Original Research Article

EVALUATION OF MEDIAN LETHAL DOSE AND SUBCHRONIC ORAL TOXICITY ASSESSMENT OF ETHANOLIC LEAF EXTRACT OF *PHYLLANTHUS AMARUS*

ABSTRACT

Aims: To determine the median lethal dose (LD_{50}) of crude ethanolic leaf extractof *Phyllanthus amarus* and evaluate its sub-chronic oral toxicity in experimental mice (BALB/$_C$ strain).
Study design: One-factor, one-control, one-test group experimental design.
Place and Duration of Study: Department of Medical Biochemistry, Delta State University, Abraka, Nigeria, between December, 2014 and November, 2015.
Methodology: Crude ethanolic leaf extract of *P. amarus* was prepared as previously described and twenty (20) Swiss albino mice (BALB/$_C$ strain) were randomly and equally divided into two (2) groups and administered 2000 mg/kg body weight (Group A) and 5000 mg/kg body weight (Group B of the prepared extract as single oral dose in line with the limit dose method of determining $LD_{50.}$ For the sub-chronic oral toxicity study, ten (10) mice were assigned into control (n=5) and experimental (n=5). The control animals were given placebo-normal saline, but the experimental mice were administered with nocebo – 300 mg/kg body weight of *P. amarus* of crude ethanolic extract for twenty one (21) days. Thereafter, the animals in each group were sacrificed and then, serum and liver homogenate were obtained for the assay of total antioxidant capacity (TAC) and oxidative damage (Malondialdehyde-MDA) Using documented methods. Liver tissue was also processed for histopathological examination using H&E stain.
Results: Data showed LD_{50} of the extract to be greater than 5000 mg/kg. Assessment of the herb's sub-chronic oral toxicity indicates that the leaf extract significantly (*P*=.03) enhanced total antioxidant capacity (TAC) in both serum (Control: TAC = 0.10±0.03 mM, Experimental: TAC = 0.33±0.05 mM) and liver (Control: TAC = 0.12±0.09 mM, Experimental: TAC = 0.34±0.06 mM) but reduced (*P* = .01) the biomarker for liver tissue (Control: MDA = 41.89±3.36 µM, Experimental: MDA = 4.67±4.04 µM). In addition, hepatic cells were invigorated by *P. amarus* treatment as suggested by the histopathological features.
Conclusion: Collectively, *P. amarus* crude ethanolic leaf extract possesses high degree of tolerance and hepatic tonic potential with no identifiable toxic or side effects.

Keywords: Phyllanthus amarus, Median Lethal Dose (LD_{50}), Sub-chronic Toxicity, Total Antioxidant Capacity (TAC), Malondialdehyde (MDA).

INTRODUCTION

The use of plants, plant extracts or plant-derived chemicals to treat diseases is a therapeutic modality that has been explored for centuries. Over 40,000 species of tropical flowering plants are known to possess medicinal properties [1] and are currently in use for various medical conditions. Majority of Africans patronize herbal or traditional medicine for their health needs. It is estimated that 70-80% of patients in Africa are treated by traditional healers and herbal practitioners [2]. Modern medicine recognizes herbalism as a form of alternative medicine based on evidence derived from scientific methods [3]. Herbal medicine is, thus, gaining popularity and one of such herbs receiving wide patronage is *Phyllanthus amarus.*

Phyllanthus amarus is an herbal plant belonging to the Euphorbiaceae family. It has approximately 800 species which are found in tropical and subtropical countries of the world [4,5]. The plant has been found in Philippine, Cuba, Nigeria and India among others. Extract of the plant has been reported to possess pharmacological effects such as antibacterial [4,6], antiviral [7], anticancer [8], antiamnesic [9], antioxidative [10], antimicrobial [11], antileptospiral [12], anticonvulsant [13] and

anti-inflammatory [14,15] activities. *Phyllanthus amarus* has been used as chemoprotective [16], antimutagenic [17], nephroprotective, cardioprotective [18], hepatoprotective [19] and hypoglycemic [20] agent. It is known to exhibit *in vivo* antiplasmodial property [21] in addition to its demonstrated ability to invigorate the pancreas [22] and restore renal function altered by *Plasmodium berghei* malarial parasite infection in experimental mice [21].

