

Full Length Research Paper

Acute and sub-acute toxicity of *Terminalia fagifolia* Mart. & Zucc. (Combretaceae) in rodents

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Terminalia fagifolia Mart & Zucc is a typical plant from Brazilian savanna, whose stem bark is used in popular medicine for relief of gastrointestinal discomfort. A phytochemical study showed the presence of large amounts of antioxidants (flavonoids and triterpenes). In acute toxicity tests, five groups of female mice were orally treated with stem bark extract from *T. fagifolia* or fractions by gavage at doses 2 g/kg/day (n = 5) and general behavior, adverse effects and mortality were recorded for up to 14 days. In sub-acute toxicity assays, animals received *T. fagifolia* ethanol extract or fractions at doses of 80, 240 or 720 mg/kg/day (n = 6) for 30 days and biochemical and morphological parameters were determined. The acute treatment in mice produced 40% of death. The estimate for the median lethal oral dose was 2 g/kg (LD₅₀ <5 g/kg). Sub-acute treatment with *T. fagifolia* failed to change body weight gain, food and water consumption and biochemical profiles or reproductive parameters in female Wistar rats. In addition, no changes in macroscopical and microscopical aspect of organs were observed. Our results showed that acute or sub-acute administration of *T. fagifolia* has no significant toxicity, suggesting that it is safe for use as herbal medicine.

Key words: Toxicity, herbal medicine, reproductive toxicity, rodents.

INTRODUCTION

One of the great legacies of miscegenation of the Brazilian population is knowledge about the use of plants to treat illnesses. The Brazilian savanna has 5% of the flora and fauna of the world. Their medicinal potential is known historically by indigenous peoples, Portuguese colonists, African slaves and the hinterland communities, which have passed their knowledge from generation to generation. This conjunction of knowledge and biodiversity, provides good scenario for the development of research aimed at discovering new drugs from native species (Ferreira et al., 2011). Vochysiaceae, Fabaceae,

and Combretaceae families are among the 59 already identified in the savannah area. The family Combretaceae includes 18 genera, *Combretum* and *Terminalia* being the most abundant, with about 370 species and 200, respectively (Almeida et al., 1998).

Terminalia fagifolia Mart. & Zucc. known as chapadeiro, cachaporra, cachaporra-do gentio and pau de bicho is a Brazilian savannah tree, its stem bark is widely used by local people for relief of gastric discomfort, treatment of tumors and ulcers (Almeida et al., 1998). Fitoquímicos and biological studies with this species showed that the

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ethanolic extract of the bark has antioxidant and cytotoxic activity, as well as the occurrence of flavonoids (flavanones, flavan and chalcones), 1,3-diarylpropanos, pentacyclic triterpenes glycosylated and non-glycosylated and the steroid sitosterol (Garcez, 2006). The ethanol extract of leaves showed antioxidant potential in the DPPH assay, this activity was higher in hydroalcoholic fraction, as evidenced by the values of effective concentration able to reduce the DPPH by 50% (EC₅₀) (Ayres, 2009).

This characterization of phytochemical and antioxidant performance of *T. fagifolia* signal their full therapeutic potential. However, it is possible that antioxidants protect a given system and another made vulnerable, or even induce lesions in other systems, thus allied with the study on the medication, it is essential to study toxicological effects according to recommendations of the OECD.

Several species of *Terminalia* and their isolated compounds have been targeted for research on pharmacological activities. Studies by Manna et al. (2006) demonstrated that the aqueous extract of *Terminalia arjuna* prevented hepatotoxic and nephrotoxic effects induced by carbon tetrachloride (CCl₄). The methanol extract of *T. arjuna* showed gastroprotective activity in trials of gastric ulcer induced by diclofenac sodium in rats (Devi et al., 2007).

