IJPSR (2012), Vol. 3, Issue 09 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 24 May, 2012; received in revised form 26 June, 2012; accepted 23 August, 2012

AN EVALUATION OF ACUTE AND SUBCHRONIC TOXICITIES OF A NIGERIAN POLYHERBAL ANTIDIABETIC REMEDY

H.I. Obi [1], E.E. Ilodigwe [1], D.L. Ajaghaku*[1], J.M. Okonta [2]

Department of Pharmacology and Toxicology [1], Department of Clinical Pharmacy [2], Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Agulu Campus, Anambra state, Nigeria

Keywords:
Polyherbal therapy,
Toxicity,
Diabetes mellitus,
Gongrenema latifolium,
Allium cepa,
Biochemical parameters,
Haematological parameters

Correspondence to Author:

D. L. Ajaghaku

Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Agulu Campus, Anambra state, Nigeria

E-mail: danlotaaja@yahoo.com

ABSTRACT

The acute and subchronic toxicities of Nigerian polyherbal antidiabetic remedy, prepared from *Gongrenema latifolium* and *Allium cepa* (1:1) leaves was investigated in Swiss albino mice and rats of both sexes. For acute toxicity study, 1000-5000mg/kg of the combined extract were administered orally to rats and observed for 24 hours for obvious toxic symptoms and mortality. The median lethal dose of the combined extract (LD_{50}) was estimated. In subchronic study, 1000-4000mg/kg of the combined extracts were orally administered daily for 90 days. Weight changes and selected biochemical and hematological parameters were determined periodically. At the end of the experiment, the liver tissues were harvested for histopathological examination. The estimated LD_{50} of the combined extracts was above 5000mg/kg. There was no sign of weakness, anorexia and no significant reduction in body weight. There were non- significant ($p>0.05$) effects on hemoglobin concentration and PCV. There were transient significant ($p<0.05$) increases in ALT and AST. Liver cells photomicrographs at the 91st day showed slight changes in histo-architecture of the hepatocytes at 4000mg/kg. These results suggest that combined aqueous extracts of *Gongrenema latifolium* and *Allium cepa* (1:1) is safe in the treatment of diabetes mellitus.

INTRODUCTION: The use of medicinal plants in the treatment of variety of ailments is widely practiced throughout the world [1, 2]. About eighty percent of the world population rely on herbal medicine for treatment of various diseases [3, 4].

Polyherbal therapy the combination of two or more plant products can be used to treat more than one disease condition [5]. Herbal medicine practitioner, use combination of different plant products to treat diabetes mellitus [6]. Studies have shown that poly-herbal therapy have pharmacological agents that produce enhanced therapeutic efficacy with minimal side effects [7]. Most of these herbal recepies are used to treat various ailments over a period. Studies have shown that prolonged use of herbal medicine is associated with toxic effects [8, 9].

Gongrenema latifolium Benth, (*Asclapiadaceae*) called "utazi" in the South Eastern part of Nigeria possess hypoglycemic activity [10, 11] and so used in the treatment of diabetes mellitus.

QUICK RESPONSE CODE

IJPSR:
ICV- 4.57

Website:
www.ijpsr.com

It also possesses hypotensive, hypolipidermic [12, 13, 14], antioxidant [10, 13] and anti-inflammatory activities [15]. Also *Allium cepa* Linn (*Liliaceae*) known as onion is grown in the Northern Nigeria. It has been shown to possess antioxidant [16], hypoglycemic [17] and hypo-lipidemic properties [18]. In the rural communities of the South East of Nigeria, the combined aqueous leaf decoctions of *Gongrenema latifolium* and *Allium cepa* in different proportions are popular among traditional healers in the management of type II diabetes.

Despite the popular use of this polyherbal antidiabetic remedy, no information exists about its safety. Since diabetes is a chronic disorder, this study evaluated the acute and subchronic toxicities of the aqueous polyherbal antidiabetic remedy.

MATERIALS AND METHODS.

Plant Materials: Leaves of *Gongronema latifolium* and bulbs of *Allium cepa* were collected from local market in Onitsha, Anambra state, Nigeria, during the month of February 2011. These plant materials were authenticated by a taxonomist, Mr. Alfred Ozioko of Bioresource Development and Conservation Project, Nsukka, Enugu State, Nigeria at the department of Botany, Nnamdi Azikiwe university, Awka.

