Research Article

Evaluation of acute oral toxicity of ethanolic extract of *Terminalia tomentosa* (Roxb.) stem bark in Swiss albino mice

Avik Das[1], Subhajit Hazra[1], Shailendra Patil[2], Kalyan Kumar Sen[1]

[1]*Department of Pharmacology, Gupta College of Pharmaceutical Sciences, Asansol, West Bengal*

[2]*Department of Pharmacology, Faculty of Pharmacy, Swami Vivekanand University, Saugor (M.P.), India*

Received: 6 December 2018 Revised: 7 January 2019 Accepted: 18 January 2019

Abstract

Background: Despite its widespread use in traditional medicine, availability of a report on the toxicity of stem bark extract of *Terminalia tomentosa* (Asan) is lacking largely. So the present study aims to provide an account of the acute toxic potential of the ethanolic extract of the dried stem bark of *Terminalia tomentosa* in accordance to OECD TG 425. **Materials and Methods:** Female mice were randomly assigned to either test or control groups (n = 5). The test animals received a single dose of 2000 mg/kg b.w. *T. tomentosa* bark extract while the control ones received vehicle of equal volume. Both the groups were kept under observation for a period of fourteen days for signs of mortality and morbidity. Blood samples were collected periodically through the observation period for assessing hematological and biochemical parameters. Animals were sacrificed at the end of the observation period and vital organs were collected for histo-pathological analysis. **Results:** The data presented is indicative of the conclusion that LD_{50} of the extract is beyond 2000 mg/kg and hence the extract under investigation can be considered practically non-toxic. The vital parameters fairly remained constant across the groups with no significant alterations observed in the animals of either groups with respect to ALT, total protein, globulin etc. though AST was slightly raised in the test groups with a dip in ALP. Hematological and biochemical parameters however didn't show significant variation between the groups. **Conclusion:** Results suggest that LD_{50} of the extract is greater than 2000 mg/kg and the putative role of the same in hepatoprotection is hinted by the elevation of AST in the test group.

***Keywords**:* Ulcerative colitis, *Terminalia tomentosa*, toxicity

Introduction

Folklore medicine still occupies a major role in the practice of rural medicine. According to a report published by World Health Organization, out of the total world's population, about 80% still relies on traditional medicine (Saleem et al., 2017). In developing countries like India, 65% of rural communities still uses the traditional form of medicine to fulfill their primary health care need (Vaidya and Devasagayam, 2007). However, in spite of being the lifeline of medical practice in developing countries, the issue of safety evaluation of these herbal medications are not given due importance owing to the general notion that herbal products have minimal side effects (Shankar et al., 2012). However, on the contrary, use of various herbal therapies has been reported to be associated with long-term side effects (Chen et al., 2006). Owing to the growing popularity of herbal products among the modern urban population too, the focus has now turned on elucidating the toxic potential of these herbal products so as to make their use more safe and effective.

Terminalia tomentosa (Roxb.) of family Combretaceae, commonly known as *Asan* or *Ain* is a shrub widely distributed in the deciduous forest of India, Burma, and other South East Asian countries. Although limited, the reports available on its chemical constituents include polyphenolic compounds like Ellagic acid, Dimethyl ellagic acid, Dimethyl flavellagic acid and beta-sitosterol (Meriga et al. 2017). Presence of 5-Aminovaleric acid, Thymin, Quercetin and Kynurenic acid have also been reported earlier (De Onis, Blössner, and Borghi 2010; Mopuri et al. 2015). Like many

***Address for Corresponding Author:**
Avik Das
Department of Pharmacology
Gupta College of Pharmaceutical Sciences, Asansol, West Bengal, India
Email: avik.tox@gmail.com

DOI: https://doi.org/10.31024/ajpp.2019.5.3.18

other members of the genus *Terminalia* the medicinal properties of *Terminalia tomentosa* are also multifarious. The spectrum includes anti-leucorrheal, antidiarrheal, anti-inflammatory, antihyperglycemic, antifungal and wound healing activities (Anjaneyulu et al. 1986; Fahmy et al. 2016; Kapoor, Vijayvergiya, and Dhawan 2014). However, in spite of having such a wide spectrum of medicinal potential, no substantial work has been reported on the evaluation of toxicity of this shrub as yet. Hence we found a good rationale behind investigating the acute oral toxicity of the methanolic extract of the dried stem bark of this plant.