The *Terminalia chebula* is used in Ayurvedic medicine for its astringent, anthelmintic, expectorant, laxative effects, among others (Pfundstein et al., 2010). Pretreatment of animals with *T. chebula* Gertner reduced the production of ferric nitrilotriacetic acid, inducer of oxidative stress and renal tumorigenesis (Prasad et al., 2007) and showed hepatoprotective activity in the model of toxicity induced by rifampicin, pyrazinamide and isoniazid in rodents (Tasduq et al., 2006). *In vitro* studies using different concentrations of extracts and fractions of this species showed a reduction in the concentration of cytochrome P450 in rat liver, revealing anti-apoptotic activity (Ponnusankar et al., 2011) and also promoted the proliferation and activation of matrix metalloproteinase-2 (MMP-2) in fibroblasts, justified by the antioxidant activity of the extract (Manosroi et al., 2010). In studies by Srivastav et al. (2010) for evaluation of reproductive toxicity in males of another plant of the same gender, were orally administered 100 mg/kg of extract of *T. chebula* for 60 days and observed a potent antispermato-genic effect and a sharp reduction of fertility in rats undergoing treatment.

Therefore, *T. fagifolia* Mart. & Zucc provide important pharmacological effects with great therapeutic potential, nevertheless no reports were found in the literature reports of toxicological studies, so this work is a pioneering study that aims to evaluate the acute and sub-acute toxicity of the extract and fractions of stem bark from *T. fagifolia*, including aspects of reproduction, thus contributing to a safer use of the plant by the population and the development of herbal medicines derived from this species.

MATERIALS AND METHODS

Plant

Samples of stem bark of *T. fagifolia* Mart. & Zucc. were collected in the savanna biome of the region between Northeastern and Northern Brazil in the municipality of Timon, Maranhão, Brazil, in November 2006. The voucher specimen was identified by Garden Maria de Souza and deposited in Graziella Barroso Herbarium of Federal University of Piauí, Brazil, with a record 21,691-TEPB.

Extraction

The stem bark of *T. fagifolia* (1950 g) were air-dried, crushed (knife mill Marconi, São Paulo, Brazil) and submitted to maceration process six times with ethanol PA (Vetec, Rio de Janeiro, Brazil) at room temperature for 72 h. After removal of the solvent on the rotary evaporator (Quimis 344B2, São Paulo, Brazil) at 55°C under reduced pressure and lyophilization, was obtained 416.7 g of the ethanolic extract of *T. fagifolia* (EETF). A portion of the extract (256.3 g) was taken for toxicological activity tests and remaining material (160 g) was suspended in 1200 ml of a mixture of methanol (Qeel São Paulo, Brazil) and water (H₂O/MeOH) (2:1) and submitted to partition with ethyl acetate PA. The organic phase was concentrated (99.66 g), suspended in MeOH/H₂O (9:1) and then was extracted with hexane providing hydroalcoholic (FHA-EETF, 91.96 g) and hexane (FHEX-EETF, 4.19 g) phases. The EETF and fractions from the partition were lyophilized. The lyophilized extract was kept at room temperature until use as a suspension in distilled water.

Animals

Adult non-pregnant female Wistar rats (*Ratus norvegicus* var. *albinus*) weighing 180 to 220 g and female mice (*Mus musculus* var. *Swiss*) created and maintained in the Animal Facility for Experimentation of the Department of Veterinary Morphophysiology, Center for Agricultural Sciences, Federal University of Piauí, Brazil. The animals were kept under 12-h light and 12 h of darkness, in individual cages in a room with air conditioning and free access to water and food (FRI-LAB Rats - Fri-Ribe).

The procedures that required euthanasia of the animals were performed according to CFMV Resolution Number 714 of 20 June 2002. The experimental procedures were approved by the Ethics Committee on Animal Experiments of UFPI, protocol number 04/2011.

The experimental protocols were elaborated and developed based on the principle of the three R's (Refine, Reduce and Redesign). That is, the lowest number of animals possible was used in order to determine statistical differences; the protocols developed do not overlap regarding the objective of the study. Moreover, the animals were handled by trained researchers in our laboratory only when necessary. They were not exposed to any kind of pain or stress caused by noise, lack of food, water, or variation in temperature.

