African Journal of Pharmacy and Pharmacology

Volume 11 Number 20, 29 May, 2017
ISSN 1996-0816

Academic Journals

ABOUT AJPP

The **African Journal of Pharmacy and Pharmacology (AJPP)** is published weekly (one volume per year) by Academic Journals.

African Journal of Pharmacy and Pharmacology (AJPP) is an open access journal that provides rapid publication (weekly) of articles in all areas of Pharmaceutical Science such as Pharmaceutical Microbiology, Pharmaceutical Raw Material Science, Formulations, Molecular modeling, Health sector Reforms, Drug Delivery, Pharmacokinetics and Pharmacodynamics, Pharmacognosy, Social and Administrative Pharmacy, Pharmaceutics and Pharmaceutical Microbiology, Herbal Medicines research, Pharmaceutical Raw Materials development/utilization, Novel drug delivery systems, Polymer/Cosmetic Science, Food/Drug Interaction, Herbal drugs evaluation, Physical Pharmaceutics, Medication management, Cosmetic Science, pharmaceuticals, pharmacology, pharmaceutical research etc. The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles published in AJPP are peer-reviewed.

Contact Us

Editorial Office: ajpp@academicjournals.org

Help Desk: helpdesk@academicjournals.org

Website: http://www.academicjournals.org/journal/AJPP

Submit manuscript online http://ms.academicjournals.me/

Editorial Board

African Journal of Pharmacy and Pharmacology

Table of Contents: Volume 11 Number 20 29 May, 2017

ARTICLES

academicJournals

Vol. 11(20), pp. 250-259, 29 May, 2017
DOI: 10.5897/AJPP2017.4730
Article Number: F0BB9D564545
ISSN 1996-0816

http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Hepatocurative effect of aqueous extract of *Hibiscus sabdariffa* on some antioxidants and haematological indices of acetaminophen-challenged Wistar albino rats

Parker E. Joshua[1]*, Chuma S. Eze[2], Chimere Y. Ukegbu[1], Joshua O. Okafor[1], Prisca C. Okoli[1], Chinelo C. Nkwocha[1], Florence O. Nduka[1], Amaechi L. Ogara[3], Chibuike S. Ubani[1]

[1]Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka, Nigeria.
[2]Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology, Enugu, Nigeria.
[3]Department of Degree Programme in Science Laboratory Technology, Faculty of Physical Sciences, University of Nigeria, Nsukka, Nigeria.

Received 10 January, 2017; Accepted 2 February, 2017

Hibiscus sabdariffa **is among the medicinal plants which have been shown to possess several medicinal properties. The present study was conducted to investigate the antioxidant and haematological properties of the aqueous leaf extract of *H. sabdariffa* on acetaminophen-challenged liver using rat model. Twenty (20) Wistar albino rats were used for this study and were divided into 4 groups of 5 rats each. Group 1 rats were the normal control; group 2 (positive control) rats were administered acetaminophen only, at a dose of 750 mg/kg b.w. ip. Group 3 rats were administered mid dose (400 mg/kg b.w) of the extract after acetaminophen-induction while group 4 rats received high dose (600 mg/kg b.w) of the extract after acetaminophen-induction. Group 2 rats showed a significant ($p<0.05$) decrease in the activities of the enzymes, catalase (CAT), superoxide dismutase (SOD) and vitamin C concentrations when compared with group 1 rats. However treatment with the extract caused a significant ($p<0.05$) increase in the activities of the enzymes, catalase (CAT), superoxide dismutase (SOD) and vitamin C concentrations when compared with group 2 animals. More so, group 2 rats treated with acetaminophen only, showed significant increase ($p<0.05$) in white blood cell, neutrophil and lymphocytes counts when compared with the group 1 rats. Conversely, a significant decrease ($p<0.05$) was observed in packed cell volume, red blood cell count and haemoglobin concentration of the group 2 rats when compare with the group 1 rats. Treatment with the aqueous extract of *H. sabdariffa* caused a dose-dependent significant increase ($p<0.05$) in the pack cell volume, red blood cell count and haemoglobin concentration of the treatment groups (groups 3 and 4) when compared with the group 2 rats. Conclusively, the results of this study suggest that *H. sabdariffa* possess antioxidant properties and could be potent in boasting the blood level in a disease state.**

Key words: *Hibiscus sabdariffa*, antioxidant, haematological properties, acetaminophen-challenged, liver.

INTRODUCTION

The liver plays a central role in transforming and clearing both endogenous and exogenous chemicals and is susceptible to the toxicity from these agents (Zahra et al., 2012). The liver performs its function through two identified processes described as phases I and II detoxification pathways. In the phase I mechanism, the liver either directly neutralizes a toxin or transforms it into an activated metabolite which is subsequently neutralized

in the phase II (Monira et al., 2012). The phase II process involves the addition (conjugation) of the toxin with certain substances so as to make the toxin more water soluble and thus easy to excrete. Drugs are an important cause of liver injury (Bray et al., 2000). More than 900 drugs and toxins have been reported to cause liver injury even at therapeutic dose and it is the most common reason for a drug to be withdrawn from the market (Laura et al., 2003). However, hepatotoxicity is a direct liver injury which can be caused by an overdose of acetaminophen and consequently the actions of its toxic metabolite N-acetyl-p-benzoquinone imine (NAPQI) (Alexander and Glyn, 2005). Its ability to cause liver damage at an overdose has made it one of the most preferred drug used in studying hepatotoxicity in animal models.