Materials and methods

Collection of plant materials and preparation of extract

The stem bark of *Terminalia tomentosa* was collected from the District range of Bankura, West Bengal and authenticated by taxonomist (Dr. P.V. Prasanna) at BSI, Sibpur, West Bengal (Ref. No. AD/GCTS/01). The bark was dried under shade and pulverized to obtain a coarse powder. The coarse powder so obtained was subjected to cold maceration, sequentially in pet ether, ethyl acetate, and ethanol. The filtrates from the respective solvents were evaporated to dryness by rotary evaporator. The ethanolic extract was chosen for toxicity testing based on the preliminary phytochemical screening and reported pharmacological activities.

Ethics committee approval and animal husbandry

All procedures involving animals were in accordance with the guidelines published by CPCSEA and were duly approved by Institutional Animal Ethics Committee, GCTS, Asansol. The study is based on OECD Test Guideline 425. So non pregnant and nulliparous female albino mice of age 8 weeks were chosen for the experiment. Animals were harbored under standard housing conditions for a span of five days.

Acute toxicity test

The animals were subjected to limit test by administering a single dose of 2000 mg/kg p.o. in accordance to OECD Test Guidelines 425. The animals were kept fasted for a period of 3 to 4 hours prior to dosing. Single female mouse was dosed according to the body weight and post-dosing the animals were kept under observation initially for 30 mins and then for 4 hours. 1-2 hours post dosing food was provided to the animals. Upon survival of the first mice the other four mice were dosed according to their respective body weights under similar conditions. Identical procedures were followed for five mice of vehicle-treated group.1% Carboxymethyl cellulose (CMC) gel was administered as vehicle for both test and control mice. Mice in both the groups were closely observed for any toxic effects, first for 24 hours and thereafter for a span of 14 days. The mice were specifically observed for the emergence of toxic manifestations and the weights of all individual animal was monitored and documented. Towards the concluding part blood samples were drawn from animals under light ether anesthesia via retro orbital puncture and serum was isolated from the collected volume of blood for biochemical and hematological examination. At the end the mice were sacrificed by decapitation and organs were separated for determination of relative organ weight and were preserved in 10% formalin for histopathological examination.

Biochemical evaluation

Biochemical evaluation was carried out to estimate the levels of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), alkaline phosphate, total protein, albumin, globulins etc. with standard kits from Sigma Aldrich.

Hematological analysis

Microfuge tubes pretreated with EDTA were used for collection of blood from the animals of both control and test group for studying hematological parameters (CBC) which included total RBC, Hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count, white blood cells (WBC) count, neutrophils, lymphocytes, eosinophils and monocytes. The parameters were estimated by autoanalyser.

Histopathological analysis

Mice were sacrificed and the isolated vital organs were fixed in neutral buffered formalin (10%). They underwent subsequent processing and were sliced into sections of 5 nm thickness before being stained with routine hematoxylin and eosin. The slides were studied under Light microscope integrated with automatic image capturing system (Magnus, India)

Statistical analysis

The values are represented as mean ± SEM. For calculating statistical significance one-way ANOVA was used followed by Tukey's multiple comparison test. Statistical significance was considered at $P < 0.05$.

Results

The animals of both groups were kept under strict observation for the first thirty minutes and thereafter for the next four hours. Subsequently, they were closely monitored for a span of fourteen days. Signs of mortality or morbidity were absent in either group through the entire observation period. The results of the other parameters are presented hereafter.

Table 1. Effects of extract (2000 mg/kg) on body weight

Groups	1st Day	2nd Day	3rd Day
	Body Weight (gms)	Body Weight (gms)	Body Weight (gms)
Vehicle Control	25.34 ± 0.23	26.12 ± 0.14	27.02 ± 0.34
SBE (2000 mg/kg)	25.44 ± 0.73	24.72 ± 0.18	23.62 ± 0.23

Body weight and behavior

A progressive increase in body weight was observed in both the groups, however the animals of the test group did not show significant shooting of weight as that of their peers in the control group. The behavioral patterns did not show any intergroup variation though table 2. However, itching could be noticed in both control and test groups during the period following administration of vehicle and the extract respectively.