Acute toxicity

Healthy female mice fasted overnight, but with access to water *ad libitum*, were randomly divided into five groups (n=5). The first group (control group) received distilled water orally. Groups 2 to 5 were orally treated with EETF, aqueous fraction (FAq-EETF), hydroalcoholic fraction (FHA-EETF) and hexane fraction (FHEX-EETF) with up to dose 2 g/kg of body weight. Animals were observed for general behavioral and body weight changes,

Table 1. Food and water consumptions of female Wistar rats treated orally with the *Terminalia fagifolia* ethanolic extract (EETF), hexane fraction (FHA-EETF) or aqueous fraction (FAQ-EETF) 30 consecutive days.

Parameter	Food (g/day)	Water (g/day)
Control	9.792±0.4382	24.88±4.218
EETF- 80 mg/kg	8.898±0.4313	23.43±3.386
EETF- 240 mg/kg	9.327±0.5142	26.78±4.315
EETF- 720 mg/kg	8.063±0.4377	19.96±3.419
FHA-EETF 240 mg/kg	9.726±2.281	21.54±3.890
FAQ-EETF 240 mg/kg	11.70±2.618	25.55±4.272

The values are expressed as mean ± SEM (n=6/group). Have no significant difference (p<0.05).

hazardous symptoms and mortality for a period of 14 days after treatment. The acute toxicity was estimated according to the method described by the Organisation for Economic Co-operation and Development (OECD) Test Guidelines (OECD, 2001).

Sub-acute toxicity

The method was performed according to the OECD Test Guidelines with no modification (OECD, 1995). Healthy female Wistar rats were randomly divided into six groups by treatment (n=6). Animals received water-vehicle orally (control group) or EETF with doses of 80, 240 or 720 mg/kg/day, FAQ-EETF and FHA-EETF with dose 240 mg/kg/day for 30 consecutive days. Food consumption and water intake were monitored daily. The body weight was recorded weekly and the percentage of weight gain of each animal was calculated by the difference of the final weight and the initial weight (g) divided by the initial weight × 100. To evaluate the toxicity of EETF and fractions during the estrous cycle all the rats were examined daily between 8:00 and 9:00 h, initially for seven days to verify whether they were cycling normally and only those with regular cycles were included in the experiment. Then the rats were submitted to the pharmacological experimental design. The stage of the estrous cycle of each rat was recorded daily during the 30 days of treatment. The interval between estrus of each rat was calculated from the number of days between estrus divided by the total days of treatment. At the end of the treatment, animals were fasted overnight, but allowed access to water *ad libitum*. They were then anesthetized with a combination of ketamine (50 mg/kg) and xylazine (11.5 mg/kg), blood collection was performed by puncturing heart, in bottles without anticoagulant (BD-Vacurette) for evaluation of biochemical parameters.

Biochemical analysis

For biochemical analysis, blood was centrifuged at 20.000 g for 5 min to obtain serum; which was stored at -20°C until following parameters were determined: aspartate aminotransferase (AST); alanine aminotransferase (ALT); and creatinine and urea. Dosages were made using semi-automated biochemical analyzer (RA-50, Bayer Germany), using kits Lab test (Labtest Diagnostica, Lagoa Santa, MG, Brazil).

Morphological study

The animals were euthanized with excess of the same anesthetic mixture previously described and necropsied. The organs: heart,

liver, kidneys, spleen, ovaries and uterus were then removed, weighed and evaluated macroscopically as the morphology and color. The absolute masses were then converted to relative masses (mg/100 g body weight).

Statistical analysis

The results are expressed as mean±standard error of mean (SEM). Variance in data for body weights, food and water consumption, hematology, serum biochemistry and relative organ weights was checked for homogeneity by analysis of variance (one-way ANOVA) followed by Dunnet post hoc test for comparison between the control and treatment groups. A probability level of less than 5% (p < 0.05) was considered significant. Statistical analyses were performed with the aid of the software GraphPad Prism® 5.0.