A number of drugs or chemicals such as melatonin, vitamin E and N-acetyl-cysteine have been used to prevent acetaminophen-induced hepatic and renal injury (Bray et al., 2000). Increased use of synthetic drug therapy leads to many side effects and undesirable hazards. Therefore, there is a worldwide trend to return to natural resources, which are culturally acceptable and economically viable (Sharida et al., 2012). The use of the leaf extracts of *H. sabdariffa* is among such natural resources. *H. sabdariffa* belongs to a family of herbal plants called malvaceae. Phytochemical analysis showed that there are some plant chemicals present in the extract such as alkaloids, tannins, saponnins, glycosides, phenols and flavonoids and quantitative result revealed their presence as follows: Tannins (17.0%), saponnins (0.96%), phenols (1.1%), glycosides (0.13%), alkaloids (2.14%) and flavonoids 20.08%) (Okereke et al., 2015). Furthermore, HPLC analysis revealed two phenolic acids, 16 flavonoids and four anthocyanins in petal of *H. sabdariffa*. The major compounds were gossypetin, hibiscetin, quercetin and sabdaretin (flavonoids) while delphinidin 3-O-sambubioside and cyanidin 3-O-sambubioside were the major anthocyanins (Obouayeba et al., 2014).

Previous works on *H. sabdariffa* suggests that it could inhibit lipid peroxidation by maintaining the levels of antioxidants in the serum of animals treated with acetaminophen (Kolawole and Maduenyi, 2004). Studies have also shown that *H. sabdariffa* can offer protective effects against paracetamol-induced hepatotoxicity in rats (Mukesh and Ashok, 2011). Low doses of ascorbic acid found in the leaves of *H. sabdariffa* were able to prevent lipid peroxidation following acetaminophen induction in rats (Norina and Hazlin, 2004).

Thus, this present study was set out to investigate the hepato-regenerative properties of an aqueous extract of. *H. sabdariffa* on some antioxidants and haematological indices of acetaminophen-challenged Wistar albino rats

MATERIALS AND METHODS

Fresh leaves of *H. sabdariffa* were purchased from Ogige market, Nsukka, Enugu State of Nigeria and were identified by Mr. Alfred Ozioko of the herbarium, Botany Department, University of Nigeria, Nsukka. The leaves were air-dried separately at room temperature and ground to powdery form using electrical grinding machine.

Animals

Adult male Wistar albino rats of 10 to 16 weeks and average weight of 160±15 g were obtained from the Animal House of the Faculty of Biological Sciences, University of Nigeria, Nsukka. The animals were acclimatized for a duration of 7 days under standard environmental conditions with a 12 h light/dark cycle maintained on a regular feed (vital feed) and water *ad libitum*.

Chemicals/reagents/samples

All chemicals used in this study were of the analytical grade and products of May and Baker, England; BDH, England and Merck, Darmstadt, Germany. Reagents used for all the assays were commercial kits and products of Randox, USA; QCA, Spain; Teco (TC), USA; Biosystem Reagents and Instruments, Spain.

Preparation of acetaminophen (paracetamol) sample

The stock concentration of acetaminophen was prepared by dissolving 600 mg of the standard drug in 2 ml of distilled water bringing the stock concentration to 60 mg/ml. Paracetamol was induced intraperitoneally at the dose of 750 mg/kg b.w. (Hiroshini et al., 1987).

Extraction of the active agents of *H. sabdariffa*

Large quantities of the leaves of *H. sabdariffa* were purchased from Ogige market in Nsukka, Enugu State of Nigeria and were identified by Mr. Ozioko of the herbarium Botany Department, University of Nigeria, Nsukka. The leaves of *H. sabdariffa* were air-dried separately at room temperature (25-30°C), then into powdery form using electrical grinding machine. The ground samples extracted with aqueous solvent (H_20), using cold maceration techniques for 48 h. The samples were filtered using Whatman filter paper No 1. The filtrates (that is, the active agents of the extract) concentrated using rotary evaporator, which then become the stock sample of the aqueous leaf extract which were used for the analysis. These extracts were stored in the refrigerator compartment to prevent microbial growth.

Experimental design

Twenty (20) male albino Wistar rats were acclimatized for seven

*Corresponding author. E-mail: parker.joshua@unn.edu.ng.

days at room temperature and vital animal feeds were used for all the rats. The rats were divided into four (4) groups of five (5) rats each as shown below: Group 1:
Normal/negative rats (Control); Group 2: positive control (Acetaminophen-induced untreated rats); Group 3: Acetaminophen-induced + 400 mg/kg b.w. of the extract *H. sabdariffa*; Group 4: Acetaminophen-induced + 600 mg/kg b.w. of the extract *H. sabdariffa*.

After the experiment, the animals were sacrificed at the end of the experiment and blood was collected for biochemical analysis.

Determination of malondialdehyde concentration

Lipid peroxidation assay was done by determining the concentration of malondialdehyde (MDA) formed using the method of Varshney and Kale (1990).

Determination of vitamin C

The concentration of vitamin C (ascorbic acid) was determined according to the method of Baker et al. (1971).

Assay of superoxide dismutase activity

Superoxide dismutase (SOD) activity was assayed using the method as described by Fridorich (1989) as contained in Randox commercial kit.

Assay of catalase activity

Catalase activity was assayed using the method of Aebi (1983).

Total white blood cell count (WBC)

This was done using standard techniques as described by Ramnik (2003).

Red blood cell count (RBC)

This was done using standard method as described by Daice and lewis (2000).

Hemoglobin estimation

Hemoglobin concentration was determined by the method described by Dacie and Lewis (2000).

Packed cell volume (PCV)

This was done using standard technique as described by Ochei and Kolharta (2008).

White blood cell differential count

The differential WBC counts was obtained using a coulter counter in a well standardized commercial laboratory.

Statistical analysis

The results were expressed as Mean±SEM and test of statistical significance was carried out using one-way analysis of variance (ANOVA). The means were separated using Duncan multiple test. The statistical packaged used was the statistical package for social sciences (SPSS), version 17.

RESULTS AND DISCUSSION

Acetaminophen belongs to a subgroup of analgesics called aniline analgesics and is very effective in relieving pain and mild fever. At therapeutic doses, it causes no discomfort. However, an overdose of the drug can result to severe hepatic damage (Linda et al., 2009). It undergoes metabolic activation by hepatic microsomal cytochrome P_{450} mixed function oxidase system (especially the enzyme CYP2E1) to N-acetyl-P-benzoquinone imine (NAPQI). The active metabolite (being highly electrophilic) quickly binds to intracellular proteins, causing a change in their structure and hence their function (Monira et al., 2012). The NAPQI is the active metabolite involved in virtually all the metabolic disorders experienced during an overdose of acetaminophen.

