IJIRR

International Journal of Information Research and Review
Vol. 04, Issue, 08, pp.4369-4372, August, 2017

RESEARCH ARTICLE

SERUM PROTEIN AND ENZYME LEVELS IN RATS FOLLOWING CO-ADMINISTRATION OF ETHANOLIC LEAF EXTRACT OF *AGERATUMCONYZOIDES*AND NIGERIAN BONNYLIGHT CRUDE OIL

***Ita, S. O. and Udotung, Y. C.**

Departments of Physiology, University of Uyo, Uyo, Nigeria

ARTICLE INFO

Article History:

Received 27th May, 2017
Received in revised form
24th June, 2017
Accepted 02nd July, 2017
Published online 30th August, 2017

Keywords:

Ageratum conyzoides,
Rats, Toxicological effect.

ABSTRACT

Background: Many environmental insults through accidental discharge, pollution of various kinds, industrial and work place exposure including NBLCO can on exposure inflict dangerous injury on organ and tissue of the body including the liver. And in such cases hepatocellular injury can ultimately cause hepatocellular damage to compromise the synthetic function of the liver as well as liberating ALT and AST to flood the extracellular compartment. A search for cheaper and readily available substance(s) with efficient potentials to ameliorate the harmful effects of these insults is novel. Many herbaceous plants have been reported to possess such property. This study is design to evaluate the ameliorating potentials of *Ageratum conyzoides* against NBLCO hazardous effects on the liver.

Materials and methods: Twenty female Wistar rats (120-150g body weight) were divided into four groups of five rats each. The rats in group I served as the control group and were oral gavaged 3ml/kg of normal saline; group II gavaged 748.33mg/kg body weight of the extract of *A. conyzoides*, which was 20% of the LD_{50} (3741.66mg/kg). This dose was calculated as 20% of the lethal dose of 14.14 ml/kg. Group IV animals were gavaged 748.33mg/kg body weight of the extract of *A. conyzoides*, and 3ml/kg body weight of NBLCO. In all cases, doses were applied daily for 31 days according to animal's most recent body weight.

Results: The results showed that NBLCO significantly increase serum albumin, globulin, ALT, AST and ALP compared with control group ($P<0.05$). Co-administration of leaf extract of *A. conyzoides*with NBLCO caused significant reduction in the aforementioned parameters when compared with crude oil group ($p<0.05$).

Conclusion: It is concluded that ethanol leaf extract of *Ageratum conyzoides* ameliorates effects of NBLCO in rats.

INTRODUCTION

The liver is pivotal to the normal development, synthetic, metabolic balance and physiological balance such that alteration in hepatic function potent great consequences to the body.Determination of serum protein including albumin is an index in assessing synthetic function of the liver as plasma proteins are synthesized in the liver. Furthermore, the assessment of ALT and AST can be used to diagnose a wide spread organic damages. ALT occur in the cytosol of the liver, while AST has cytosolic and mitochondrial forms and is present in various tissues including the liver, heart, skeletal muscle, kidneys, brain, pancreas and indeed blood cells (Batzakis and Briere, 1979).

****Corresponding author: Ita, S. O.,***
Departments of Physiology, University of Uyo, Uyo, Nigeria

Hepatic damage is usually associated with elevated serum ALT, AST and bilirubin concentration (Kew, 2000; Green and Flamm, 2002; Collier and Bassendine, 2002). Many environmental insults through accidental discharge, pollution of various kinds, industrial and work place exposure including NBLCO can on exposure inflict dangerous injury on organ and tissue of the body. And such hepatocellular injury can ultimately cause hepatocellular damage to compromise the synthetic function of the liver as well as liberating ALT and AST to flood the extracellular compartment. The modern world is technologically driven and quest for industrial expansion remain unabated and as such the emission of these insults with tendency to cause tissue and cellular injury will remain unabated for a long time. A search for cheaper and readily available substance(s) with efficient potentials to ameliorate the harmful effects of these insults is novel. Many herbaceous plants have been reported to possess such property; among this

