Annals of Phytomedicine 5(2): 108-112, 2016
Journal homepage: www.ukaazpublications.com
Print ISSN : 2278-9839 and Online ISSN : 2393-9885

ANNALS OF PHYTOMEDICINE
An International Journal

Original article

Acute toxicological studies of leaf extracts of *Morus indica* L. in rats

P. Vanitha Reddy, N. Sreenivas* and Asna Urooj

Department of Studies in Food Science and Nutrition, University of Mysore, Mysore-570006, Karnataka, India
**Mysore Medical College, Mysore-570001, Karnataka, India*

Received August 20, 2016: Revised September 5, 2016: Accepted September 10, 2016: Published online December 30, 2016

Abstract

Morus indica L. (MI-S36) is a potent source of phytochemicals and its antioxidant activity at *in vitro* and *ex vivo* level is very well studied. Further to proceed with the animal studies, the safety and efficacy of the different solvent extracts has to be evaluated at maximum dosage, *i.e*., 2 g/kg body weight (BW) as per Organisation for Economic Co-operation and Development (OECD) guidelines.The acute toxicity study recorded no mortality or any toxic reaction in any group after 14 d. of extracts administration at 2 g/kg BW. The extracts aqueous-MAq; dechlorophylised-MDc; 80% methanol-M8M did not cause any behavioral or physical changes in experimental rats. There was no significant ($p \leq 0.05$) difference in the biochemical parameters, analysed between the groups. Slight elevation in activity of alanine transaminase (ALT) and alkaline phosphatase (ALP) in MDc treated groups was observed, but did not exert any deleterious effect on the normal metabolism which was supported by the histopathology of liver. Extracts did not induce any oxidative stress which is indicated by, no significant difference ($p \leq 0.05$) in the control and experimental lipid peroxides (LPO) and glutathione values. The serum total cholesterol and triglycerides were less than the control group. Histopathological studies showed no remarkable changes in hepatocytes after 14 d. of oral administration of MAq, MDc and M8M extracts. The study contributes in establishing the non-toxic quality parameters of *M. indica* leaf and the results indicate the non toxic effect of the extracts.

Key words: *Morus indica* leaves, acute toxicity, solvent extracts, hepatic enzymes, histopathology

1. Introduction

Internationally, the issue of medicinal plants is of great concern in many heathcare systems and a gradual increase in medicinal plant sales is observed globally,which was proven by WHO survey, estimating about 80% of people living in developing countries for their basic healthcare needs, rely on medicinal plants that have been used traditionally (Fransworth, 1993; Klink, 1997; Mukherjee, 2002). Chemical substances of medicinal plants especially phytochemicals such as polyphenols, flavonoids, carotenoids, *etc*., produce a definite physiological action that can treat chronic as well as infectious diseases in human body (Pillai *et al*., 2011). However, these bioactive compounds might have serious side effects on vital organs of the body and especially the dose-related toxicity is not well documented. To explore a medicinal plant or its extracts, it is of importance to have confirmation on safety levels and toxicity effect to use them as a new therapeutic agent. Therefore, to spread the use of medicinal plants as alternative medicine, studying the potential, safety levels and toxicological effects of the plants becomes imperative for developing formulations for clinically efficient remedies (Taylor *et al*., 2001; Jebasingh *et al*., 2013).

M. indica L. (Mulberry tree) of the family Moraceae has been widely cultivated in countries all over the world including temperate to tropical areas. Plant is well explored for antihyperglycemic potency in streptozotozin induced diabetic rats (Andallu and Varadacharyulu, 2003; Devi and Urooj, 2008). Also, as a good source of phytochemicals, plants antioxidant properties in food and biological substrates, lipid lowering properties by bile acid binding and HMG CoA reductase inhibition using *in vitro* and *ex vivo* methods are reported (Reddy and Urooj, 2013; Reddy and Urooj, 2013a; Reddy and Urooj, 2014). Further to evaluate the plant activity as antihyperlipidemic, anti-inflammatory, anticancer, *etc*., with clinical studies, there is a need to study *M. indica* toxicity effect in animals to confirm the extract strength and its dosage.

2. Materials and Methods

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, albumin, urea, creatinine, total bilirubin, triglycerides, total cholesterol assay kits were purchased from Aggappe Diagnostics, Ernakulam, India. Reduced glutathione (GSH), 5, 5-dithio(bis) nitro benzoic acid (DTNB) were purchased from Sigma-Aldrich, Bangalore, India.