Relative organ weights

Isolated organs were found to be free of any lesion and no significant intergroup variation could be figured upon examination. Summary of relative weights of the isolated vital organs has been represented in table 3.

Biochemical parameters

Biochemical parameters like urea and creatinine were tested as indices for renal function. None of the parameters showed any significant variation between the groups table 4. Several biomarkers for hepatic damage were tested. Among them, AST showed an increase while ALP was significantly decreased in the animals of the test group. None of the other hepatic parameters showed a significant variation between the groups in table 5.

Table 2. Behavioral patterns of animals treated with extracts (2000 mg/kg) or vehicle

Parameters	Behavioral patterns in different groups									
	30 mins		24 hrs		48 hrs		7 Days		14 days	
	VC	SEB	VC	SEB	VC	SEB	VC	SEB	VC	SEB
Fur & Skin	N	N	N	N	N	N	N	N	N	N
Eyes	N	N	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N	N	N
Respiration	N	N	N	N	N	N	N	N	N	N
Urination (color)	N	N	N	N	N	N	N	N	N	N
Faecal Consistency	N	N	N	N	N	N	N	N	N	N
Somatomotor activity & Behavioral Pattern	N	N	N	N	N	N	N	N	N	N
Sleep	N	N	N	N	N	N	N	N	N	N
Mucous Membrane	N	N	N	N	N	N	N	N	N	N
Convulsions & Tremors	Nf	Nf	Nf	Nf	Nf	Nf	Nf	Nf	Nf	Nf
Itching	Nf	P	Nf	P	Nf	Nf	Nf	Nf	Nf	Nf
Coma	Nf	Nf	Nf	Nf	Nf	Nf	Nf	Nf	Nf	Nf
Mortality	Nf	Nf	Nf	Nf	Nf	Nf	Nf	Nf	Nf	Nf

Key: VC : Vehicle Control; SBE: Stem bark extract; N- Normal; Nf : Not Found P: Present

Table 3. Effect of extracts (2000 mg/kg p.o) on relative organ weight

Organs	Vehicle Control Group (1% CMC gel)	Acute Toxicity Group (SBE 2000 mg/kg)
Heart	0.711 ± 0.041	0.635 ± 0.012
Kidney	1.365 ± 0.021	1.421 ± 0.257
Liver	6.126 ± 0.041	7.156 ± 0.156

Values are represented in the form of ± SEM. SBE – Ethanolic extract of stem bark

Table 4. Effect of extract (2000 mg/kg) on renal function

Parameters	Vehicle control group (1% CMC gel)	Acute toxicity group (SBE 2000 mg/kg)
Serum Creatinine (mg/dl)	0.711 ± 0.041	0.725 ± 0.012
Serum Urea (mg/dl)	1.365 ± 0.021	1.384 ± 0.257

Table 5. Effect of extract (2000 mg/kg) on hepatic function

Parameters	Vehicle control group (1% CMC gel)	Acute toxicity group (SBE 2000 mg/kg)
SGOT (U/L)	192 ± 1.081	195 ± 1.001
SGPT (U/L)	323 ± 3.404	322 ± 1.004
Alk. Phosphatase (U/L)	160 ± 0.008	135 ± 1.012
Total Bilirubin (mg/dl)	0.85 ± 0.123	1.85 ± 0.141
Total Protein (G/dl)	6.8 ± 0.156	7.0 ± 0.152
Albumin (G/dl)	4.3 ± 0.124	4.2 ± 0.004
Globulin (G/dl)	3.5 ± 1.154	3.2 ± 0.031

Values represented as Value ± S.E.M.; SBE – Ethanolic extract of *Terminalia tomentosa* stem bark.