From Figure 1 and Table 1, intraperitoneal (ip) induction of Acetaminophen at the dose of 750 mg/kg b.w. caused a significant increase ($p<0.05$) in the malondialdehyde (MDA) concentration which is a product of lipid peroxidation of the group 2 animals as compared to the normal control group 1. This increase in MDA concentration is as a result of increase in lipid peroxidation by the actions of the toxic metabolite NAPQI. However, treatment with *H. sabdariffa* aqueous extract caused a significant decrease ($p<0.05$) in the MDA concentration of the treatment groups (groups 3 and 4) as compared to the untreated group 2 animals. Thus, these decreases could be as a result of the actions of the phytochemical constituents of the extract such as flavonoids in inhibiting the actions of the toxic metabolite NAPQI and also stabilizing the cell membranes of the intracellular proteins and other compounds. This result is consistent with the finding of Bray et al. (2000) who observed a decrease in the above mentioned parameters following treatment with ethanoic stem extracts of *H. sabdariffa* after acetaminophen-induction.

From Figure 2 and Table 2, induction of acetaminophen (ip) caused a significant decrease ($p<0.05$) in vitamin C concentrations, superoxide dismutase (SOD) activity and catalase activity of the group 2 rats when compared with the normal control group (group 1). This may be attributed to the metabolite, N-acetyl-P-benzoquinone imine (NAPQI), which induces lipid peroxidation and free radical generation. However, the free radicals generated caused the significant decrease ($p<0.05$) in the vitamin C concentration, SOD activity and catalase activity of the group 2 animals as compared to the normal control

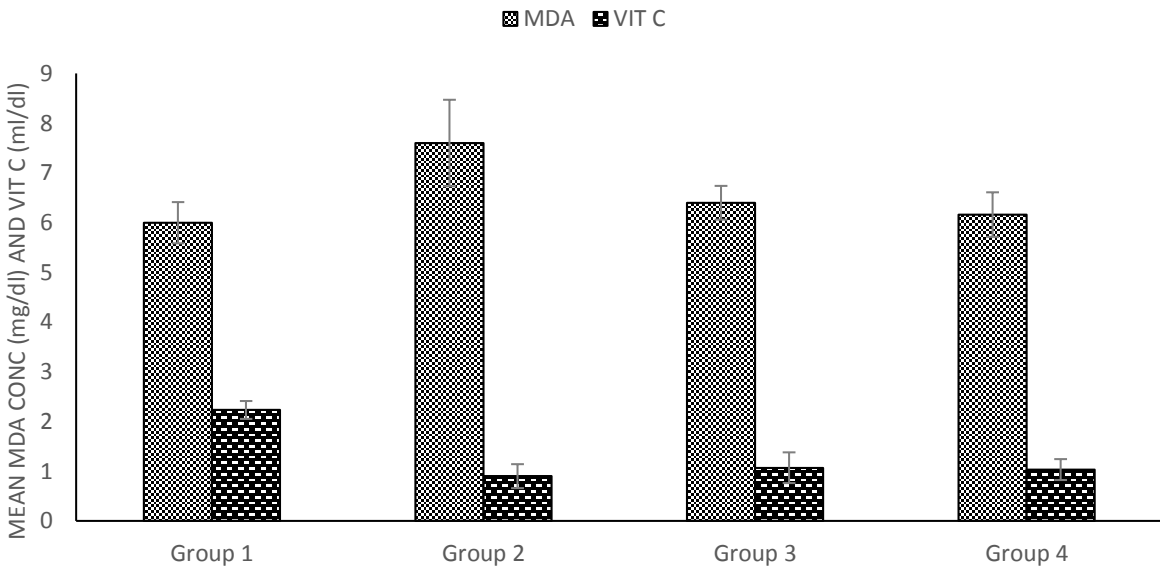

Figure 1. Effect of aqueous leaf extract of *Hibiscus sabdariffa* on malondialdehyde and vitamin C concentrations against acetaminophen-induced liver damage in Wistar Albino rats.

Table 1. Effect of aqueous leaf extract of *H. sabdariffa* on malondialdehyde and vitamin C concentrations against acetaminophen-induced liver damage in Wistar albino rats.

Groups	MDA (mg/dl)	VIT C (ml/dl)
Group 1 Normal (Control)	6.00 ± 0.42*	2.33 ± 0.18*
Group 2 Positive Control (untreated rats)	7.60 ± 0.87	0.90 ± 0.24
Group 3 Acetaminophen + 400 mg/kg b.w. of extract	6.40 ± 0.34*	1.07 ± 0.31
Group 4 Acetaminophen + 600 mg/kg b.w. of extract	6.16 ± 0.45*	1.03 ± 0.21

Values are mean ± SEM; n = 3 animals in each group; * = significantly (P<0.05) different when compared with paracetamol control (positive control) group using one way analysis of variance.