RESULTS

Acute toxicity

In the experimental model adopted for acute toxicity with EETF, FHA-EETF, EETF FAQ-or-FHEX EETF at doses of 1 and 2 g/kg, mortality was not observed. The acute treatment has not altered other evaluated parameters such as weight gain, water consumption and feed intake. The estimate for the median lethal oral dose was LD₅₀>2 g/kg.

Sub-acute toxicity

No toxicity signs (such as piloerection, alteration in the locomotor activity or diarrhea) or deaths were recorded during the 30 consecutive days of treatment via oral route with EETF with doses of 80, 240 or 720 mg/kg/day, FAQ-EETF and FHA-EETF with dose 240 mg/kg/day. No significant differences were found between the initial and final body weight of the rats treated with *T. fagifolia* extract ethanolic and control rats (Figure 1). A similar absence of toxic effect was observed in the case of food and water consumption (Table 1).

The treatment with ethanolic extract and fractions of *T. fagifolia* for 30 days did not change the biochemical profile of female rats, as show in Table 2. In biochemical studies, all values in control and treated groups were within the reference range for the species.

Morphological analysis

The relative organ weights were not altered by extract and fractions of *T. fagifolia* treatment (Table 3). The macroscopic analysis of the target organs of the treated animals did not show significant change in color and texture when compared with the control group.

DISCUSSION

As shown in the results of acute toxicity test, the stem

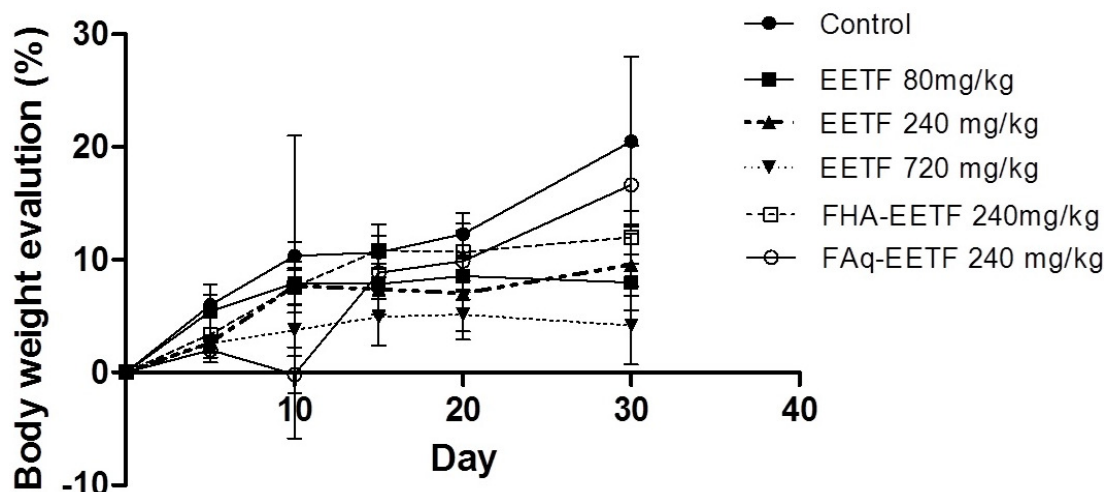


Figure 1. Body weight gain curves of female Wistar rats treated orally with the *Terminalia fagifolia* ethanolic extract (EETF), hexane fraction (FHA-EETF) or aqueous fraction (FAQ-EETF) for 30 consecutive days. The values are expressed as mean $\pm$ SEM (n=6/group). Have no significant difference (p<0.05).

Table 2. Effects of *Terminalia fagifolia* ethanolic extract (EETF), hexane fraction (FHA-EETF) and aqueous fraction (FAQ-EETF) by oral route on Biochemical parameters in female Wistar rats treated for 30 consecutive days.