Lack of knowledge of the mechanisms and side effects of some herbal preparations as well as safety regulations for their usage may have serious consequences [23]. Many consumers believe that herbal medicines are "safe" because they are "natural", but, several adverse effects of herbs have been reported including allergic reactions, hepatotoxicity [24,25,26], nephrotoxicity [27,28,29], cardiac toxicity [30,31], neurotoxicity [32,33], and even death [34].

Since *Phyllanthus amarus* is currently gaining recognition in alternative medical practice, it has therefore become pivotal to evaluate the median lethal dose and subchronic toxicity of the ethanolic leaf extract of the plant cultivar wildly grown in the tropical rain forest zone of Abraka, Delta State, Nigeria. This freely growing variety of the plant is common and easily harvested in our environment for medicinal use.

MATERIALS AND METHODS

Harvesting and preparation of plant extract: Fresh whole plants of *Phyllanthus amarus* wildly growing in uncultivated land space in Abraka, Ethiope East Local Government Area of Delta State, Nigeria were obtained in July, 2015 and authenticated (No: FHI: 109728) in the Herbarium Unit, Forestry Research Institute of Nigeria, Ibadan. Crude ethanolic leaf extract of the harvested fresh plant was prepared as earlier described [21]. The leaves were washed, air-dried and pulverized using a sterile Electric blender (Kenwood Ltd, Hertfordshire, U.K) to produce a fine powder. The ethanolic extract of the plant sample was prepared by soaking 100 g of dry powdered sample in 200 ml of ethanol for 24 hours. The extract was filtered using whatman filter paper and the filtered extract were concentrated using the Soxhlet apparatus (Corning, U.S.A). The extract was evaporated to dryness using rotary evaporator (Buchi R-210 Hana, China) under reduced pressure and dissolved in distilled water which was then stored in a refrigerator until required for analysis.

Experimental mice: Forty (40) Swiss albino $BALB/_c$ mice of mixed sexes weighing between 21.1 to 28.2 g were used for the entire study. They were maintained at the Laboratory Animal Centre, Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria. The mice were fed on growers' mash (Top Feeds, Sapele, Delta State, Nigeria), and were given clean drinking water *ad libitum.* The animals were housed in plastic cages, under controlled condition of 12 hr light/12 hr dark cycle at a temperature of $29\pm2^{o}C$. The animals were maintained in accordance with the guidelines provided by the Research and Bioethics Committee of the Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria.

Evaluation of lethal and effective doses (LD_{50} and ED_{50}): LD_{50} and ED_{50} were determined by the limit dose method [35]. A total of thirty (30) mice (20 for LD_{50} and 10 for ED_{50}) were used. In the phase of LD_{50} determination, the mice were divided into two groups of ten (10) mice each. They were treated with ethanolic leaf extract of *Phyllanthus amarus* at doses of 2000 and 5000 mg/kg body weight as oral single dose. The animals were observed for 24 hours first and then, for twenty one (21) days for any sign of toxicity and mortality.

Subchronic Study: For the subchronic study, the remaining ten (10) mice were divided into Control (n = 5) and Experimental (n = 5) Groups. The Experimental Group was administered 300 mg/kg/d *P. amarus* ethanolic leaf extract as single daily dose for 21 days. The dosing regimen was based on previous experience [22]. The animals were observed for any physical signs of toxicity, morbidity and mortality. Body weights were measured weekly throughout the 21-day study period.

Animal Sacrifice and Collection of Sample: On the 21st day of the experiment, the mice were fasted overnight and sacrificed the next day under chloroform anesthesia. The liver was excised and whole blood was collected by heart puncture and centrifuged (Cent 80D, Serico, China) to obtain serum which was used for the biochemical analyses of total antioxidant capacity (TAC) and malondialdehyde (MDA) levels. The excised liver was fixed in 10% formol saline for histological processing and examination. However, a portion (0.5 g) was homogenized and then, prepared for biochemical assay.