Table 6. Effects of vehicle (CMC 1% gel) and extract (2000 mg/kg) on hematological profile

Parameters	Unit	Vehicle control group	Acute toxicity group
		(1% CMC gel)	(SBE 2000 mg/kg)
Hb	g/dl	10.06 ±0.062	11.05 ±0.132
Total RBC	x 10 ^ 12/l	7.21 ± 0.126	7.56 ± 0.052
HCT	%	23.45 ± 0.134	34.63 ±0.024
MCV	FI	42.5 ± 0.123	56 ± 0.014
MCHC	g/dl	32.4 ± 0.134	25.47 ± 0.012
Platelet Count	x 10 ^ 12/1	270 ± 3.42	432 ± 7.2
WBC count (TLC)	x 10 ^ 19/1	3.42 ±0.102	4.82 ±0.215
Neutrophils	%	12 ± 0.012	10.45 ± 0.062
Lymphocytes	%	84 ± 1.452	87 ± 0.067
Monocytes	%	3 ± 0.123	4 ± 0.146
Eosinophils	%	2 ± 0.154	3 ± 0.152
MCH	Pg	17.4 ± 0.154	18.2 ± 0.164

Values represented as Value± S.E.M.; SBE – Ethanolic extract of *Terminalia tomentosa* stem bark.

Hematological profile

The hematological profile was tested in both the groups by estimating the complete blood count (CBC) (table 6). There was a significant increase in total leukocyte count and differential lymphocyte count though all other parameters did not show any significant differences between the groups.

Discussion

Herbal therapies are the buzzwords of modern medicine. Although plants have been exploited for their medicinal properties from time immemorial, growing awareness among the consumers regarding the safety of these products have brought a vast majority of such products under the scan (Ridtitid et al. 2008). Hence global regulatory authorities like WHO and FDA are increasingly demanding safety characterization of the plants envisaged to be having medicinal values. The shrub *Terminalia tomentosa* (Roxb.) in spite of possessing a plethora of medicinal properties, as evident from the literature, the shrub *Terminalia tomentosa* (Roxb.) lacks sufficient literary support on the basis of which its safety profile can be authenticated. Moreover, to ascertain a safer range of dose for systematic evaluation of pharmacological properties, determination of acute oral toxicity is an essential step. Hence we found a good rationale behind conducting the present study to evaluate the acute toxicity of the ethanolic extract of *T. tomentosa* stem bark in accordance to the OECD Test Guidelines 425 (In 2008; Saleem et al. 2016).

Mice have been chosen as a preferred species for toxicity studies because of higher fidelity and sensitivity than rats. Alteration in behavioral patterns serves as one of the prime indices of toxicity. In our study, there was no record of mortality or significant alteration with regard to the rate of respiration, neuromotor activity, convulsions or tremor though responses like itching were observed in significant number of animals in the treatment group during initial 24 hours. Through the entire 14-day period of toxicity

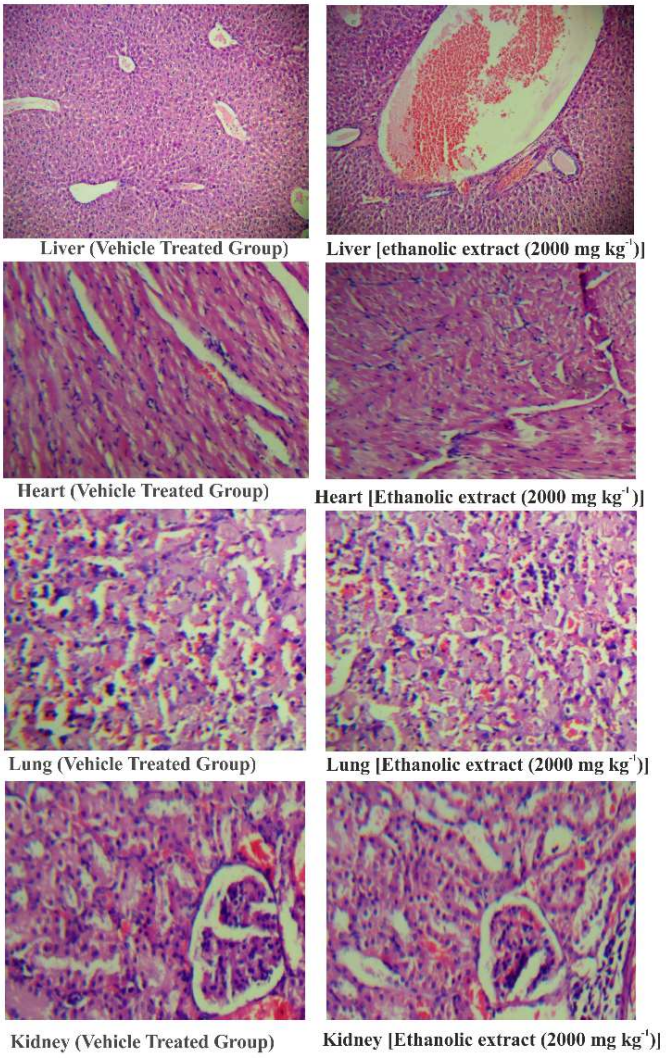

Figure 1. Histopathology of different vital organs of the animals treated with either vehicle or limit dose of ethanolic extract (2000 mg/kg)