group of herbaceous plant with therapeutic potentials is *Ageratum conyzoides*. *Ageratum conyzoides* is an herbaceous plant used in several countries of the world for its medicinal and therapeutic purposes. The use of this herb as a bacteriocide, antidysentric and antibiotic has been reported forcommunities in Asia, Africa, India and South America (Almagboul, 1985; Ekundayo *et al*, 1988; Borthakar and Baruah, 1987). In some local communities in Nigeria, the leaves of *Ageratum conyzoides*are used in the management of fever, pneumonia and rheumatism. Various parts of this plant contain many secondary metabolites (Kong *et al*, 2004) which are responsible for its reported medicinaland therapeutic effects. The ethanolic extract of the roots and aerial parts of *A. conyzoides* have been reported to exhibit gastroprotective activity via Ca^{2+} channel blocking and antiserotogenic properties (Achola *et al*., 1994). Ita *et al* (2005) have reported the ability of the aqueous leaf extract of *A. conyzoides* to stimulate gastric acid secretion. In another study Ita *et al* (2007) have also reported the advantageous effect of the ethanolic leaf extract on haematological indices of rats as a blood booster. The medicinal and therapeutic benefits of *A. conyzoides* have been attributed to its phenolic content. The hydroxytyrosol content of this plant can prevent cardiovascular disease by reducing the expression of adhesion molecules on endothelial cells and preventing the oxidation of low density lipoprotein (Rafehi *et al*., 2012).

One of the organs usually affected by ingestion of xenobiotic insults because of its cetral role in synthetic, metabolic and detoxification activities is the liver. Generally, hepatic injuryis often associated with alterations in the serum and liver levels of some enzymes notably ALT, AST and ALP (Whitby *et al*, 1984) and studies with medicinal plant extracts have shown the varying effects of phytochemicals on serum and liver enzyme levels. While some phytochemicals are hepatotoxic, others are hepato-protective. Many researchers in this fields have reported changes in ALT, AST, ALP, GGT (gamma glutamyl transferase) activities in animals treated with plant extracts (Nada *et al*. 1997; Udosen and Ojong, 1998, Bumah *et al*, 2005 Akpanabiatu *et al*, 2005). This study is design to evaluate the ameliorating potentials of *Ageratum conyzoides* against NBLCO hazardous effects on the liver.

MATERIALS AND METHODS

The crude petroleum used in this study was obtained from the Exxon Mobil laboratory, Ibeno, Nigeria.

Collection of plant material

The whole plant was obtained from the Botanical farm of the Department of Pharmacognosy and Natural Medicine, University of Uyo, Uyo, Nigeria. Specimen of the leaves was authenticated by Dr. (Mrs.) Uduak Aniema Essiett of the Department of Botany and Ecological Studies, University of Uyo, Uyo. A voucher specimen (UUH 3517) was deposited at the Herbarium.

Preparation of leave extract

The leaves of *A. conyzoides* were rinsed with distilled water and dried under shade. The dried leaves were ground into powder with an electric blender. Four hundred grammes of the blended leaves sample was macerated in 700ml 70% ethanol, agitated for 10 minutes with an electric blender and left overnight in a refrigerator at 4^{o}C. The mixture was filtered with a cheese cloth and the filtrate obtained concentrated under reduced pressure using a rotary evaporator (at 37^{o}C) to about 10% of its original volume. The concentrate was then allowed in a water bath at 37^{o}C for complete evaporation to dryness yielding 40.64g (10.15%) of the extract.

Acute toxicity test

Acute toxicity study (LD_{50}) was estimated using Lorke's method (Lorke, 1993). A total of 25 mice weighing between 15-22g were divided into five groups with five mice per group. Mice in the five groups were administered 3000mg/kg, 3500mg/kg, 4000mg/kg, 4500mg/kg and 5000ml/kg of body weight respectively (intraperitoneally). All experimental animals were observed for physical signs of toxicity such as gasping, palpitation, writhing, decreased respiratory rate, body limb and death after 24 hours. The median lethal dose of *Agerantum conyzoides* was calculated as geometrical means of the maximum (most tolerable) dose producing 0% mortality (a) and the minimum (least tolerable) dose producing 100% mortality (b) using the formula:

$LD_{50}= \sqrt{ab}$

$LD_{50}= \sqrt{3500 \times 4000}$

$= 3741.66mg/kg$

The acute toxicity test for the NBLCO also involved 25 mice weighing between 15-22g were divided into five groups with five mice per group. Mice in the five groups were administered intraperitoneally 10ml/kg, 15ml/kg, 20ml/kg, 25ml/kg and 30ml/kg of body weight respectively.