Biochemical Assay: Total antioxidant capacity, TAC in serum and liver homogenate as determined by the Trolox Equivalent Antioxidant Capacity (TEAC) method described by Miller *et al.*[36] and MDA levels were estimated by the Thio-Barbituric Acid Reacting Substances (TBARS) method earlier described by Ohkawa *et al.*[37]. TAC provides information on degree of antioxidant defense, and MDA indicates a measure of membrane lipid peroxidation, and hence, oxidative stress/damage.

Histological Studies: The portion of the liver tissue fixed in 10% formol saline was processed overnight using histokinette and embedded in paraffin wax. Three sections - four micron in thickness - were cut from each paraffin block.

Light Microscopic Examination: One section from each sample was stained with Heamatoxylin and Eosin (H&E) stain by the standard method for light microscopic (histological) examination.

Ethical Approval: The study was conducted in compliance to the guidelines provided by the Research and Bioethics Committee of the Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria – the body that approved the study.

Statistics: Data were presented as Mean ± S.D and analyzed by the Student's *t*-Test using SPSS software package version 20. Significant difference was set at *P*=.05

RESULTS

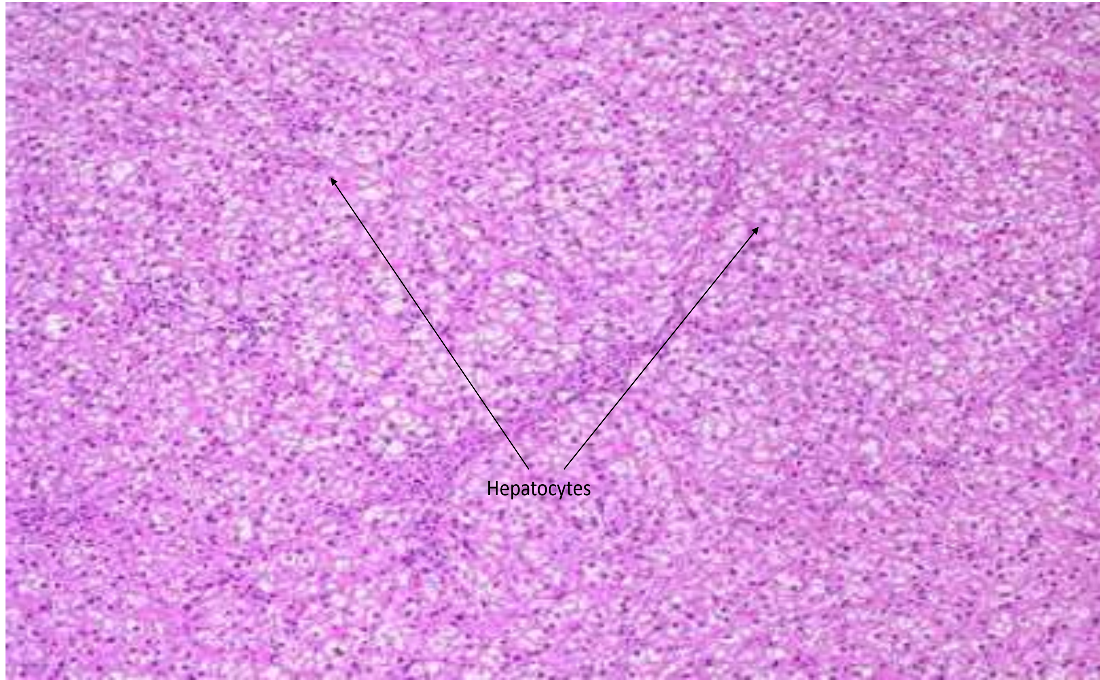
Results obtained from evaluation of median lethal dose (LD_{50}) and subchronic oral toxicity study of the ethanolic leaf extract of *Phyllanthus amarus* grown freely in uncultivated land space in Abraka, Ethiope East Local Government Area of Delta State, Nigeria, are shown in Tables 1-2 and Figures 1-2.