assessment, no significant difference was observed in food or water intake between the control and test groups though the same was slightly decreased in the tst group. The effect was duly reflected in the trend of variation of body weights in both the groups. This may well be indicative of an anorexigenic potential of the extract the mechanistic pathway of which still remains elusive.These consistencies are suggestive of a normal metabolic status in the animals across the groups (Iversen and Nicolaysen 2003; Klaassen 2013). The relative organ weights of the animals in the test group did not show any significant difference with that of the controls. The macroscopic or histopathological examination also did not reveal any pathological lesions in the vital organs like liver, kidney, and heart. Taken together these data indicate no systemic toxicity in the animals treated with the limit dose of the extract.

In the present study, the health status of the animals was also evaluated by estimating a variety of serum biomarker (Friedman, Martin, and Munoz 1996; Ramaiah 2011; Ozer et al. 2008). Hepatic damage often associated with a plethora of drugs was tested by estimating systemic levels of various biomarkers like AST, ALP, total protein, globulin etc. Among these, Aspartate transaminase (AST) also known as SGOT is pivotal in indicating hepatocellular damage and hike in systemic Alkaline phosphatase (ALP) is a gold standard for determination of biliary tract obstruction. In our study, we did not find any significant variation in either of these markers in between control and test groups though AST was slightly raised in the animals of the test groups. Renal function, often an important indicator of drug toxicity was determined by systemic biomarkers like serum urea and creatinine. In our study, we did not find any significant difference in any of these markers in between the control and test animals.

Toxic stress induced by xenobiotics often get reflected in the altered hematological profile of animals (Jain, Sharma, and Sharma 2009) . In this study, we found a significant increase in platelet count of the treated groups compared to controls which may be indicative of a probable hemostatic potential of the extract. Along with a significant rise in mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV), a decline in mean corpuscular hemoglobin concentration (MCHC) were observed. With regard to cellular counts, we found a significant increase in TLC and lymphocytes with a decrease in neutrophils in the treated groups which may correlate with its antimicrobial and anti-inflammatory potential already reported by other authors (Adedapo, Abatan, and Olorunsogo 2004; Chunlaratthanaphorn et al. 2007; Sillanaukee 1996).

Conclusion

Globally Harmonized Classification System (GHS) is a gold standard for classifying chemicals on the basis of the median lethal dose. Our study revealed that ethanolic extract of *Terminalia tomentosa* stem bark can be a potential agent to be placed in category 5 (LD_{50}> 2000 mg/kg) of the GHS scheme. However, the results presented in this work are still preliminary ones which are expected to pave the way for follow up confirmatory studies in the future.

Conflicts of interest: Not declared.

References

Adedapo AA, Abatan MO, Olorunsogo OO. 2004. Toxic Effects of Some Plants in the Genus Euphorbia on Haematological and Biochemical Parameters of Rats. Veterinarski Arhiv. https://hrcak.srce.hr/index.php?id_clanak_jezik=101133&show=clanak.

Anjaneyulu ASR, Raghava Reddy AV, Mallavarapu GR, Chandrasekhara RS. 1986. 3-Acetylmaslinic Acid from the Root Bark of Terminalia Alata. Phytochemistry

25(11):267071.

Chen X, Zhou H, YB Liu, JF Wang HLi, Ung CY, Han LY, Cao ZW, Chen YZ. 2006. Database of Traditional Chinese Medicine and Its Application to Studies of Mechanism and to Prescription Validation. British Journal of Pharmacology 149(8):10921103.

Chunlaratthanaphorn S, Nirush L, Umarat S, Amornnat T, Anongnad N, Nadthaganya S, Kanjana J. 2007. Acute and Subchronic Toxicity Study of the Water Extract from Root of Citrus Aurantifolia (Christm. et Panz.) Swingle in Rats. Songklanakarin Journal of Science and Technology 29(1):12539.