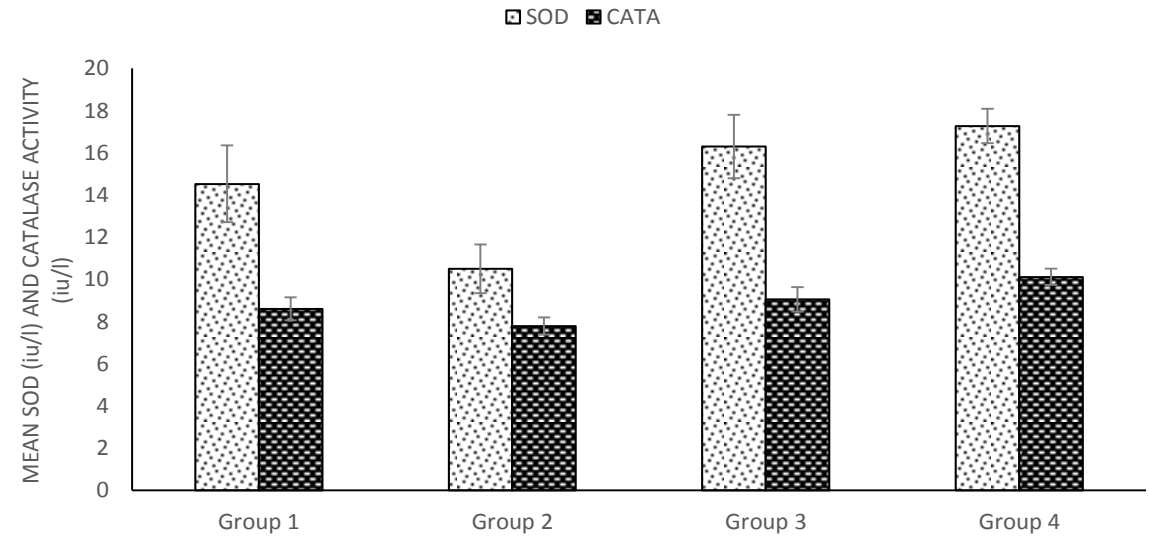

Figure 2. Effect of aqueous leaf extract of *Hibiscus sabdariffa* on superoxide dismutase and catalase activity against acetaminophen-induced liver damage in Wistar Albino rats.

Table 2. Effect of aqueous leaf extract of *Hibiscus sabdariffa* on superoxide dismutase and catalase activity against acetaminophen-induced liver damage in Wistar albino rats.

Groups	SOD (IU/L)	CATA (IU/L)
Group 1 Normal (Control)	14.53 ± 1.82*	8.60 ± 0.54*
Group 2 Positive Control (untreated rats)	10.50 ± 1.16	7.79 ± 0.41
Group 3 Acetaminophen + 400 mg/kg b.w. of extract	16.30 ± 1.50*	9.05 ± 0.58*
Group 4 Acetaminophen + 600 mg/kg b.w. of extract	17.27 ± 0.82*	10.12 ± 0.38*

Values are mean ± SEM; n = 3 animals in each group; * = significantly (P<0.05) different when compared with paracetamol control (positive control) group using one way analysis of variance.

Table 3. Effect of aqueous leaf extract of *H. sabdariffa* on packed cell volume and hemoglobin concentration against acetaminophen-induced liver damage in Wistar albino rats.

Groups	PCV (%)	HB (G/DL)
Group 1 Normal (Control)	44.33 ± 4.45*	12.33 ± 3.50*
Group 2 Positive Control (untreated rats)	33.33 ± 3.76	8.50 ± 2.67
Group 3 Acetaminophen + 400 mg/kg b.w. of extract	52.66 ± 4.74*	13.67 ± 3.43*
Group 4 Acetaminophen + 600 mg/kg b.w. of extract	53.00 ± 2.67*	13.33 ± 4.6*

Values are mean ± SEM; n = 3 animals in each group; * = significantly (P<0.05) different when compared with paracetamol control (positive control) group using one way analysis of variance.

group 1. Administration with both low (400 mg/kg b.w) and high (600 mg/kg b.w) doses of the extract after acetaminophen-induction however significantly increased ($p<0.05$) the activities of CAT and SOD as compared to the untreated group 2 animals. However, there was a slight non-significant ($p > 0.05$) increase in the Vitamin c concentration of the treated group as compared to the untreated group 2 animals. This is probably due to the antioxidant components of the extract. A high level of vitamin C is a good body mechanism for fighting not only free radicals but also diseases.

These antioxidants include beta-carotene, vitamin C and niacin. SOD, being an enzyme which converts superoxide radical ($O_2^{\cdot}$) to hydrogen peroxide and molecular oxygen. CAT, an enzyme which decomposes the hydrogen peroxide generated during lipid peroxidation, is also decreased following acetaminophen-induction without treatment with extract. However, the increase experienced could be as a result of the antioxidant and vitamin contents of the extract being able to mop up the free radicals generated as a result of overdose of acetaminophen. This result is consistent with the findings of Monira et al. (2012) who observed an increase in vitamin C concentration following carbon tetrachloride-induction and the findings of Sharida et al. (2012) who observed a restoration of the above parameters to their normal value following treatment with methanol leaf extract of *H. sabdariffa* after cyclophosphamide-induction Table 3.

Administration of acetaminophen alone to all the groups significantly increased ($p<0.05$) the white blood count cell (wbc) of the group 2 rats when compared with the normal control of rats of group 1 as can be seen in Table 4 and Figure 3. Acetaminophen overdose initiates fast mobilization of total white blood cells for the initial defense against drug toxicity, this is in response to the metabolite, N-acetyl-p-benzoquinone imine (NAPQI), which causes reactive metabolite formation, GSH depletion and mitochondrial oxidant stress, which contributes directly to the mitochondrial membrane permeability transition pore opening and collapse of the membrane potential and indirectly through release of inter-membrane proteins, to nuclear DNA damage. Treatment with various doses (400 and 600 mg/kg b.w.) of the extract caused a dose dependent significant decrease in the white blood cell count of the treated groups (3 and 4) as compared to the untreated group 2 rats. These results suggest that the extract was able to restore normalcy in the rat after treatment and this could be as a result of the actions of some active phyto-constituents of the plant extract in stimulating the immune system to fight the disease state. This result also indicates that the extract has an immune-stimulatory effect on the component of the immune cells. Thus, it could be deduced that aqueous extract of *H. sabdariffa* has immuno-modulatory potentials. Thus, this result of Ademola et al. (2015) where the protective effect of pretreatment of rats with Calyx extract of *H. sabdariffa* against carbon tetrachloride-induced hemato-toxicity.