2.1 Collection and preparation of samples

M. indica leaves (MI-S36) were collected from Centre for Sericulture Research and Technical Institute (CSRTI), Mysore district of Karnataka, India and subsequently identified by Dr. G. R. Shivamurthy, Department of Studies in Botany, University of Mysore, Mysore, India. The samples were thoroughly washed under running water, dried overnight (50ºC), powdered, passed through 60 mesh sieve (BS) and stored in airtight container at 4ºC till further use.

Author for correspondence: Dr. P. Vanitha Reddy
Assistant Professor : Division of Nutrition and Dietetics, Faculty of Life Sciences, JSS University, Mysore-570015, Karnataka, India
E-mail: vanithareddy@jssuni.edu.in
Tel.: +91-9590481932

DOI: 10.21276/ap.2016.5.2.14

2.2 Preparation of extracts

Three different extracts, aqueous (MAq), 80% methanol (M8M), and dechlorophylised (MDc) extracts were prepared and studied for their toxicological effects in animals following the Organisation for Economic Co-operation and Development guidelines (OECD, 2001). The cold aqueous extract was prepared by extracting powdered material with cold water (RT) in a mechanical shaker (6 h), filtered and freeze dried (Thermo Modulyo D, Hong Kong). 80% methanol extract was prepared by extracting 15 g sample, with 100 ml of 80% methanol (methanol : water - 8:2 ratio) in a mechanical shaker (6 h). The extracts were evaporated at 40ºC under reduced pressure to dryness in a rotary evaporator (Superfit, India) and stored in air tight container at 4ºC until further use. To avoid the interference of chlorophyll, one batch of 80% methanol extract was dechlorphyllised by following the method of Rich and Rich (1964). Briefly, hexane was added to the 80% methanol extract, shaken for 30 min and the chlorophyll-rich hexane top layer was separated. The remaining extract was further evaporated (Rotary evaporator) and oven dried (50°C) and stored in airtight container at 0°C until used.

2.3 Experimental animals

Adult Wistar strain albino rats of weighing around 140-180 g were housed in the polyacrylic cages, maintained at 25 ± 2ºC, 45 to 60 % RH and 12 h photo period. Acclimatization was done for 14 d., where the animals were observed for general conditions every day and standard pellet diet (Amrut feeds, Pune, India) and water *ad libitum* were provided during the experimental period. The experimental protocol of the study was approved by the Institutional Animal Ethical Committee (IAEC) for the purpose of control and supervision of experiments on animals (MGZ/2620/2011-12; dated: 31.01.2012).

2.4 Acute toxicity studies

The animals were grouped into 4 as Group I-Ctrl; Goup II-MAq; Group III-MDc; Group IV-M8M; consisting of 6 animals each (3 male, 3 female), using Randomized Block Design. According to OECD 420 guidelines (2001), the animals were administered with 2 g/kg BW of extracts as suspensions for 14 d. The animals were observed individually after the initiation of dose. During the study period, the physical or behavioral changes such as skin and fur, eyes and mucous membrane, respiratory, circulatory, autonomic and central nervous systems (ANS and CNS, respectively) and somatomotor activity, behavior pattern and mortality of individual animal was recorded. Observations were also made for presence of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma. At the end of the study period, animals were euthanized and decapitated.

2.5 Biochemical estimations

Activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were determined in serum of each animal along with total protein, albumin, urea, creatinine, total bilirubin, total cholesterol, triglycerides (TGL) using respective standard kits. Glutathione (GSH) and thiobarbituric acid reactive substances (TBARS) as markers of lipid peroxidation were also determined by following Ellman (1959) and Ohkawa *et al*. (1994) method in serum, liver and kidney homogenates, respectively.

2.6 Histopathological procedures

Weights of the various organs - liver, kidney, heart, brain and spleen were noted after washing with phosphate buffered saline. Small portions of liver were fixed in 10 % formaldehyde, then dehydrated in graduate ethanol (50 - 100 %), cleared in xylene and embedded in paraffin. The sections (4 - 5 µm) were stained with haemotoxylin and eosin (H-E) dye and examined with photomicroscope (400x) for any histopathological changes.

2.7 Statistical analysis

The values are expressed as Mean ± SD. The data were subjected to One-way ANOVA followed, by Tukey's multiple comparisons test for significant difference ($p \leq 0.05$) using SPSS 11.5 software.