Table 1 shows the cage side physical observations of the control and experimental mice used in the determination of LD_{50}, while, Table 2 presents the biochemical data (TAC and MDA) obtained from both serum and liver tissues of the animals used to assess subchronic oral toxicity. Then, Figures 1-2 are the histological features of the liver tissues excised from Control (Fig. 1) and *P. amarus* (300 mg/kg/d for 21 days) treated mice (Fig. 2).

Table 1: Cage side physical observations during the LD_{50} evaluation of *P. amarus* ethanolic leaf extract

	Considerations	Cage side physical observations after 24 hours and 21 days					
		2000 mg/kg		5000 mg/kg		Control (0 mg/kg)	
		24 hours	21 days	24 hours	21 days	24 hours	21days
1	Condition of fur	Normal	Normal	Normal	Normal	Normal	Normal
2	Skin appearance	Normal	Normal	Normal	Normal	Normal	Normal
3	Subcutaneous swelling	Nil	Nil	Nil	Nil	Nil	Nil
4	Abdominal distension	Nil	Nil	Nil	Nil	Nil	Nil
5	Eye dullness	Nil	Nil	Nil	Nil	Nil	Nil
6	Eye opacity	Nil	Nil	Nil	Nil	Nil	Nil
7	Pupil diameter	Normal	Normal	Normal	Normal	Normal	Normal
8	Colour/consistency of faeces	Normal	Normal	Normal	Normal	Normal	Normal
9	Teeth condition	Normal	Normal	Normal	Normal	Normal	Normal
10	Gait	Normal	Normal	Normal	Normal	Normal	Normal
11	Weight gain (%)	0.3	5.0	0.5	7.0	0.1	3.0
12	Mortality	0	0	0	0	0	0

Evidence from observations (Table 1) indicates that the LD_{50} of P. amarus crude ethanolic leaf extract is greater than 5000 mg/kg. Trial doses cannot be increased beyond 5000 mg/kg because that is the limit dose. Effective dose (ED_{50}) = 200 mg/kg. Hence, therapeutic index, TI (LD_{50} / ED_{50}) = 25.0



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129 **Fig. 1:** *Photomicrograph of liver tissue from control mouse showing normal hepatocytes.*
130 *Magnification ×100 (H & E stain).*
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132 **Table 2: Changes in total antioxidant capacity (TAC) and malondialdehyde levels (MDA)**
133 **induced by subchronic oral toxicity study of *P. amarus* crude ethanolic leaf**
134 **extract.**
135

Sample	Assay	Control	*P. amarus* (300 mg/kg/d)	P- value
SERUM	TAC (mM)	0.10±0.03	0.32±0.05*	.03
	MDA (µM)	40.33±3.36	21.02±1.59*	.02
LIVER	TAC (mM)	0.12±0.09	0.34±0.06*	.03
	MDA (µM)	41.89±2.27	4.67±4.04*	.01

136
137 *Data are presented as Mean ±SD for n=5*
138 **Significantly different from comparable control values at P<0.05*
139 *TAC-Total antioxidant capacity, MDA-Malondialdehyde.*
140 *The subchronic oral toxicity of P. amarus crude ethanolic leaf extract was studied by administering*
141 *300 mg/kg/d of the plant extract to experimental BALB/$_c$ mice for 21 days.*