Furthermore, from Figure 4 and Table 5 administration of acetaminophen alone to groups 2 rats significantly increased ($p<0.05$) the neutrophil count when compared

Table 4. Effect of aqueous leaf extract of *Hibiscus sabdariffa* on red blood cell and white blood cell count of acetaminophen-induced liver damage in Wistar albino rats.

Groups	WBC (mm^{-3})	RBC ($x10^{6}mm^{-3}$)
Group 1 Normal (Control)	4716.67 ± 538*	423.67 ± 35*
Group 2 Positive Control (untreated rats)	6450.00 ± 832	320.00 ± 56
Group 3 Acetaminophen + 400 mg/kg b.w. of extract	4766.67 ± 445*	396.00 ± 12*
Group 4 Acetaminophen + 600 mg/kg b.w. of extract	4966.67 ± 438*	366.67 ± 35*

Values are mean ± SEM; n = 3 animals in each group; * = significantly (P<0.05) different when compared with paracetamol control (positive control) group using one way analysis of variance.

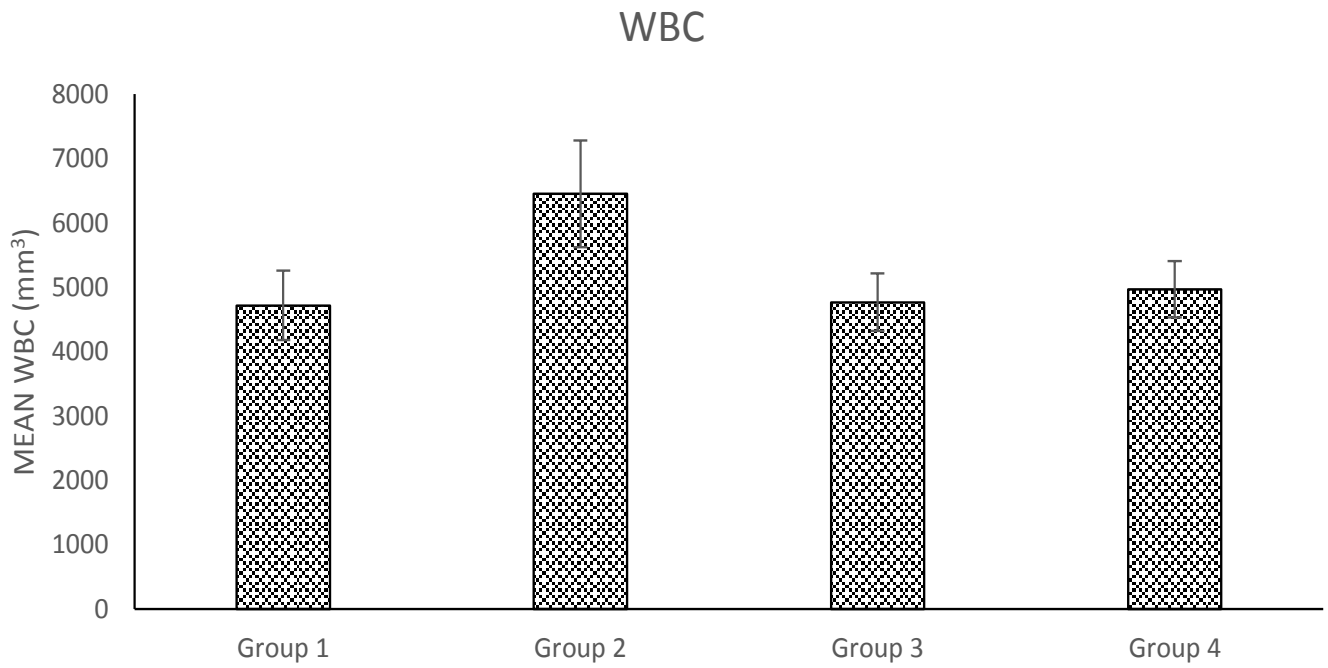

Figure 3. Effect of aqueous leaf extract of *Hibiscus sabdariffa* on white blood cell count against acetaminophen-induced liver damage in Wistar Albino rats.

with the normal group 1 rats, however, there was no significant increase (p>0.05) in the lymphocyte counts of the treated groups when compared with the untreated group. Neutrophils are the primary white blood cells that respond to infection or any form of cell toxicity or cell inflammation while lymphocytes are also a part of the white blood cells but specific to acute viral infections such as viral hepatitis, cytomegalovirus and others such as protozoal infections. Thus, treatment with various doses (400 and 600 mg/kg b.w.) of aqueous extract of *H. sabdariffa* caused a significant decrease (p<0.05) in the neutrophil count of the treatment groups (3 and 4) as compared to the untreated group 2 rats. The results of this research are in line with the works of olatunji et al. (2005) where the hematological effect of *H. sabdariffa* petals on rats was determined.

Eosinophil is a white blood cell containing granules and an eosinophil count typically helps to confirm a diagnosis, it have two distinct functions in the immune; they destroy invading germs like viruses, bacteria or parasites and they create inflammatory response especially when an allergy is involved. Thus, drug induced liver injury is not associated with eosinophil as can be seen in Figure 5 and Table 6 that induction of acetaminophen did not cause any significant increase or decrease (p>0.05) since it is mostly concern with allergy responses.