3. Results and Discussion

The results of the acute toxicological effects of *M. indica* L. (MI-S36) extracts in the animals are presented below:

3.1 Behavioral and other observations

The record sheet of data on toxic symptoms, behavioral and other changes are presented in Table 1. All the extracts did not show any notable toxic symptom during study period. There were no significant changes in behavior, ANS or CNS and no mortality was observed in any of the animal. The weight of the vital organs of control and Morus treated groups were within the normal in relation to their body weights (Table 2).

3.2 Biochemical parameters

The ALP and AST activity of Morus treated and control groups was significantly ($p \leq 0.05$) same in all the groups, respectively (Figure 1). Although the activity of ALT was high in MDc and M8M, the values were within the normal range. These results indicate that Morus extracts did not affect the normal metabolism or behavioral pattern of the animals. The biochemical data of on serum, total protein, albumin, creatinine, total bilirubin and urea is shown in Table 3. No significant difference was observed in total protein, albumin, total bilirubin and urea other than M8M treated group. The creatinine levels of MAq was higher than other groups. Figure 2 shows protein and albumin of vital organs, *viz*, liver, brain and kidney. The Morus extracts did not affect the protein metabolism and only total protein of control and MDc of control was high. Figure 3 depicts the Morus effect on lipid metabolism in comparison with control. The total cholesterol levels of Morus treated groups were comparable with the control and triglycerides level was high in control compared to less in Morus treated groups (Figure 3A). The serum LPO of dechlophyllised treated group was higher than the control, aqueous and 80% treated groups, whereas the serum glutathione (GSH) values of control, aqueous and 80% methanol extract treated group were comparable (Figure 3B). The GSH of the organs was in the order of kidney < liver and brain, however, LPO were high in kidney of control and aqueous treated group (Figure 3C and 3D). The treatment with Morus extracts did not show any adverse effects on cellular defense mechanisms against oxidative stress.

3.3 Histopathological procedures

The histopathological sections of the control and extract treated groups are represented in Figure 4. There were no detectable changes in cellular morphology of hepatocytes. The hepatic architecture was normal with well-defined central vein. No necrosis, steatosis, chronic inflammatory infiltration or degenerative changes were observed in any of the extract treated animals.

Table 1: Acute oral toxicity record sheet of the rats treated with *M. indica* extracts

	Toxicity			Additional observations																
	ANS/CNS												Behavioral observations							
Drug P.O	onset	stop	Time of death	skin & fur	eye lacri	sali	Diah	Resp	Leth	Sleep	Con	Coma	Ste	Tre	Cat	Geo	Hal	Retr	Stu	Exe
Ctrl	nil	nil	0	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
MAq	nil	nil	0	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
MDc	nil	nil	0	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
M8M	nil	nil	0	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x

ctrl-control; MAq-Morus Aqueous; MDc-Morus Dechlorophyllised extract; M8M- Morus 80% methanol extract; Lacri:Lacrimation, Sali: salivation: Diah:Diarrhoea, Resp: Respiratory distress, Leth:Lethargic. Con: Convulsions, Ste: Stereotype, Tre: tremors, Cat: Catalepsy, Geo: Effect of positive geotropism, retr: retropulsion, Stu: Stupor, Exe: excitement, x-absence of symptoms, "-Presence of symptom.

Table 2: Body weights and organ weights of the animals during toxicological studies treated by *M. indica* extracts

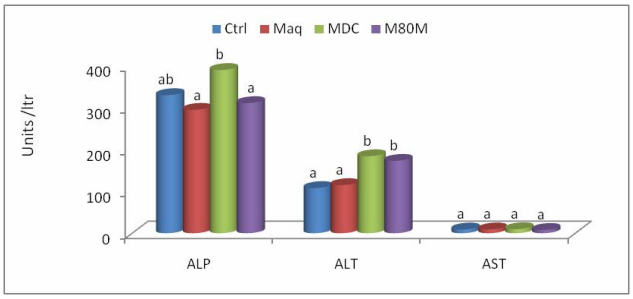
Group	B.Wt.	Liver	Kidney	Heart	Brain	Spleen
Ctrl	134±8.42a	4.28±0.36a	1.06±0.06a	0.459±0.036a	1.35±0.13a	0.33±0.03a
MAq	168 ±49.07a	5.37±0.99a	1.32±0.35a	0.604±0.15a	1.48±0.21a	0.36±0.06a
MDC	143±10.40a	4.52±0.15a	1.18±0.27a	0.598±1.44a	1.52±0.12a	0.34 ±0.036a
M8M	174±6.18a	4.91±0.40a	1.33±0.18a	0.633±0.07a	1.50±0.05a	0.40±0.04a

ctrl-control; MAq-Morus Aqueous; MDc-Morus Dechlorophyllised extract; M8M-Morus 80% methanol extract;