$LD_{50}= \sqrt{10 \times 20}$

$= 14.14ml/kg$

Experimental animals

Female Albino Wistar rats weighing between 150-180g were obtained from the Animal House of the Faculty of Basic Medical Sciences University of Uyo, Uyo, Nigeria and were kept in a well-ventilated section of the Animal House. They were allowed access to feed (Chow: vital feeds, Grand Cereals Ltd, Jos) and water *adlibitum*. The animals were kept in separate experimental room and allowed to acclimatize for a period of one week before commencement of studies.

Experimental design and treatment of animals

A total of twenty (20) adult female AlbinoWistar rats were randomly divided into four groups (group I, II, III and IV) of five (5) rats each. Group I served as the control and was oral gavaged 3 ml/kg body weight of normal saline. Group II was oral gavaged 748.33mg/kg body weight of ethanolic leaf extract of *Ageratum conyzoides*, this dose was calculated as 20% of the lethal dose (LD_{50}) of 3741.66mg/kg. Group III was oral gavaged 3 ml/kg body weight of NBLCO. This dose was calculated as 20% of the lethal dose (LD_{50}) of 14.14 ml/kg,

while group IV in addition to 3 ml/kg body weight of NBLCO, were supplemented with 748.33 mg/kg body weight of ethanolic leaf extract of *Ageratum conyzoides*. In all cases, the doses were based on the rat's most recently recorded body weight. The calculated volume in milliliter (ml) was applied daily for thirty one (31) days. The experimental procedures involving the animals and their care were conducted in conformity with the approved guidelines by the Research and Ethical Committee of the Faculty of Basic Medical Sciences, University of Uyo, Uyo. Nigeria.

Collection of blood sample for analysis

After the thirty one (31) days of administration, the rats were anaesthetized with chloroform soaked in swap of cotton wool in a killing chamber. Blood was collected by cardiac puncture with a 5ml sterile syringe and needle. The total volume of blood collected was 4 ml, which was transferred into plain sample bottles. This was allowed to stand for 2 hours to clot after which the serum was separated by centrifugation (RM-12 micro centrifuge, REMI, England) at 4000 rpm for 10 minutes. The serum obtained was stored at –20^{o}C until required for analysis.

Collection of blood samples

After the thirty one (31) days of administration, the rats were anaesthetized with chloroform soaked in swap of cotton wool in a killing chamber. Blood was collected by cardiac puncture with a 5ml sterile syringe and needle. The total volume of blood collected was 4 ml, which was transferred into plain sample bottles. This was allowed to stand for 2 hours to clot after which the serum was separated by centrifugation (RM-12 micro centrifuge, REMI, England) at 4000 rpm for 10 minutes. The serum obtained was stored at –20^{o}C until required for analysis.

Determination of Albumin

Albumin was estimated with albumin reagent from Dialab, France as described by Tietz (1994).

Determination of Globulin

Serum globulin concentration = total protein - serum albumin as described by Tietz (1995).

Statistical analysis

Data were expressed as the mean ± standard error of the mean. Statistical analysis was carried out using window SPSS package (SPSS 22.00 version). Data were analyzed using one way analysis of variance (ANOVA), results obtained were further subjected to test for least significant difference (LSD). Values of $P<0.05$ were considered significant.

RESULTS

Some plasma proteins (albumin and globulin)

As could be observed in table 1, NBLCO ingestion significantly increased albumin level compared to groups I and II ($p<0.05$). The co-administration of *A. conyzoides* with NBLCO to group IV animals significantly reversed the albumin level with respect to NBLCO-treated group (group III) ($p<0.05$). Neither administration of NBLCO alone nor co-administration of NBLCO with *Ageratum conyzoides* alters globulin level significantly.