Parameter	Control	EETF 80 mg/kg	EETF 240 mg/kg	EETF 720 mg/kg	FHA-EETF 240 mg/kg	FAQ-EETF 240 mg/kg
Creatinine (mg/dl)	0.288 $\pm$ 0.0441	0.312 $\pm$ 0.0227	0.288 $\pm$ 0.0295	0.233 $\pm$ 0.0422	-	-
AST (U/L)	350.6 $\pm$ 94.4	180.7 $\pm$ 13.1	238.7 $\pm$ 42.1	167.6 $\pm$ 18.2	-	-
ALT (U/L)	57.9 $\pm$ 6.10	54.3 $\pm$ 2.95	45.1 $\pm$ 7.84	38.4 $\pm$ 5.84	52.4 $\pm$ 3.75	52.5 $\pm$ 4.40
Alkaline phosphatase	96.4 $\pm$ 27.2	194.7 $\pm$ 76.9	165.8 $\pm$ 27.7	132.6 $\pm$ 64.9	85.6 $\pm$ 24.18	77.37 $\pm$ 30.8
Urea	105.6 $\pm$ 7.0	90.3 $\pm$ 6.54	88.4 $\pm$ 10.30	102.2 $\pm$ 12.29	40.43 $\pm$ 2.51	41.0 $\pm$ 2.68

Values represent the mean $\pm$ SEM (n=6/group). AST, aspartate aminotransferase; ALT, alanine aminotransferase. Have no significant difference (p<0.05).

Table 3. Effects of *Terminalia fagifolia* ethanolic extract (EETF), hexane fraction (FHA-EETF) and aqueous fraction (FAQ-EETF) on relative organ weight (mg/100g) in female Wistar rats treated by oral route for 30 consecutive days.

Treatment	Liver	Kidney	Spleen	Heart	Uterus	Ovary	Stomach
Control	3254 $\pm$ 99.44	677.2 $\pm$ 15.46	341.2 $\pm$ 18.81	343.4 $\pm$ 8.640	242.1 $\pm$ 27.18	51.61 $\pm$ 3.322	640.9 $\pm$ 27.16
EETF 80 mg/kg	3246 $\pm$ 121.7	675.4 $\pm$ 25.76	385.6 $\pm$ 23.34	329.4 $\pm$ 10.61	279.1 $\pm$ 34.05	55.62 $\pm$ 3.833	686.5 $\pm$ 31.10
EETF 240 mg/kg	3579 $\pm$ 164.6	664.8 $\pm$ 25.73	374.2 $\pm$ 23.03	324.5 $\pm$ 12.94	228.7 $\pm$ 30.26	48.44 $\pm$ 2.878	677.4 $\pm$ 22.88
EETF 720 mg/kg	3525 $\pm$ 131.3	708.4 $\pm$ 19.39	358.5 $\pm$ 10.41	330.3 $\pm$ 8.244	206.8 $\pm$ 15.80	44.17 $\pm$ 3.851	742.3 $\pm$ 29.74
FHA-EETF 240 mg/kg	3384 $\pm$ 141.8	735.1 $\pm$ 53.18	416.0 $\pm$ 28.18	328.8 $\pm$ 12.34	261.8 $\pm$ 38.79	54.25 $\pm$ 3.714	592.0 $\pm$ 28.06
FAQ-EETF 240 mg/kg	3226 $\pm$ 43.65	657.2 $\pm$ 24.52	344.1 $\pm$ 24.33	300.2 $\pm$ 9.607	211.8 $\pm$ 6.336	43.05 $\pm$ 4.65	661.8 $\pm$ 6.60

Values represent the mean $\pm$ SEM (n=6/group). Have no significant difference (p<0.05).

bark extract and fractions of *T. fagiflora* Mart. & Zucc were practically non-toxic, because of the result of the dose of 2 g/kg and the document of OECD does not recommend the test for acute toxicity using a dose of 5000 mg/kg, as a way to protect the animal's life (OECD, 2001).