Table 3: Influence of *M. indica* extracts on serum biochemical parameters during toxicological studies

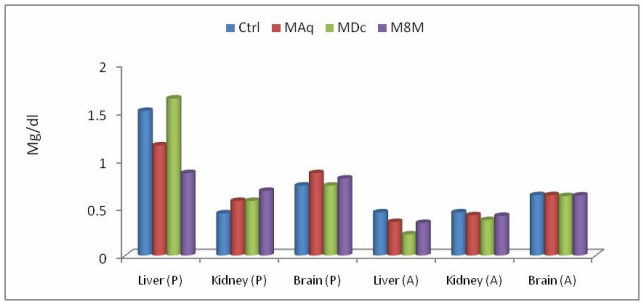
	TP	Ab	Creatinine	T.Bil	Urea
Ctrl	4.38±0.84a	3.94±1.21b	1.2±0.43b	0.43±0.04a	74±9a
MAq	3.78±0.75a	3.84±0.46b	1.9±0.07c	0.3±0.03a	73.98±11.98a
MDC	4.53±0.18a	3.71±0.27b	0.74±0.17a	0.41±0.06a	60.81±11.47a
M8M	4.7325±0.45a	2.57±0.19a	0.89±0.10ab	1.0905±0.04b	51.25±9.53a

ctrl-control; MAq H-Morus Aqueous hot extract; MDc-Morus Dechlorophyllised extract; M8M M-Morus 80% methanol extract; TP-Total protein, Ab-albumin, T.Bil-total bilirubin. Mean values carrying different superscripts a, b, c… differ significantly ($p \leq 0.05$).



ctrl-control; MAq- Morus Aqueous; MDc-Morus Dechlorophyllised extract; M8M- Morus 80% methanol extract; ALP- Alkaline phosphatase; ALT (SGPT) - Alanine transaminase; AST (SGOT) - Aspartate transaminase;

Figure 1: Activity of hepatic enzymes in serum of different groups (U L^{-1}) treated with *M. indica* extracts.

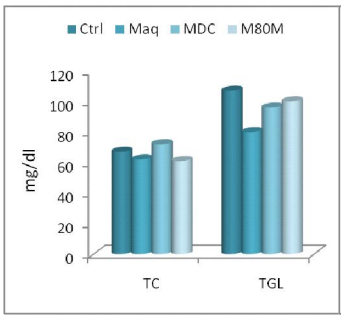


ctrl-control; MAq- Morus Aqueous; MDc – Morus dechlorophyllised extract; M8M- Morus 80% methanol extract;P-protein; A-albumin.

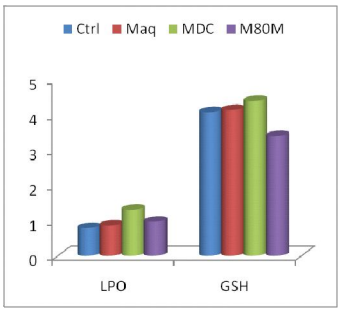
Figure 2: *Morus indica* extracts influence on the protein and albumin content of the vital organs.

Medicinal / herbal plants and their preparations are being used from thousands of years in all types of traditional medicinal practices; due to their non-toxic effect. As sources of numerous

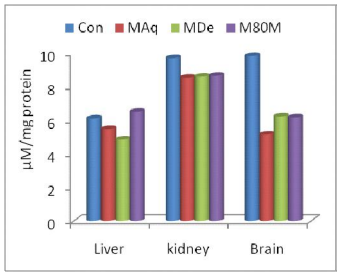
bioactive components, they prevent, treat and help in the management of several disease / disorders. Although the biological effects and bioactive components are identified, there is no scientific documentation of their toxicological effects. Till date, only few plants have been explored for their safety levels and potential pharmacological activities. Similarly, *M. indica* plant and its extracts have been reported for its antioxidant and antidiabetic potential through *in vitro*, *ex vivo* and *in vivo* studies, however, safety evaluation of the plant is not documented.