De Onis M, Blössner M, and Borghi E. 2010. Global Prevalence and Trends of Overweight and Obesity among Preschool Children. The American Journal of. https://academic.oup.com/ajcn/article-abstract/92/5/1257/4597558.

Fahmy NM, Eman AS, Mohamed MAD, Maarit K, Abdel NS. 2016. Protective Effect of Terminalia Muelleri against Carbon Tetrachloride-Induced Hepato and Nephro-Toxicity in Mice and Characterization of Its Bioactive Constituents. Pharmaceutical Biology 54(2):30313.

Friedman LS, Martin P, Munoz SJ. 1996. Liver Tests and the Objective Evaluation of the Patients with Liver Diseases. Hepatology. A Textbook of Liver Disease.

In O. 2008. OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects. Test No. 425: Acute Oral Toxicity: Upanddown Procedure.

Iversen PO, Nicolaysen G. 2003. Water--for Life. Tidsskrift for Den Norske Laegeforening: Tidsskrift for Praktisk Medicin, Ny Raekke. https://europepmc.org/abstract/med/14713981.

Jain N, Sharma P, Sharma N. 2009. Haemato-Biochemical Profile Followi G Sub Acute Toxicity of Malathio I Male Albi O Rats. Avicenna J. .

Kapoor D, Rajesh V, Veena D. 2014. Terminalia Arjuna in Coronary Artery Disease: Ethnopharmacology, Pre-Clinical, Clinical & Safety Evaluation. Journal of Ethnopharmacology 155 (2):102945.

'Klaassen C. 2013. Casarett & Doulls Toxicology: The Basic Science of Poisons, Eighth Edition. McGraw Hill Professional.

Meriga B, Parim BN, Ganjayi M, Gen HK, Ramavat RN, Suresh P. 2017. Ethanolic fraction of Terminalia tomentosa attenuates biochemical and physiological derangements in diet induced obese rat model by regulating key lipid metabolizing enzymes and adipokines. Pharmacognosy Magazine 13(51):38592.

Mopuri R, Muniswamy G, Kruthika SB, Brahma NP, Balaji M. 2015. "Evaluation of Anti-Obesity Activities of Ethanolic Extract of Terminalia Paniculata Bark on High Fat Diet-Induced Obese Rats. BMC Complementary and Alternative Medicine 15 (March): 76.

Ozer J, Marcia R, Martin S, Wendy B, Shelli S. 2008. The Current State of Serum Biomarkers of Hepatotoxicity. Toxicology 245 (3):194205.

Ramaiah, Shashi K. 2011. Preclinical Safety Assessment: Current Gaps, Challenges, and Approaches in Identifying Translatable Biomarkers of Drug-Induced Liver Injury. Clinics in Laboratory Medicine 31(1):16172.

Ridtitid W, Wong CS, Reanmongkol W, Wongnawa M. 2008. Antinociceptive Activity of the Methanolic Extract of Kaempferia Galanga Linn. in Experimental Animals. Journal of Ethnopharmacology 118(2):22530.

Saleem U, Bashir A, Mobasher A, Alia E, Khalid H, Nadeem IB. 2016. Is Folklore Use of Euphorbia Helioscopia Devoid of Toxic Effects? Drug and Chemical Toxicology 39(2): 23337.

Saleem U, Amin S, Ahmad B, Azeem H, Fareeha Anwar, Sunita Mary. 2017. Acute Oral Toxicity Evaluation of Aqueous Ethanolic Extract of Saccharum Munja Roxb. Roots in Albino Mice as per OECD 425 TG. Toxicology Reports 4 (October):58085.

Shankar R, Lavekar GS, Deb S, Sharma BK. 2012. Traditional Healing Practice and Folk Medicines Used by Mishing Community of North East India. Journal of Ayurveda and Integrative Medicine 3(3):12429.

Sillanaukee P. 1996. Laboratory Markers of Alcohol Abuse. Alcohol and Alcoholism 31(6):61316.

Vaidya, Ashok DB, Thomas PA, Devasagayam. 2007. Current Status of Herbal Drugs in India: An Overview. Journal of Clinical Biochemistry and Nutrition 41(1):111.