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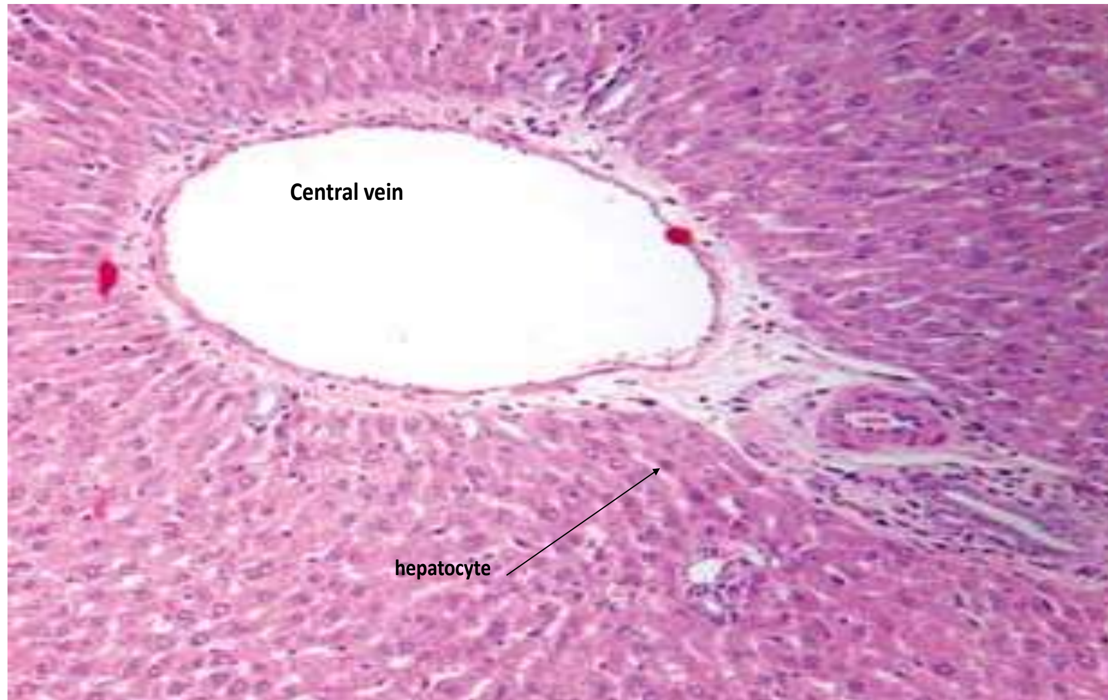

144 **Fig. 2:** *Photomicrograph of liver tissue obtained from mouse administered 300mg/kg body weight of*
145 *crude ethanolic leaf extract of P. amarus for 21 days, indicating normal histological features of*
146 *invigorated hepatocytes and central vein. Magnification × 100 (H & E stain).*

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148 DISCUSSION

149 This study attempted to evaluate the LD_{50} and subchronic oral toxicity of the crude ethanolic
150 leaf extract of *Phyllanthus amarus*. Result of the limit dose test indicates that the LD_{50} of *P. amarus*
151 crude ethanolic leaf extract is well above 5000 mg/kg with an ED_{50} of 2000 mg/kg and hence,
152 therapeutic index of 25. These observations show that the herb possesses very high phytotherapeutic
153 efficacy with no demonstrated toxicity. These findings suggest that *Phyllanthus amarus* is safe and
154 non-toxic with very high remedy potential in experimental mice. This agrees with previous documents
155 [38].
156 Chronic toxicity study identifies and provides information on drugs that could possibly cause
157 harm and pose health challenges [39]. The subchronic oral toxicity assessment of *P. amarus* crude
158 ethanolic leaf extract during this study, reveals that the extract significantly (P = *.03*) boosted
159 antioxidant defense activity in both blood and liver tissue with associated reduction (P = *.01*) in overall
160 membrane damage. The liver is the organ involved in several metabolic functions and is therefore
161 prone to xenobiotic-induced injury because of its central role in xenobiotic metabolism [40].