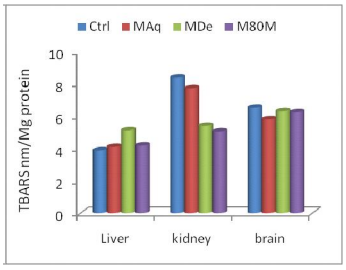
3A. Serum



3B. Serum



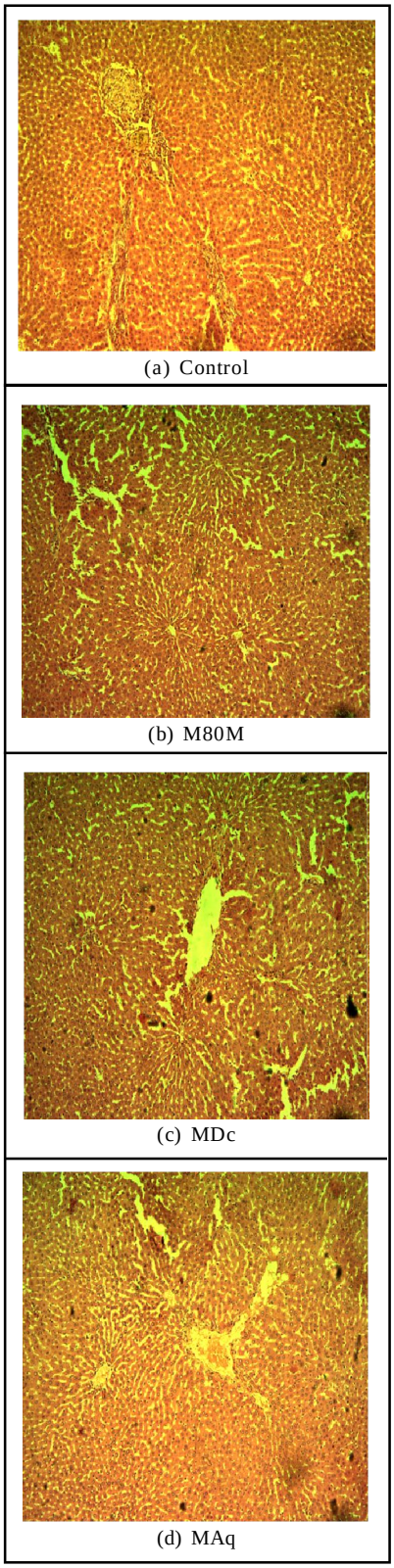
3C. Glutathuione



3D. LPO

ctrl-control; MAq-Morus Aqueous; MDc-Morus dechlorophyl-lised extract; M8M- Morus 80% methanol extract;LPO expressed as nMdl; GSH expressed as µM/ldl

Figure 3: Influence of *M. indica* extracts on lipid metabolism during toxicological studies.



(a) Control

(b) M80M

(c) MDc

(d) MAq

Figure 4: Histopathological sections of liver treated with *M. indica* extracts.

In the present acute toxicological study, the aqueous, dechlorophylised and 80% MeOH extracts of Morus (MI-S36) did

not show any signs or symptoms of toxicity in experimental animals. During the study period, dosage of MAq, MDc and M8M at 2 g/kg BW, did not show any physical or behavioral changes, which are the simple observations to assert the toxic effects of the extracts. Although the incidence of side effects from natural products is relatively low, they are not entirely free of serious risks (Sniderman, 2004). The phytochemicals of the medicinal plants show positive influence on lipid metabolism, especially reducing lipid levels and lipid peroxides, which was proven in the present study with less TGL in the Morus treated groups than the control groups.

Liver is the vital organ, involved in the maintenance of metabolic function and detoxification of drugs. Disturbance to normal metabolic function is hampered due to hepatic damage and to monitor the hepatic injury, serum biochemical markers like ALT, AST, ALP and bilirubinetc, were assessed, whose elevation in the serum indicate the degree of damage on liver by the plant extract (Homolka, 1969; Young *et al*., 2007; Payasi *et al*., 2010). In the present study, hepatic enzyme activities and the biochemical parameters analysed proved the nontoxic nature of the plant extracts. Here, ALP and ALT activity was high in MDc and M8M than control. However, this did not exert any remarkable changes on normal metabolic processes of the liver, as the elevation was marginal in MDc and M8M extract treated groups. Further, the normal condition of the liver was confirmed by the other biochemical parameters and histopathology of liver which did not show any evidence of steatosis, necrosis or degenerative changes. From the results it is inferred that, although there was significant differences in few biochemical markers in rats treated with *M. indica* (MI-S36) extracts, the liver histopathology indicate the safety of the plant.

