaceutical and Biological Evaluations*, 3(1), 50-59.

Jacob, RA, 1995. The Integrated Antioxidant System. *Nutrition Research*, 15, 755–766.

Jadwiga, JL, Marek, M, Elzbeita, B, 2000. Effect of Sesquiterpene Lactones on Antioxidant Enzymes and some Drug Metabolizing Enzymes in Rat Liver and Kidney. *Planta Medica*, 66**,** 199-205.

Liu, J, Edamatsu, R, Kabuto, H, Mori, A, 1990. Antioxidant Action of Guilingji in the Brain of Rats with $FeCl_3$-Induced Epilepsy. *Free Radical Biology & Medicine*, 9, 451-454.

Maxwell, SR, 1995. Prospects for the use of Antioxidant Therapies. *Drugs*, 49(3), 345-361.

Meister, A and Anderson, ME, 1983. Glutathione. *Annual Review of Biochemistry*, 52,711-760

Mergort, I, Heilmann, J, Weis, M, Pietta, P, Gardana, C, 1996. Radical Scavenger Activity of three Flavonoid Metabolites Studied by Inhibition of Chemiluminescence in Human PMNs. *Planta Medica*, 62, 289-292.

Mukherjee, PK, 2013. Quality Control of Herbal Drugs - A Taxonomic and Ethno-Medical study of species from Moraceae family in Bangladesh. Evaluation of Herbal Medicinal Products, Business Horizon Pharmaceutical Publishers. India. 1(3), 53-57

Niedernhofer, LJ, Daniels, JS, Rouzer, CA, Greene, RE, Marnett, LJ, 2003. Malondialdehyde, a product of Lipid Peroxidation is Mutagenic in Human Cells. *Journal of Biological Chemistry,* 278, 31426–31433.

Orrenius, S and Moldeus P, 1984. The Multiple Roles of Glutathione in Drug Metabolism*. Trends in Pharmacological Sciences,* 5, 432-438.

Rai, S, Wahile, A, Mukherjee, K, Saha, BP, Mukherjee, PK, 2006. Antioxidant Activity of *Nelumbo nucifera* (sacred lotus) seeds. *Journal of Ethnopharmacology*, 104, 322-327.

Rajlakshmi, D, Banerjee, SK, Sood, S, Maulik, SK, 2003. *In vitro* and *in vivo* antioxidant activity of Different Extracts of the Leaves of *Clerodendron colebrookianum* Walp in the rat. *Journal of Pharmacy and Pharmacology*, 55, 1681-1686.

Saikarthik, J, Saraswathi, I, Vijayakumar, J, Vijayaraghavan R, 2017. Phytochemical analysis of methanolic extract of seeds of *Mucuna pruriens* by gas chromatography mass spectrometry. *International Journal of Pharmaceutical Sciences and Research*. 8(7), 2916-2921.

Vayalil, PK, 2002. Antioxidant and Antimutagenic Properties of Aqueous Extract of Date Fruit (*Phoenix dactylifera* L, Arecaceae). *Journal of Agricultural and Food Chemistry*, 50, 610-617.

Verma, SC, Vashishth E, Singh R, Pant P and Padhi MM, 2014. A review on phytochemistry and pharmacological activity of parts of *Mucuna pruriens* used as an ayurvedic medicine. *World Journal of Pharmaceutical Research*, 3(5), 138-158.

Visioli, F, Keaney, JF, Halliwell, B, 2000. Antioxidant and Cardiovascular Disease; Panacea or Tonics for tired sheep. *Cardiovascular Research*, 47(3), 409.