Preparation of Aqueous Extracts: Dried leaves of *Gongronema latifolium* and bulbs of *Allium cepa* were reduced to coarse powder using domestic grinder. The dried powder of the plant materials (500g each) were separately macerated with 2 litres of distilled water for one hour. The mixtures were filtered cold through muslin cloth. Filterates were concentrated and dried using freeze drier to yield constant weights of 48.9g and 38.40g respectively. The extracts were stored till further use. During administration, the crude extracts were redissolved in normal saline.

Acute Toxicity (LD_{50}): Thirty albino mice were divided into six groups of 5 mice per group after overnight fasting. Mice in groups 1-5 received oral doses of the combined aqueous extracts of *Gongronema latifolium* and *Allium cepa* (in the ratio of 1:1) at 2000, 3000, 4000, 5000 and 6000mg/kg. Mice in group 6 received normal saline. The animals were observed for 24 hours for obvious toxic symptoms and mortality.

The median lethal dose of the combine extract (LD_{50}) was estimated using up and down method [19].

Subchronic Toxicological Studies: Sixty rats were randomly divided into four groups of 15 rats per group. After pretreatment studies, groups 1-3 received (1000, 2000, and 4000mg/kg respectively) oral doses of the combined aqueous extracts of *Gongronema latifolium* and *Allium cepa*. Group 4 served as control and received normal saline (oral). Toxic manifestations and mortality were monitored daily and the body weight changes were recorded every 7 days.

At the 91st day, blood was collected from the retro-orbital puncture into two tubes, one with the anticoagulant ethylenediamine-tetraacetate (EDTA), and the other without the anticoagulant additive. The blood without the anticoagulant was allowed to clot before centrifugation (4000 rpm for 10 minutes) to obtain the serum which was analyzed for biochemical parameters: blood urea nitrogen (BUN) [20], alanine aminotransferase (ALT) and aspartate amino-transferase (AST) [21]. The anticoagulated blood was analyzed immediately for hematological parameters: packed cell volume (PCV) and hemoglobin concentration (Hb) [22]. At the end of the experiment, the liver and kidney tissues were harvested for histopathological examination.

Statistical Analysis: Significant difference between the control and experimental groups of animals were assessed by student's t-test using SPSS statistical program. All data were expressed as Mean±SEM. P values< 0.05 were considered significant.

RESULTS AND DISCUSSION: No death was recorded in the acute toxicity testing for all the animals that received 1000-6000 mg/kg of the combined extracts. No sign of weakness or anorexia was observed within 24 hours of post-administration and there was no significant (P>0.05) change in the body weight of the animals (**Table 1**) during the sub-chronic administration of the poly-herbal mixture. *Allium cepa* have been reported to produce heamolytic anemia in some animal species like sheep [23] and dog [24] owing to their high content of organosulfoxides which are metabolized to highly reactive oxidants [25].

However, the combined extract of *A. cepa* and *G. latifolium* did not produce signigicant decrease (p>0.05) in heamoglobin (**Table 2**) and PCV (**Table 3**). The non elevation of these heamatological parameters might be as a result of anti-lipid peroxidative [26] and free redical scarvenging activities [27] of *G. latifolium* which might have annulled the heamolitic ability of *A. cepa.* The non signigicant effects on these heamatological parameters indicates the unlikelihood of the polyherbal remedy to cause anemia [22].

G. latifolium have been reported to have hepatoprotective activity [28]. The combined extract however produced significant (p<0.05) increase in ALT at 2000-4000 mg/kg at the 31st day and at 2000 mg/kg in the 61st day (**Table 5**). Also a signigicant increase in AST at 2000 mg/kg at the 91st day was recorded (**Table 4**). Increased serum ALT and AST reflect major liver permeability, congestion or cell rupture [5].