4. Conclusion

The present study proves the *M. indica* plant extracts are safe and suitable for using as alternative medicine in different disease conditions. The plant investigated is an inexpensive medicinal species with potential to be developed into adjuncts against degenerative or the non-communicable diseases. As a rich source of phytochemicals, food products or capsules of *M. indica* plant leaves or its extracts, can be developed and fed as nutraceutical supplement to the subjects with disease condition. However, the chronic toxicity effect has to be studied before prescribing *M. indica* as a therapeutic agent in long term prescription.

Conflict of interest

We declare that we have no conflict of interest.

References

Andallu, B. and Varadacharyulu, N.C. (2003). Antioxidant role of mulberry (*Morus indica* L.) leaves in streptozotocin-diabetic rats. J. Clin. Chem. Acta., **338**:3-10.

Devi, V. and Urooj, A. (2008). Hypoglycemic potential of *Morus indica* L. and *Costus igneus* Nak : A preliminary study. Indian J. Exptl. Biol., **6**:614-616.

Ellman, G.L. (1959). Tissue sulfhydryl groups. Arch. Biochem. Biophy., **82**:70-72.

Fransworth (1993). Ethnopharmacology and future drug development: The North American experience. J. Ethnopharma, **38**:145-152.

Homolka, J. (1969). Clinic Biochemistry. Publ. House SZN, Prague, pp:434 in Czech

Jebasingh, D.; Jackson, D. D.; Venkataraman, S. and Emerald, B.S. (2013). Physiochemical and toxicological studies of the medicinalplant *Cyperus rotundus* L. (Cyperaceae) Intl. J. Applied Res. Natural Products, **5**(4):1-8.

Klink, B. (1997). Alternative medicines is natural really better. J. Drug Top., **141**:99-100.

Mukherjee, P. K. (2002). Quality control of herbal drugs: An approach to evaluation of botanicals. Business Horizon, 1st ed. New Delhi.

OECD (2001). Guidelines for testing of chemicals. Acute Oral Toxicity -Fixed Dose Procedure-420.

Ohkawa, H.; Ohoshi, N. and Yagi, K. (1994). Assay for lipid peroxides in animal tissues by thiobarbituric reaction. Ann. Biochem. **95**:351-358.

Payasi. A.; Chaudhary, M.; Singh, B.M.; Gupta, A. and Sehgal, R. (2010). Sub-acute toxicity studies of paracetamol infusion in albino Wister rats. Intl. J. Pharma. Sci. Drug Res., **2**(2):142-145.

Pillai, P.G.; Suresh, P.; Aggarwal, G.; Doshi, G. and Bhatia, Vidhi (2011). Pharmacognostical standardization and toxicity profile of the methanolic leaf extract of *Plectranthus amboinicus* (Lour) Spreng, J. Appl. Pharm. Sci., **1**(2):75-81.

Reddy, P. V. and Urooj, A. (2013). *Morus indica* L.: Antioxidant activity in food and biological systems, Intl. J. Pharma. Biol. Sci., **4**(3):706-715.

Reddy, P. V. and Urooj, A. (2013a). Proximate, phytochemical profile and antioxidant activity (*in vitro* and *in vivo*) of *Morus indica* L. varieties. Intl. J. Pharma. Sci. Res., **4**(4):1626-1634.

Reddy, P. V. and Urooj, A (2014). Inhibition of 3-Hydroxy -3-Methylglutaryl Coenzyme A (HMG Co A) Reductase (*ex vivo*) by *Morus indica* plant extracts. Chinese J. Biol. pp:5.

Rich, A and Rich, C. (1964). Extraction of chlorophyll and carotenes from spinach and analysis by thin layer chromatography. Physiological chemistry-extraction of chlorophyll from spinach. Toni Bell, Bloomsburg University, pp:1-6.

Sniderman, A. D. (2004). Is there value in liver function test and creatinine phosphokinase monitoring with statin use? Amer. J. Cardiol., **94**:30-34.

Taylor, J. L.S.; Rabe, T.; McGraw, L.J.; Jäger, A.K. and Staden, J. (2001). Towards the scientific validation of traditional medicinal plants. J. Plant Growth Regulation, **34**:23-37.

Young, T. A.; Tang, H. S.; Lee, H. S.; Hsiong, C. H. and Hu, O. Y. (2007). Effects of hyperglycemia on quantitative liver functions by the galactose load in diabetic rats. Metabolism, **6**:1265-1269.