Table 1. Comparison of albumin and globulin levels in rats following exposure to NBLCO and ethanol leaf extract of *Ageratum conyzoides*

Groups	Albumin (g/dL)	Globulin (g/dL)
I (Normal saline)	3.59 ± 0.13	2.70 ± 0.88
II (*Ageratum conyzoides*)	4.17 ± 0.11a	2.55 ± 0.12
III (NBLCO)	6.16 ± 0.22a,b	3.42 ± 0.21
IV (NBLCO + *Ageratum conyzoides*)	4.97 ± 0.90a,b,c	2.85 ± 0.13

Legend:
a = significantly different from group I ($p<0.05$)
b = significantly different from group II ($p<0.05$)
c = significantly different from group III ($p<0.05$)

Serum enzymes

As could be observed in table2, NBLCO ingestion significantly increased AST, ALT and ALP activities compared to groups I and II ($p<0.05$). The co-administration of *A. conyzoides* with NBLCO to group IV animals significantly reversed the aforementioned parameters with respect to NBLCO-treated group (group III) ($p<0.05$).

Table 2. Comparison of serum enzymes (AST, ALT and ALP) activities in rats following exposure to NBLCO and ethanol leaf extract of *Ageratum conyzoides*

Groups	AST (u/L)	ALT (u/L)	ALP (u/L)
I (Normal saline)	71.88 ± 2.17	25.00 ± 2.03	39.00 ± 3.16
II (*Ageratum conyzoides*)	83.83 ± 1.40	40.17 ± 1.54a	68.83 ± 4.71a
III (NBLCO)	236.83 ± 6.93a,b	66.67 ± 4.30a,b	169.35 ± 4.11a,b
IV (NBLCO + *Ageratum conyzoides*)	187.00 ± 3.42a,b,c	47.83 ± 1.42a,c	140.67 ± 5.13a,b,c

Legend:
a = significantly different from group I ($p<0.05$)
b = significantly different from group II ($p<0.05$)
c = significantly different from group III ($p<0.05$)

Evaluation of serum activities of AST, ALT and ALP

Serum activities of ALT, AST and ALP were estimated using laboratory kits obtained from Randox laboratory Ltd., United Kingdom and absorbance were read using a uv-vis spectrophotometer (DREL 300 HACH).

DISCUSSION

In many regions of the world medicinal plantscontribute immensely to the healthcare of thepopulation. Many rural dwellers dependin many ways on a variety of plant materials for their wellbeing.These plants do not only supply essential

nutrientsbut contain secondary metabolites which haveproven to be efficacious against many diseases. In our localcommunities *Ageratum conyzoides* commonlyreferred to as "goat weed" is used for the treatmentof fever, rheumatism, pneumonia, wounds, dysentery, etc. This study examined the ameliorating potentials of ethanol leaf extract of*Ageratumconyzoides* on serum levels of albumin, globulin, AST, ALT and ALP activities. The results of this study provide evidence that NBLCO has a damaging effect on the hepatocellular cells. Such injurious effects underscore the significantly higher globulin and albumin levels as well as the high activities of AST, ALT and ALP observed in this present study. It is important to note that AST in particular is a mitochondrial enzyme (Ajon *et al*., 1981), the flooding of plasma by enzyme that ordinarily is restricted to hepatocellular mitochondrial compartment is suggestive of toxic injury caused by the ingested NBLCO. This finding is corroborated by similar injurious effect on erythrocyte membrane where oral ingestion of NBLCO damages erythrocyte membrane to cause haemolysis resulting in anaemia (Ita and Udofia, 2011; Ita *et al*., 2011; Ita *et al*., 2013). It is also important to point out that NBLCO is toxic such that a minute concentration in the environment can cause a lot of physiological changes (Ezenwaji *et al*., 2012). The co-administration of the ethanolicleaf extract of *Ageratum conyzoides*revealed the efficacy of the plant extract to significantly reverse the aforementioned parameters. This observation agrees with previous findings of the plant's efficacy as antibiotic, anti-inflammatory agent (Durodola, 1977; Ekundayo *et al*., 1988),

Conclusion

It is concluded that co-administration of ethanolic leaf extract of *Ageratum conyzoides* has demonstrated the potential to efficaciously ameliorates the hazardous effects of NBLCO on the hepatocellular cells to prevent injury to the liver.