However, the acute toxicity experiment guided the

choice of doses to be used in the sub-acute toxicity protocols. We used three doses (80, 240 and 720 mg/kg) in a geometric progression of ratio, in order to establish the highest dose that does not induce toxic effect level (NOAEL) and lowest dose that induces a toxic effect without leading to death (LOAEL), and obeying 1000 mg/kg the limit for toxicological testing in accordance with

OECD 407 (OECD, 1995).

In nulliparous rats, treatment with EETF, FHA-EETF and PAq-EETF for 30 days did not cause changes in length, sequence of phases of the estrous cycle and repeatability of the following cycles, compared to control group. These data indicate that the treatment did not cause disturbances in the hypothalamic-pituitary-ovarian preserving reproductive function (Andrews et al., 2002).

Signs of systemic toxicity were evaluated from the reduction of body weight of experimental animals, reduced water consumption and food intake, behavioral changes, apathy and other changes that may signal, for example, liver injury. Only EETF females treated with doses of 80 and 720 mg/kg showed a reduction in weight gain body mass compared to vehicle group, no change in the consumption of food and water could be seen. It was also observed that the intermediate dose (240 mg/kg) caused no reduction in mass gain, showing that the effect was not dose dependent. Considering that in the experiment with males (data not shown) there was no reduction of the gain in the same doses of the experiment with females and not observed any signs of significant systemic toxicity within 30 days of treatment with EETF and fractions, it can be considered that the doses studied did not induce significant toxic effects.

Toxic effects on biological systems can be observed by the standard serum urea, creatinine (mg/dl) whose changes provide evidence of renal overload, acute renal failure, or even increase in protein catabolism (Vijayalakshmi et al., 2000; Adebayo et al., 2003). Changes in aminotransferases enzymes (AST and ALT U/L) and alkaline phosphatase are important indicators of injury in liver cells. A drug does not cause liver damage without interfering with normal activity of their enzymes (Alía et al., 2003). The biochemical profile of animals showed statistical similarity between groups and that all parameters evaluated were within the reference values (Rodrigues et al., 2006), which does not indicate significant levels of hepatic or renal toxicity in sub-acute treatment.

The ethanol extract of stem bark of *T. fagifolia* showed low systemic toxicity and no effect was observed on estrous cycle within the limits of doses used in this experiment, which are also of toxicological limits.

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ABREVIATIONS

AST, Aspartate transaminase; **ALT**, alanin transaminase; **ALP**, alkaline phosphatase; **EETF**, ethanolic extract of *Terminalia fagifolia*; **Faq-EETF**, aqueous fraction of

ethanolic extract of *Terminalia fagifolia*; **FHA-EETF**, hydroalcoholic fraction of ethanolic extract of *Terminalia fagifolia*; **NOAEL**, no observed adverse effect level; **LOAEL**, lowest observed adverse effect level; **OECD**, organization for economic cooperation and development; **LD₅₀**, median lethal dose.