162 Histopathological examination of the liver shows that *P. amarus* administered at 300 mg/kg/d body
163 weight for 21 days invigorated liver cells. Hepatotoxic drugs could cause peroxidation of liver cell
164 membrane lipids and increase the amount of end products such as MDA [39].
165 Data suggest that *Phyllanthus amarus* extract has a measure of health benefits as shown by
166 the significant decrease in malondialdehyde (MDA) levels and associated increase in total antioxidant
167 capacity, TAC (Table 2). The decrease in malondialdehyde level may be as a result of the increased
168 antioxidant activities of *Phyllanthus amarus* [41]. Increased antioxidant activity in cells causes a
169 decrease in free radicals thereby reducing lipid peroxidation and malondialdehyde production. The
170 reduction in both blood and liver malondialdehyde levels suggests that the extract may contain
171 mixture of biomolecules with hydroxyl groups that perhaps prevented the abstraction of hydrogen
172 atom from the double bond of lipid bilayers thereby preventing lipid peroxidation. This suggestion
173 corroborates previous report on the *in vitro* analysis of the plant extract [42].
174 Phytochemical studies of *Phyllanthus amarus* extract have shown that the plant contains
175 chemicals such as flavonoids, tannins, saponins, alkaloids, terpenoids, glycosides and phenols
176 [42,21]. Flavonoids present in the plant have been shown to possess several pharmacological
177 properties such as antioxidant activities and anti-inflammatory activities [20,43]. Flavonoid as an
178 antioxidant has a rejuvenating effect on cells and tissues [44], Tannin has demonstrated high activities