REFERENCES

Achola, K. J., Munenge, R. W. and Mwaura, A.M. 1994. Pharmacological properties of rootand aerial extracts of *Ageratum Conyzoides* onisolated ileum and heart. *Fitoterapia.* 66: 322-325.

Akpanabiatu, M.I. , Umoh, I.B., Eyong, E.U. andUdoh, F.V. 2005. Influence of Nauclea onrats fed on Coconut oil and non-coconut oilmeals. *Pharmaceut. Biol.* 43 (2): 53-157.

Almagboul, A.Z., Farroq, A.A. and Tyagi, B.R.1985. Antimicrobial activity of certainSudanese plants used in folkloric medicine;screening for antibacterial activity, part II*Fitoterapia,* 56; 103-109.

Batzakis, K. G. and Briere, R. O. 1979. Interpretative Enzymology. Thomas Nelson, Iiiinois, Springfield, pp. 225-242.

Borthakur, N. and Barauh, A.K. 1987. Search forprecocenes in *Ageratum Conyzoides* L. ofNorth-East India. *J. Indian Chem. Soc.* 64:540-581.

Bumah, V.V., Essien, E.U., Agbedahunsi, J.M. andEka, O.U. 2005. Effect of *Khayagrandifoliala* (Melianceae) on somebiochemical parameters in rats. *J.Ethnopharmacol. 102(3): 446-449.*

Collier, J. and Bassendine, M. 2002. How to respond to abnormal liver function tests. *Clin Med*., 26:606-614.

Durodola, J. J. 1977. Antibacterial property ofcrude extracts from herbal wound healingremedy – *Ageratum Conyzoides Planta Med.*32:385-390.

Ekundayo, O., Sharma, O.S., Rao, E.V. 1988.Essential Oil of *Ageratum Conyzoides. PlantaMed*. I54:55-57.

Green, R. M. and Flamm, S. 2002.AGA technical review of the evaluation of liver chemistry tests. *Gastroenterology*, 123:1367-1384.

Ita, S.O. Ettarh, R.R. Antai, A.B., Akpagomeh, B.A. 2005. The Stimulatory effect of Intragastricadministration of *Ageratum conyzoides* ongastric acid secretion in rats. *Niger. J.HealthBiomed. Sci.* 4(2): 121-124..

Ita, S.O., Etim, O.E., Ben, E.E. and Ekpo, O.F.2007. Haematopoietic properties of ethanolicleaf extract of *Ageratum conyzoides* in albinorats. *Niger. J. Physiol. Sci.* 22:(1-2) 83-87.

Kew, M. C. 2000. Serum aminotransferase concentration as evidence of hepatocellular damage. *Lancet*, 355:591-592.

Kong, C.H., Hu, F., Hu, X. H., Liang, W.J. andZhang, C.X. 2004. Allelopathic plants. XV.*Ageratum Conyzoides* L. *Allelopathy J.* 14: 1-12.

Lorke, D. (1993). A New Approach to Practical Acute Toxicity Testing. *Arch Toxicol*., 54:275-287.

Nada, S. A., Bashandy, S. A.E. and Negam, A. A.1997. Evaluation of the hypoglycaemicactivity of traditional herbal preparation inmale diabetic rats. *Fitoterapia,* 68 (3): 240-244.

Rafehi, H., Vervaris, K. and Karagiannis, T. C. 2012. Mechanisms of action of phenolic compounds in Olive. *J. Diet Suppl*., 9((2):96-109.

Tietz, N. W. 1994. Textbook of Clinical Chemistry. 2nd Ed. W.B. Saunders Company, Philadelphia.p. 703.

Tietz, N. W. 1995. Clinical guide to laboratory tests. 3rd Ed. W.B. Saunders Company, Philadelphia. p.105.

Udosen, E.O. and Ojong, A.S. 1998. Hepatotoxicactivity of *Sacoglottis gabonensis* in rats.*Pharmaceut. Biol.* 36(5): 368-371.

Whitby, L.G., Percy-Robb, I.W. and Smith, A. F.1984. Enzymes test in diagnosis. In: *Lecturenotes on Clinical Chemistry,* 3rd ed. BlackwellSci. Public

****