REFERENCES

- Adebayo JO, Yakubu MT, Eqwim EC, Owoyole VB, Enaibe BU (2003). Effect of ethanolic extract of *Khaya senegalensis* on some biochemical parameters of rat kidney. *J. Ethnopharmacol.* 88:69-72.
- Alía M, Horcajo C, Bravo L, Goya L (2003). Effect of grape antioxidant dietary fiber on the total antioxidant capacity and the activity of liver antioxidant enzymes in rats. *Nutr. Res.* 23:1251-1267.
- Almeida SP, Proença CEB, Sano SM, Ribeiro JF (1998). Cerrado - Espécies vegetais úteis. Embrapa: Planaltina. p.464.
- Andrews P, Freyberger A, Hartmann E, Eiben R, Loof I, Schmidt U, Temerowski B, Folkerts A, Sthal B, Kayser M (2002). Sensitive detection of the endocrine effects of the estrogen analogue ethinylestradiol using a modified enhanced subacute rat study protocol (OECD Test Guideline no. 407). *Arch. Toxicol.* 76:194-202.
- Ayres MCC, Chaves MH, Vilegas DRW, Vieira Jr GM (2009). Chemical constituents and antioxidant activity from leaves extracts of *Terminalia fagifolia* Mart. et Zucc. *Quím. Nova* 32:1509-1512.
- Devi CSS, Sundaresan R, Narayan S, Vani G (2007). Gastroprotective effect of *Terminalia arjuna* bark on diclofenac sodium induced gastric ulcer. *Chem. Biol. Interact.* 167:71-83.
- Ferreira BES, Costa RC, Nunez CV (2011). Some dimensions of biodiversity market in Manaus-AM. *Ateliê Geográfico.* 5:103-117.
- Garcez FR, Garcez WS, Santana ALBD, Alves MM, Matos MFC, Scalante AM (2006). Bioactive flavonoids and triterpenes from *Terminalia fagifolia* (Combretaceae). *J. Brazil Chem. Soc.* 17:1223-1228.
- Manna P, Sinha M, Sil PC (2006). Aqueous extract of *Terminalia arjuna* prevents carbon tetrachloride induced hepatic and renal disorders. *BMC. J. Altern. Complem. med.* 30:33.
- Manosroi A, Jantrawut P, Akihisa T, Manosroi W, Manosroi J (2010). *In vitro* anti-aging activities of *Terminalia chebula* gall extract. *Pharm. Biol.* 48:469-481.
- OECD (1995). Guideline for testing of chemicals No. 407: Repeated Dose 28- day Oral Toxicity Study in Rodents. Organization for Economic Co-operation and Development, Paris, France, 8 pp
- OECD (2001). Guideline for the testing of chemicals No. 414: Prenatal developmental toxicity study. Organization for Economic Co-operation and Development, Paris, France, 6 pp
- Pfundstein B, Eldesouky SK, Hullwe HR, Erben G, Owen R (2010). Polyphenolic compounds in the fruits of Egyptian medicinal plants (*Terminalia bellerica*, *Terminalia chebula* and *Terminalia horrida*): characterization, quantitation and determination of antioxidant capacities. *Phytochemistry.* 71:1132-1148.
- Ponnusankar S, Pandit S, Venkatesh M, Bandyopadhyay A, Mukherjee PK (2011). Cytochrome P450 Inhibition Assay for Standardized Extract of *Terminalia chebula* Retz. *Phytother. Res.* 25:151-154.
- Prasad L, Khan TH, Jahangir T, Sultana S (2007). Abrogation of DEN/Fe-NTA induced carcinogenic response, oxidative damage and subsequent cell proliferation response by *Terminalia chebula* in kidney of Wistar rats. *Pharmazie.* 62:790-797.
- Rodrigues ER, Moreti DLC, Martins CHG, Kasai A, Stoppa MA, Alves EG, Paz K, Lopes RA, Sala MA, Petenusci SO (2006). Estudo de parâmetros bioquímicos em ratos sob a ação de planta medicinal. [XI. *Chiococca Alba* (L.) Hitchc]. *Rev. Bras. Plantas med.* 8:169-172.
- Srivastav A, Chandra A, Singh M, Jamal F, Rastogi P, Rajendran SM, Bansode FW, Lakshmi V (2010). Inhibition of hyaluronidase activity of human and rat spermatozoa *in vitro* and antispermatoxic activity in rats *in vivo* by *Terminalia chebula*, a flavonoid rich plant. *Reprod. Toxicol.* 29:214-224.
- Tasduq SA, Singh K, Satti NK, Gupta DK, Suri KA, Johri RK (2006). *Terminalia chebula* (fruit) prevents liver toxicity caused by sub-

chronic administration of rifampicin, isoniazid and pyrazinamide in combination. Hum. Exp. Toxicol. 25:111-118.

Vijayalakshmi T, Muthulakshami V, Sachadanandam S (2000). Toxic studies on biochemical parameters carried out in rats with serankottai Nei, a siddha durg-milk extract of *Semecarpus anacardium* Nut. J. Ethnopharmacol. 69:9-15.