179 against viral and bacterial infections as well as acting as strong antioxidant [45]. The antioxidant
180 activity of this plant phytochemicals may have contributed to the decrease in MDA levels observed in
181 this study. These findings are concurrent with previous studies conducted on the toxicological
182 assessment of *Phyllanthus amarus* [46].

183 **CONCLUSION**

184 Findings indicate that *Phyllanthus amarus* plant materials have no significant toxic effect in
185 Swiss albino mice.

186 **RECOMMENDATION**

187 Put together, the crude ethanolic leaf extract of *Phyllanthus amarus* is bestowed with very
188 high phytotherapeutic efficacy and vitalizing property with no recognizable toxic effect. Therefore, the
189 phytochemicals and nutrient quality of *P. amarus* need to be characterized for functional analysis.

190 **REFERENCES**

191 1. Idu M, Timothy O, Omogbai EKI, Ameachina F. Hypothensive effects and acute toxicity property
192 of methanol extract of Baissea axillaries Hau. J Biol Sci. 2008;8:675-678.
193 2. Nyika A. Ethical and regulatory issues surrounding African traditional medicine in the context of
194 HIV/AIDS. Dev World Bioeth. 2007;7:25-34.
195 3. Talalay P. The importance of using scientific principles in the development of medicinal agents
196 from plants. Academic Med. 2001;76(3):238-247.
197 4. Mazumder A, Mahato A, Mazumder R. Antimicrobial potentiality of *Phyllanthus amarus* against
198 drug resistant pathogens. Natural Product Res. 2006;20(4):323–326.
199 5. Tahseen M, Mishra G. Ethnobotany and Diuretic Activity of Some Selected Indian Medicinal
200 Plants. The Pharm Innovation. 2013;2:112.
201 6. Kloucek P, Polesny Z, Svobodova B, Vlkova E, Kokoska L. Antibacterial screening of some
202 Peruvian medicinal plants used in Calleria District. J Ethnopharmacol. 2005;99:309-312.
203 7. Tan W, Jaganath I, Manikam I. Evaluation of antiviral activities of four local Malaysian *Phyllanthus*
204 species against Herpes simplex viruses and possible antiviral target. Int J Med Sci.
205 2013;10(13):1817-1892.
206 8. Rajeshkumar NV, Joy KL, Kuttan G, Ramsewak RS, Nair MG, Kuttan R. Antitumor and
207 anticarcinogenic activity of *Phyllanthus amarus* extract. J Ethnopharmacol. 2002;81(1):17-22.
208 9. Joshi H, Parle M. Pharmacological evidence for antiamnesic potentials of *Phyllanthus amarus* in
209 mice. African J Biomed Res. 2007;10:165.
210 10. Lim Y, Murtijaya J. Antioxidant properties of *Phyllanthus amarus* extracts as affected by different
211 drying methods. Food Sci Technol. 2007;40(9):1664-1669.
212 11. Oluwafemi F, Debiri F. Antimicrobial Effect of *Phyllanthus amarus* and Parquetina nigrescens on
213 Salmonella typhi. African J Biomed Res. 2008;11(2):215-219.
214 12. Chandan S, Umesha S, Balamurugan V. Anti Leptospiral Antioxidant and DNA damaging
215 properties of Eclipta alba and *Phyllanthus amarus*. Open Access Scientific Reports. 2012;1(4):1-
216 8.
217 13. Manikkoth S, Deepa B, Joy AE, Rao S. Anticonvulsant activity of *Phyllanthus amarus* in
218 experimental animal models. 2011;4:144-149.
219 14. Evi PL, Degbeku K. Antidiabetic Activity of *Phyllanthus amarus* Schum and Thonn on Alloxan
220 induced diabetes in Male Wistar Rats. J Appl Sci. 2011;11(16):2968-2973.
221 15. Adeolu AA, Sunday OO. Antiinflammatory and analgesic activities of soft drink leaf extract of
222 *Phyllanthus amarus* in some laboratory animals. Br Biotech J. 2013;3:191-204.
223 16. Kumar K, Kultan R. Chemoprotective activity of an extract of *Phyllanthus amarus* against
224 cyclophosphamide induced toxicity in mice. Phytomedicine. 2005;12:494-500.
225 17. Raphael KR, Ajith TA, Joseph S, Kuttan R. Anti-mutagenic activity of *Phyllanthus amarus* in vitro
226 as well as in vivo. Teratog Carcinog Mutagen. 2002;22 285-291.
227 18. Obianime AW, Uchie FI. The phytochemical screening and the effects of methanolic extract of
228 *Phyllanthus amarus* leaf on the biochemical parameters of male guinea pigs. J Appl Sci
229 Environmental Management. 2008;12(4):73-77.
230 19. Pramyothin P, Ngamtin C, Poungshompoo S, Chaichantipyuth C. Hepatoprotective activity of
231 *Phyllanthus amarus* extract in ethanol treated rats: In vitro and *in vivo* studies. J Ethnopharmacol.
232 2007;114(2):169-173.
233 20. Kassuya CA, Silestre AA, Rehder V, Calixto JB. Anti-allodynic and anti-oedematogenic properties
234 of the lignin from *Phyllanthus amarus* in models of persistent inflammatory and neuropathic pain.
235 Eur J Pharm. 2003;478:145-153.
236 21. Onyesom I, Onumaedu IF, Ehiwario J, Dagana R. Antiplasmodial activity *Phyllanthus amarus*
237 preserves renal function. Eur J Medicinal Plant. 2015;5(1):109-116.