The significant elevation noted for AST and ALT were transient. However, photomicrograph of liver at 4000 mg/kg at the 91st day of dosing of the polyherbal remedy indicates slight changes in the histo-architecture of the hepatocytes (**Fig. 4**). The gram equivalent of 4000 mg/kg in an average adult man would translate to 280,000mg/kg dose of the poly-herbal remedy. This is a very high value and makes the preparation safe for use. There was non significant (p>0.05) elevation of serum blood urea nitrogen (BUN) (**Table 6**), an indication that the polyherbal remedy is unlikely to cause kidney damage [29].

TABLE 1: EFFECTS OF EXTRACT ON BODY WEIGHT

	Body weight changes (g)			
Treatment (mg/kg)	**Pre-treatment**	**31st day**	**61st day**	**91st day**
1000	114.92± 14.95	127.50 ±10.71	129.94± 23.53	149.76± 28.33
2000	110.32± 6.50	103.32± 10.82	100.94± 8.63	105.88± 18.89
4000	128.92± 25.01	120.18 ±25.25	109.12± 19.61	119.54± 22.09
control	102.82 ±13.87	107.86 ±12.03	111.30± 12.63	125.98± 14.49

Dose of extract in mg/kg, n/gp = 5

TABLE 2: EFFECTS OF EXTRACT ON HEMOGLOBIN CONCENTRATION

	Hemoglobin Concentration (g/dl)			
Treatment (mg/kg)	**Pre-treatment**	**31st day**	**61st day**	**91st day**
1000	11.00± 1.91	12.29± 1.68	11.48± 0.92	10.14± 2.24
2000	10.20±2.00	9.46± 1.37	10.76± 1.76	12.22± 1.87
4000	11.28± 0.66	10.06± 1.27	9.86± 0.98	11.74± 1.05
control	10.14 ±1.41	10.02± 1.42	9.46± 3.27	9.40± 4.82

Dose of extract in mg/kg, n/gp = 5

TABLE 3: EFFECTS OF EXTRACT ON PACKED CELL VOLUME (PCV)

	Packed Cell Volume (%)			
Treatment (mg/kg)	**Pre-treatment**	**31st day**	**61st day**	**91st day**
1000	33.00 ± 5.76	36.80 ± 5.04	34.40 ± 2.73	30.40 ± 6.83
2000	30.60 ± 5.99	28.40 ± 4.13	32.20 ± 5.27	36.60 ± 5.61
4000	33.80 ± 1.94	30.20 ± 3.87	29.60 ± 2.87	35.20 ± 3.19
control	30.40 ± 4.22	30.00 ± 4.24	31.27 ± 3.27	32.39± 4.82

Dose of extract in mg/kg, n/gp = 5

TABLE 4: EFFECTS OF EXTRACT ON SERUM ASPARTATE AMINOTRANSFERASE (AST)

	Serum Aspartate Aminotransferase (U/L)			
Treatment (mg/kg)	**Pre-treatment**	**31st day**	**61st day**	**91st day**
1000	187.0±121.10	157.02±57.58	108.7±38.91	70.70±16.98
2000	203.0±82.44	174.76±20.57	109.94±54.06	84.04±30.52*
4000	188.0±127.26	99.26±58.20	74.10±43.41	52.60±8.75
control	136.0±54.63	218.6±19.02	87.66±38.98	58.96±25.34

Dose of extract in mg/kg, n/gp = 5

TABLE 5: EFFECTS OF EXTRACT ON SERUM ALANINE AMINOTRANSFERASE (ALT)

Serum Alanine Aminotransferase (U/L)				
Treatment (mg/kg)	**Pre-treatment**	**31st day**	**61st day**	**91st day**
1000	42.32±16.88	80.78±42.98	56.10±32.32	30.07±8.40
2000	41.44±16.24	111.52±5.61*	82.84±19.47*	28.00±12.61
4000	38.80±20.17	108.4±7.68*	40.10±16.98	26.09±4.80
control	23.62±18.69	81.62±49.72	53.00±42.65	15.20±3.97

Dose of extract in mg/kg, n/gp = 5

TABLE 6: EFFECTS OF EXTRACT ON BLOOD UREA NITROGEN (BUN).