The results shown in Figure 6 and Table 4 shows that after induction of acetaminophen, there was a significant decrease (p<0.05) in the red blood cell count of the untreated group as compared to the normal control group 1. This suggests that the high level of free radical actions could lead to increase in the breakdown of the red cell or

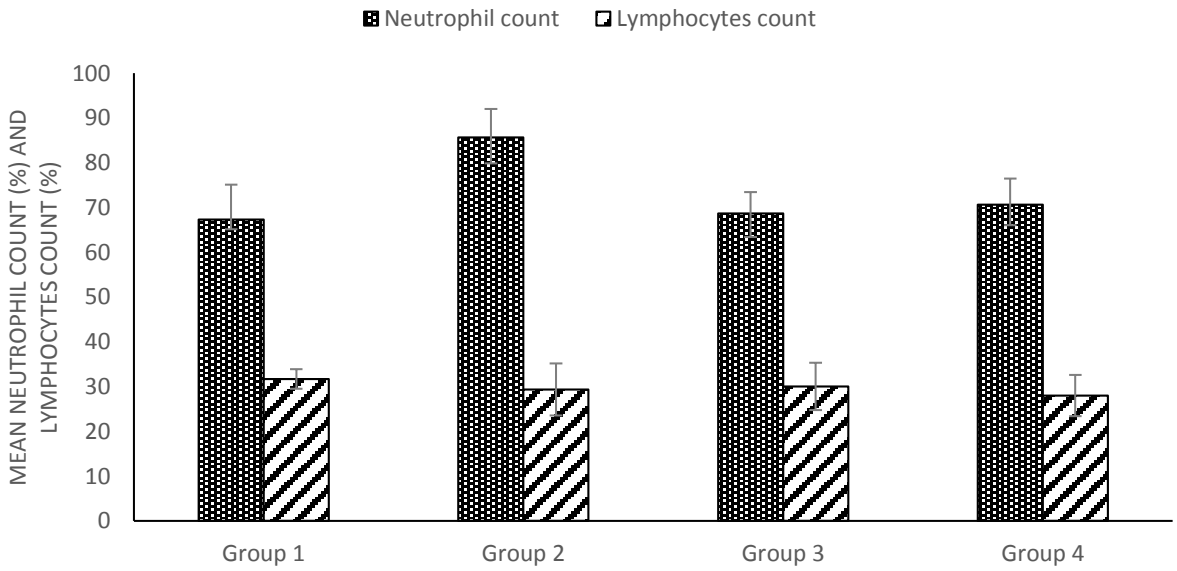

Figure 4. Effect of aqueous leaf extract of *Hibiscus sabdariffa* on neutrophil count and lymphocytes count against acetaminophen-induced liver damage in Wistar Albino rats.

Table 5. Effect of aqueous leaf extract of *Hibiscus sabdariffa* on neutrophil count and lymphocytes count against acetaminophen-induced liver damage in Wistar albino rats.

Groups	NEU. CNT (%)	LYM. CNT (%)
Group 1 Normal (Control)	67.330 ± 7.76*	31.67 ± 2.18
Group 2 Positive Control (untreated rats)	85.667 ± 6.35	29.33 ± 5.81
Group 3 Acetaminophen + 400 mg/kg b.w. of extract	68.667 ± 4.80*	30.00 ± 5.29
Group 4 Acetaminophen + 600 mg/kg b.w. of extract	70.667 ± 5.76*	28.00 ± 4.60

Values are mean ± SEM; n = 3 animals in each group; * = significantly (P<0.05) different when compared with paracetamol control (positive control) group using one way analysis of variance.

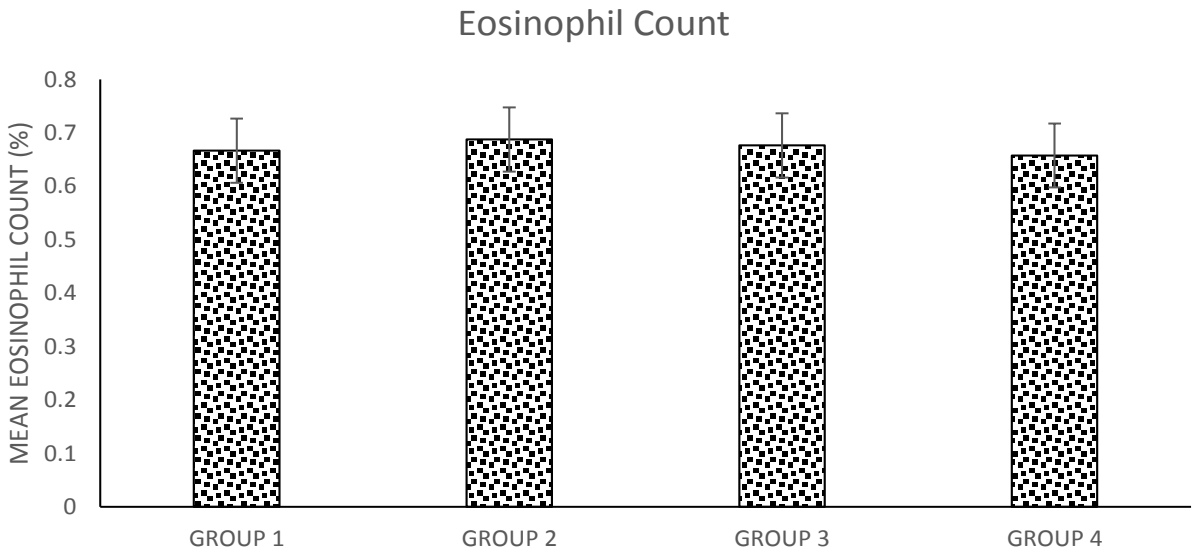

Figure 5. Effect of aqueous leaf extract of *Hibiscus sabdariffa* on eosinophil count against acetaminophen-induced liver damage in Wistar Albino rats.

Table 6. Effect of aqueous leaf extract of *H. sabdariffa* on eosinophil count against acetaminophen-induced liver damage in Wistar albino rats.

Groups	Eosinophil CNT (%)
Group 1 Normal (Control)	0.6667 ± 0.06
Group 2 Positive Control (untreated rats)	0.6876 ± 0.06
Group 3 Acetaminophen + 400 mg/kg b.w. of extract	0.6766 ± 0.06
Group 4 Acetaminophen + 600 mg/kg b.w. of extract	0.6576 ± 0.06

Values are mean ± SEM; n = 3 animals in each group; * = significantly (P<0.05) different when compared with paracetamol control (positive control) group using one way analysis of variance.