238 22. Onyesom, I, Adu, F. *Phyllanthus amarus*possesses malarial curative and pancreatic tonic
239 potentials in experimental mice. J Chem Pharm Res. 2015;7(5):7 – 15.
240 23. Boullata JI, Nace AM. Safety issues with herbal medicine. Pharmacother. 2000;20:257-269.
241 24. Saad B, Azaizeh H, Abu-Hijleh G, Said O. Safety of traditional Arab herbal medicine. Evidence
242 Based Complementary and Alternative Medicine. 2006;3:433-439.
243 25. Larrey D, Faure S. Herbal medicine hepatotoxicity: a new step with development of specific
244 biomarkers. J Hepatol. 2011;54:599-601.
245 26. Shaw D, Graeme L, Pierre D, Elizabeth W, Kelvin C. Pharmacovigilance of herbal medicine. J
246 Ethnopharmacol. 2012;140:513-518.
247 27. Colson CR, De Broe ME. Kidney injury from alternative medicines. Adv Chronic Kidney Dis.
248 2005;12:261-275.
249 28. Kwan TH, Tong MK, Leung KT, Lai CK, Poon WT, Chan YW. Acute renal failure associated with
250 prolonged intake of slimming pills containing anthraquinones. Hong Kong Med J. 2006;12:394–
251 397.
252 29. Zhu YP. Toxicology of the Chinese herb mu tong (*Aristolochia manshuriensis*). What history tells
253 us? Adverse Drug Reaction Toxicol Rev. 2002;21:171–177.
254 30. Moritz F, Compagnon P, Kaliszczak IG, Kaliszczak Y, Caliskan V, Girault C. Severe acute
255 poisoning with homemade Aconitum napellus capsules: toxicokinetic and clinical data. Clin
256 Toxicol. 2005;43:873–876.
257 31. Gaibazzi N, Gelmini GP, Montresor G, Canel D, Comini T, Fracalossi C *et al*. Long QRS
258 tachycardia secondary to Aconitum napellus alkaloid ingestion. Ital Heart J Suppl. 2002;3:874–7.
259 32. Ernst E. Herbal Medicines: balancing benefits and risk. Novarties Foundation Symposium.
260 2001;282:154-167.
261 33. Benjamin J, Muir T, Briggs K, Pentland B. A case of cerebral haemorrhage - can Ginkgo biloba be
262 implicated? Postgrad Med J. 2001;77:112–113.
263 34. Jensen WI, Allen JP. Naturally occurring and experimentally induced castor bean (Ricinus
264 communis) poisoning in ducks. Avian. Dis. 1981;5:184-94.
265 35. Bruce RD. An up-and-down procedure for acute toxicity testing. Fundam Appl Toxicol.
266 1985;5(1)151-157.
267 36. Miller NJ, Johnston JD, Collis CS. Serum total antioxidant activity after myocardial infarction.
268 Annals Clin Biochem. 1993;34: 85-90.
269 37. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxidation in animal tissues by thiobarbituric
270 acid reaction. Annals Biochem. 1979;95:351-358.
271 38. Shirish S P, Shrikant SS. Acute Toxicity Study of *Phyllanthus amarus*. Int J Pharm Sci Rev Res.
272 2011;9(1):81-84.
273 39. Kumar G, Sharmila BG, Vanitha PP, Sundararajan M, Rajeskara PM. Hepatoprotectetive activity
274 against of Trriantherma portulacastrum L. against paracetamol and thioacetamide intoxication in
275 albino rats. J Ethnopharmacol. 2004;92:37-40.
276 40. Sturgill MG, Lambert GH. Xenobiotics-induced hepatotoxicity; Mechanism of Liver injury and
277 method of monitoring hepatic function. J Clin Chem. 1997;43:1512-1526.
278 41. Faremi TY, Suru SM, Fafunso MA, Obiola UF. Hepatoprotective potentials of *Phyllanthus amarus*
279 against ethanol-induced oxidative stress in rats. Food Chem Toxicol. 2008;4(1):41-48.
280 42. Chandewar A, Dhongade H. Pharmacognostical phytochemical studies of *Phyllanthus amarus*
281 leaves. Int J Biomed Adv Res. 2013;4:383-389.
282 43. Adeneye AA, Benebo AS, Agbaje EO. Protective effect of the aqueous leaf and seed extract of
283 *Phyllanthus amarus* on alcohol-induced hepatotoxicity in rats. West Africa J Pharmacol Drug Res
284 2006;22(3):42-50.
285 44. Foo LY. Amariinic acid and related ellagitanins from *Phyllanthus amarus*. J Phytochem.
286 1995;39(8):217-224.
287 45. Maryam J, Bushra M, Abida Y, Mir AK. Pharmacological activities of selected plant species and
288 their phytochemical analysis. J Med Plants Res. 2012;6(37):5013-5022.
289 46. Calixto JB, Santos ARS, Cechinel-Filho V, Yunes RA. A Review of the plant of the genus
290 *Phyllanthus*: Their Chemistry, Pharmacology and Therapeutic potential. Med Res Rev.
291 1998;18:225-258.
292