Blood Urea Nitrogen (mg/dl)				
Treatment (mg/kg)	**Pre-treatment**	**31st day**	**61st day**	**91st day**
1000	47.88±7.30	60.16±14.64	63.24±19.35	59.36±17.07
2000	56.02±12.51	50.16±31.31	66.32±19.50	64.16±15.98
4000	54.30±7.75	46.08±7.36	47.01±9.89	52.04±7.33
control	37.26±17.30	48.08±16.90	48.80±17.88	49.10±20.08

Dose of extract in mg/kg, n/gp = 5

Histopathological Result:

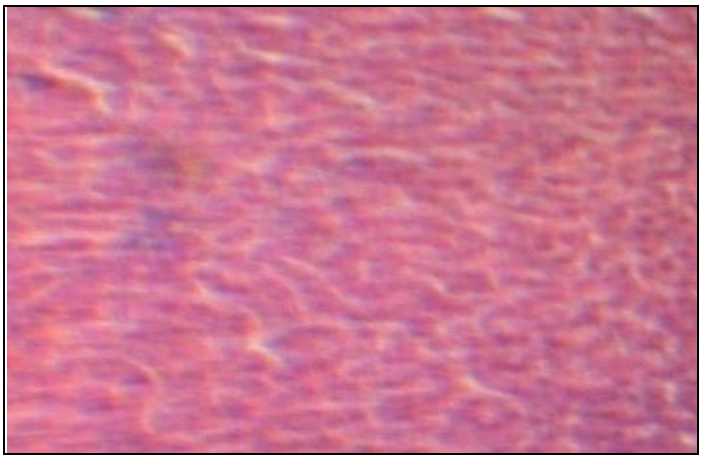

FIGURE 1: HISTOLOGICAL SECTION OF THE LIVER THAT SERVED AS CONTROL SHOWING NO HEPATIC INJURY

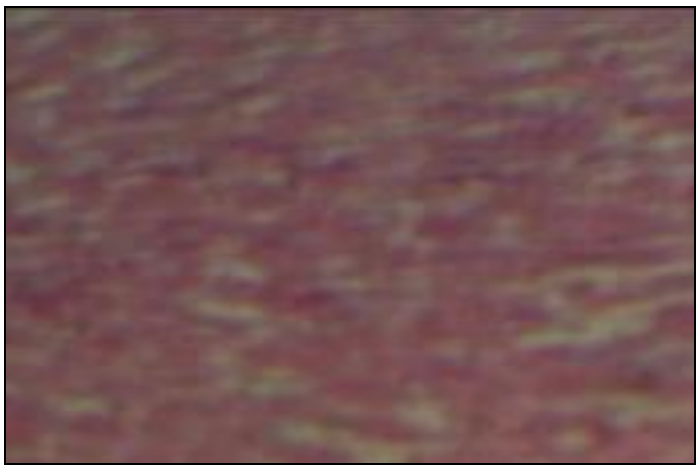

FIGURE 3: HISTOLOGICAL SECTION OF THE LIVER AT DOSE 2000mg/kg FOR THE 3RD MONTH SHOWING NO DAMAGE TO THE LIVER.