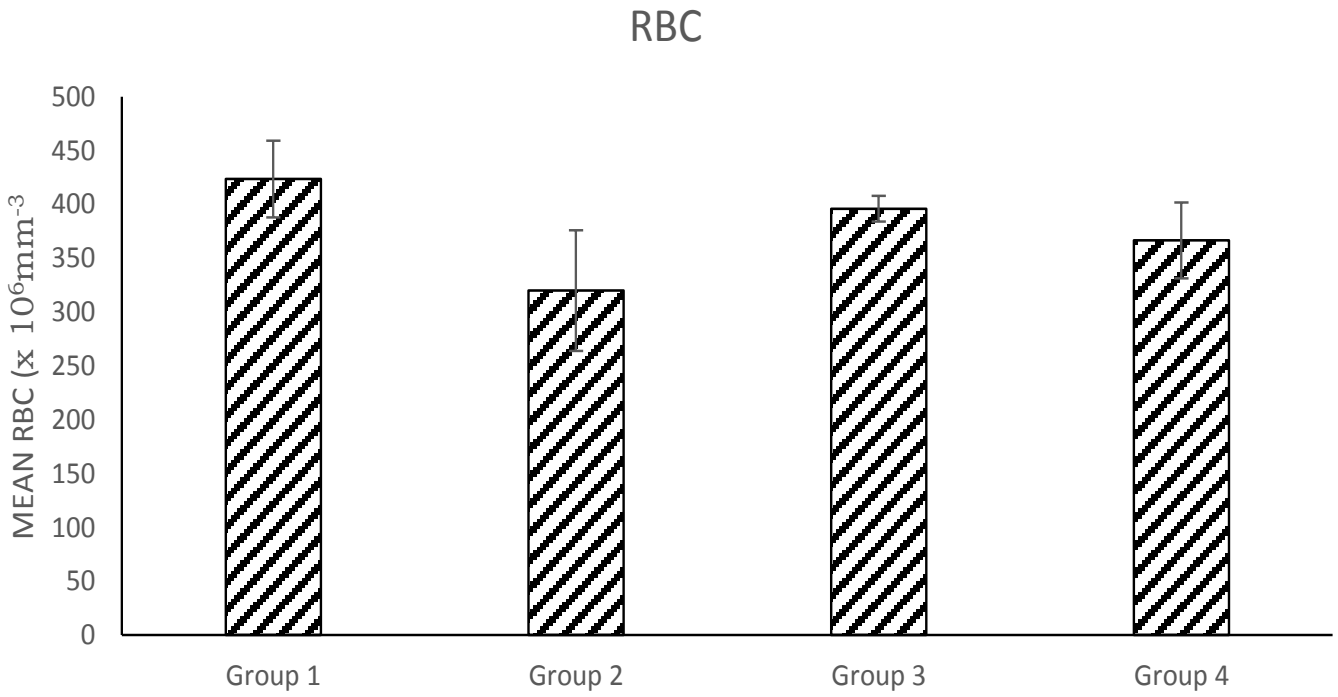

Figure 6. Effect of aqueous leaf extract of *Hibiscus sabdariffa* on red blood cell count against acetaminophen-induced liver damage in Wistar Albino rats.

other unknown actions of the toxic metabolite in the body. However, after treatment with the extract at various doses (400 and 600 mg/kg b.w), there was a significant increase ($p<0.05$) in the red blood cell count of the treatment groups (3 and 4) when compared with the untreated group 2 rats. This suggests that the extract could contain some compounds that are potent in boasting the production of the red cells by the bone marrow. However, this result is in concordance with the works of Ademola et al. (2015) where the protective effect of pretreatment of rats with calyx extract of *H. sabdariffa* against carbon tetrachloride-induced hematotoxicity induction of acetaminophen significantly decreases ($p<0.05$) the packed cell volume (PCV) count and the hemoglobin estimation of group 2 rats compare with normal control (group 1). As shown in Table 3 and Figure 7. This is probably due to the significant reduction in the weight of thymus and spleen, and this decrease could also be attributed to the low levels of hemoglobin which is a clinically condition characterized by low levels of PCV. The implication of this is suppression of the innate immune responses. This result is consistent with Fakeye et al. (2008), who observed a decrease in the above parameter after acetaminophen induction. However, administration of *H. sabdariffa* flower aqueous extract after acetaminophen administration significantly increased ($p<0.05$) PCV and hemoglobin estimation of the treated rats of group 3 and 4 as compared to the untreated rats of group 2. Thus, this suggests that the aqueous extract of *H. sabdariffa* flower could contain

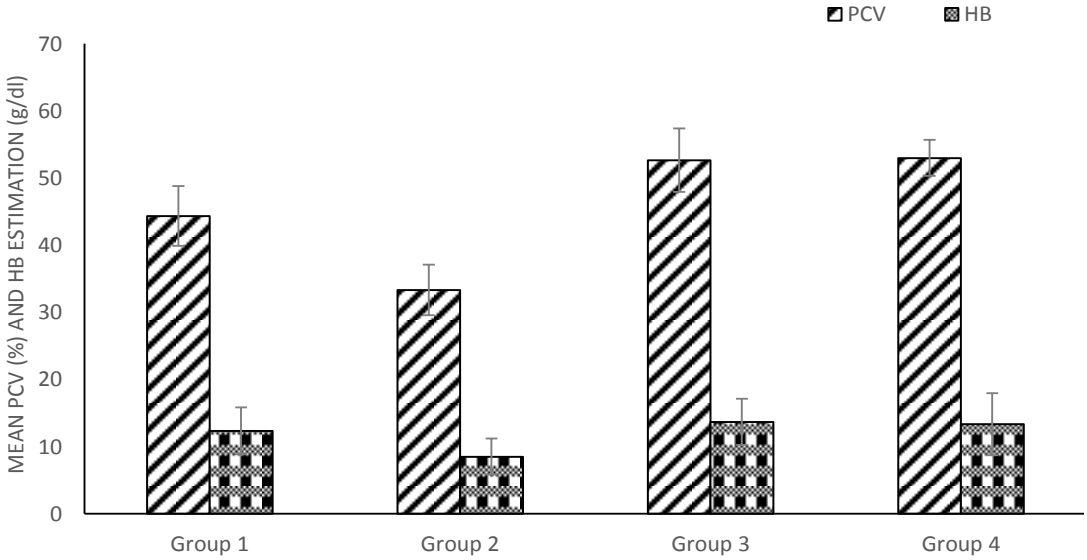

Figure 7. Effect of aqueous leaf extract of *Hibiscus sabdariffa* on packed cell volume and hemoglobin concentration against acetaminophen-induced liver damage in Wistar Albino rats.

some active compounds that will be effective against anemia and other blood related disorders. However, the results of this research is in line with the works of Ahmed et al. (2013) where the effect of aqueous extract of *H. sabdariffa* seed on hematological parameters against anemic rats.