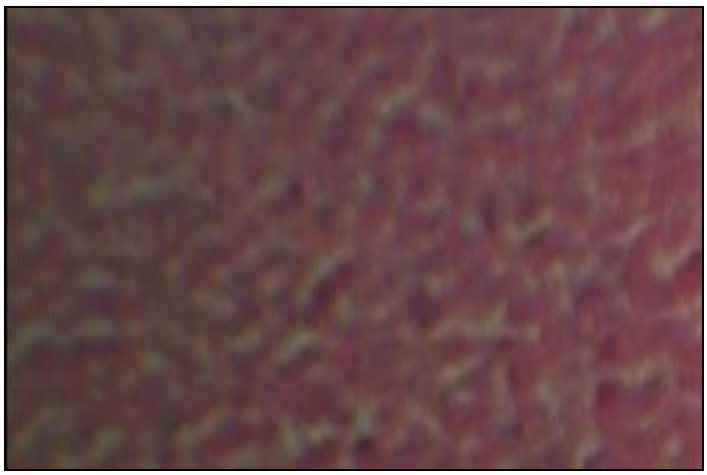

FIGURE 2: HISTOLOGICAL SECTION OF THE LIVER AT DOSE 1000mg/kg FOR THE 3RD MONTH SHOWING NO DAMAGE TO THE LIVER

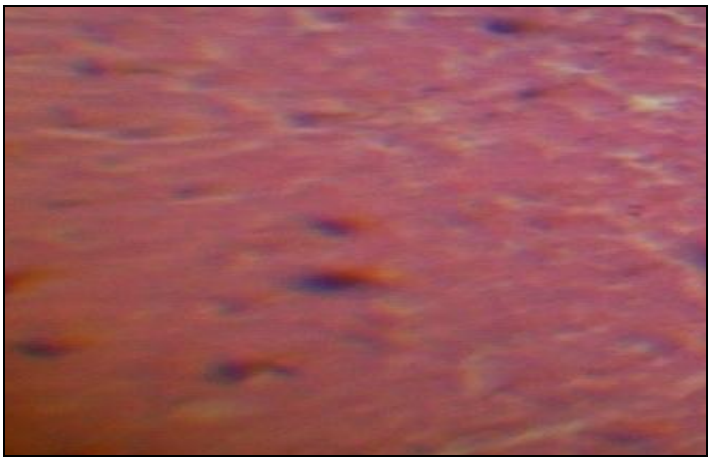

FIGURE 4: HISTOLOGICAL SECTION OF THE LIVER AT DOSE 4000mg/kg FOR THE 3RD MONTH SHOWING MILD HEPATIC DAMAGE

CONCLUSION: Based on the findings from the acute and sub-chronic toxicity of the combined extract of *A. cepa* and *G. latifolium*; their use as a polyherbal antidiabetics remedy is encouraged however, other toxicological parameters need to be evaluated to ascertain their overall toxicological status.

REFERENCES:

1. Hostettmann K, Marston A, Ndjoko K and Wolfender J. The pontential of African plants as a source of drug. Curr. Org. Chem 2000., 4: 973-1010.
2. Ahmad I, Agil F and Owais M. Modern phytomedicine: Turning medicinal plants into drugs. West-Sussex England: John Wiley and Sons 2006, pp 2-22.
3. Akah PA. Indigenous knowledge and medical practice. In: Ethnopharmacology, Akah PA Edn, Research Signpost, Kerela, india 2008. pp 1-13.
4. Ogbonnia S, Adekunle AA, Bosa MK and Enwuru VN. Evaluation of acute and subacute toxicity of Alsonia congensis Engler (Apocynaceae) back and Xylopia aethiopica (Dunal) A. Rich (Annonaceae) fruits mixtures used in the treatment of diabetes. Afr. J. Biotechnol 2008., 7(6):701-705.
5. Pieme CA, Pendlap VN, Nkegoum B, Taziebou CL, Tekwu EM, Etoa FX and Ngonggang J. Evaluation of acute and subacute toxicities of aqueous ethanolic extract of leaves of Senna alata (L) Roxb Ceasalpiniaceae. Afr. J. Biotechnol 2006. 5(3): 283-289.
6. Ilonzo FIN. . Herbs and your health: Questions and answers. MasterPRINT (Nig) 2006, pp 41-43.
7. Tiwari AK and Rao JM. Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. Curr. Sci 2002., 84(1): 30-37.
8. Tedong L, Dzeufiet PDD, Dimo T, Asongalem EA, Sokeng SN, Flejou JF, Callard P and Kamtchouing P. Acute and subchronic toxicity of Anacardium occidentale Linn (Anacardiaceae) leaves hexane extract in mice. Afr. J. Tradit. Altem. Med 2008., 4(2): 140-147.
9. Ilodigwe EE, Akah PA and Nworu CS. Evaluation of the acute and subchronic toxicities of Spathodea campanulata P. Beauv. International J. Applied research in natural products 2010, 3(2):17-21.
10. Ogundipe OO, Moody JO, Akinyemi TO and Raman A. Hypoglycemic potentials of methanolic extracts of selected plant foods in alloxanized mice. Plant Foods Hum Nutr 2003. 58(3): 1–7.
11. Ugochukwu NH, Babady NE, Cobourne M and Gasset SR. The effect of Gongronema latifolium extracts on serum lipid profile and oxidative stress in hepatocytes of diabetic rats. J. Biosci 2003., 28: 1–5.
12. Ugochukwu NH and Babady NE. Antihyperglcemic effect of aqueous and ethanolic extracts of Gongrenema latifolium leaves on glucose and glycogen metabolism in livers of normal and streptozotocininduced diabetic rats. Life Sci 2003., 73 (150): 1925-1938.
13. Nwanjo HU. Effect of aqueous extract of Gongrenema latifolium leaves on blood glucose level of rats. Alvana J. Sci 2005., 1 (5): 84-89.
14. Nwanjo HU and Alumanah EO. Effect of aqueous extract of Gongrenema latifolium leaves on some indices of liver function. blood glucose level of rats antioxidant. Global J. Med. Sci 2005., 4 (1): 29-32.
15. Morebise O, Fafunso MA, Makinde JM, Olaetjide OA and Awe EO. Anti-inflammatory property of Gongrenema latifolium. Phytother. Res 2002., 16: 575-577.
16. Dhanprakash BN and Garima U. Antioxidant and free radical scavenging activities of phenols from onion (Allium cepa). Food Chemistry 2007, 102: 1389-1393.
17. Compos KE, Diniz YS, Cataneo AC, Faine LA, Alves MJ and Nobeli EL. Hypoglycemic and antioxidant effect of onion (Allium cepa) on diabetic rats. Int. J. Food Sci. Nutr 2003., 54: 241-6.
18. Lata S, Saxena KK, Bhasin V, Saxena RS, Kumar A and Shrivastva VK. Beneficial effect of Allium sativum, Allium cepa and Comphora mukul on experimental hyperlipidemia and arthrosclerosis- A comparative evaluation. Indiann J. Pharmacol 1991., 37: 132-5.
19. Bruce RD. An up-and-down procedure for acute toxicity testing. Fundamental Applied Toxicology 1985, 5: 151-157.
20. Tietz NM. Textbook of clinical chemistry. 2nd End. W.B Saunders Company, Piladelphia, PA 1994, pp 703.
21. Reitman S and Frankel S. A calorimetric method for determination of serum glutamic oxaloacetic acid and glutamic pyruvic transaminases. Am. J. Clin. Pathol 1957., 28: 56-63.
22. Kelly WR. Veterinary clinical diagnosis. Balliere Tindall, London 1977, pp 271-282
23. Aslani MR, Mohri M, Movassaghi AR. Heinz body anaemia associated with Onion (*Allium capa*) toxicosis in flock of sheep. Comp Clin Pathol 2005; 14 (2): 118-120
24. Tang X, Xia Z, Yu J. An experimental study of hemolysis induced by Onion (*Allium cepa*) poisoning in dogs. J Vet Pharmacol Ther 2008; 31 (2); 143-149.
25. Amagase H, Petesch BL, Matsuura H, Kasuga S and Itakura Y. Intake of garlic and its bioactive components. J Nutr 2001; 131: 955s-962s.
26. Nwanjo HU, Okafor MC and Oze GO. Anti-lipid peroxidative activity of *G. latifolium* in streptozotocin-induced diabetic rats. Nigerian Journal of Physiological Sciences 2006; 21 (1-2): 61-65
27. Gupta SK, Prakash J and Srivastava S. validation of traditional claim of Tulsi, *Ocimum Sanctun* Linn as a medicinal plant. Ind J Exp Biol 2002; 40:765-773.
28. Nnodim J and Emejulu A. The protective role of *Gongronema latifolium* in acetaminophen induced hepatic toxicity in wister rats. Asian Pasific Journal of Tropical Biomedicine 2011; 151-154
29. John Timbrell: A text book of Principles of Biochemical Toxicology. Taylor and Francis publishers, third edition, 2000.

How to cite this article:
Obi HI, Ilodigwe EE, Ajaghaku DL, Okonta JM: An Evaluation of Acute and Subchronic Toxicities of a Nigerian Polyherbal Antidiabetic Remedy. *Int J Pharm Sci Res*, 2012; Vol. 3(9): 3131-3135.