Conclusion

Conclusively, the results of this study suggest that flowers of *H. sabdariffa* contains some compounds that are effective against drug induced liver toxicity and more so has some phyto-constituents that could help to boast hematological parameters in an anemic condition.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Ademola CF, Shedrach CK, Victor NO, Maduka LN (2015). Protective Effect of Pretreatment of Rats with Calyx Extract of *Hibiscus sabdariffa* against Carbon Tetrachloride-induced Hematotoxicity. J. Bio. Sci. 15(3):138-143.

Aebi HE (1983). Catalase in Methods of Enzymatic Analysis.3rd Ed (Bermeyer, H. U., *et al*. Ed). pp. 273-285.

Ahmed RH, Mohammed HWA, El-Hadi MH, El-khalifa MY (2013). Effect of aqueous extract of *Hibiscus sabdariffa* seed on hematological parameters against anemic rats. Inter. J. phytomed. 5:278-281.

Alexander VK, Glyn M (2005). Dose- and time-dependent oval reaction in acetaminophen-induced murine liver injury. Hepatol. 41:1251-1261.

Baker EM, Hodges RE, Hood J (1971). Metabolism ^{14}C and ^{3}H-labelled L-ascorbic acid in human scurvy. Am. J. Clin. Nutr. 24:444-480.

Bray BJ, Inder RE, Rosengren RJ (2000). Retinol-mediated effects on the activation and detoxification pathways of paracetamol. Aust. J. Ecotoxicol., 6:75-79.

Dacie JV, Lewis SM (2000). Practical Haematology. 9th Edn Churchill Livingstone, Longman Group. England. pp. 837-857.

Fakeye T (2008). Toxicity and immune-modulatory activity of fractions of *Hibiscus sabdariffa* Linn (family malvaceae) in animal models. AJTCAM. 5(4):394-398.

Fridovich I (1989). Superoxide dismutases: An adaptation to a paramagnetic gas. J. Bio. Chem. 264:7761-7764.

Hiroshini A, Toshiharu H, Masahiro SH (1987). An alteration in liver microsomal membrane of the rat following paracetamol overdose. J. Pharmacol. 39:1047-1049.

Kolawole JA, Maduenyi A (2004). Effect of zobo drink (*Hibiscus sabdariffa* aqueous extract) on the pharmacokinetics of acetaminophen in human volunteers. Eur. J. Drug Metabol. Pharmacokin. 29:25-29.

Laura PJ, Philip RM, Jack AH (2003). Acetaminophen induced hepatotoxicity. Drug Metabolism Disposition 31:1499-1506.

Linda J, Chun BS, Myon J (2009). Acetaminophen hepatotoxicity and acute liver failure. J. Clin. Gastroenterol., 43:342-349.

Monira A, Kader AE, Naima ZM (2012). Evaluation of protective and antioxidant activity of *Hibiscus sabdariffa* extract on paracetamol-induced toxicity in rats. Aust. J. B. App. Sci. 6:467-474.

Mukesh T, Ashok S (2011). Antoxidant and hepatoprotective activity of *Hibiscus sabdariffa* on paracetamol induced toxicity. Inter. J. Pharmaceut. Stud. Res. 2:110-121.

Norina A, Hazlin AH (2004). Protective effects of the ethanol extract of *Hibiscus sabdariffa* on paracetamol-induced hepatotoxicity in rats. J. Sains Kesihatan Malay. 2:85-95.

Obouayeba AP, Djyh NB, Diabate sékou, Djaman AJ, N'guessan JD, Kone M, Kouakou TH (2014). Phytochemical and Antioxidant Activity of Roselle (*Hibiscus Sabdariffa* L.) Petal Extracts. Res. J. Pharmaceut. Bio. Chem. Sci. 5(2):1453-1465.

Ochei J, Kolhatkar A (2008). Medical Laboratory Sciences: Theory and Practice. Tata McGraw Hill, New York. pp. 663-665.

Okereke CN, Iroka FC, Chukwuma MO (2015). Phytochemical analysis and medicinal uses of *Hibiscus sabdariffa.* Int. J. Herb. Med. 2(6):16-19.

Olatunji LA, Adebayo JO, Akinola OB, Olatunji VA, Adekoya A, Badaki OJ, Soladoye AO (2005). Haematological effects of *Hibiscus sabdariffa* petals in rats. The Trop. J. Afr. Heal. Sci. 12:39-41.

Ramnik S, (2003). Haematology for Students and Practitioners. 5th Edn. Jaypee Brothers Medical Publishers Ltd., New Delhi. pp. 108-181.

Sharida F, Syzana AS, Palanisamy A (2012). *Hibiscus sabdariffa* hydroethanolic extracts effectively alleviate acetaminophen-induced hepatotoxicity in experimental rats through their antioxidant nature. Molecules 17:8334-8350.

Varshney R, Kale RK (1990). Effects of calmodulin antagonists on radiation induced lipid peroxidation in microsomes. Inter. J. Rad. Biol., 58:733-743.

Zahra K, Malik MA, Mughal MS (2012). Hepatoprotective role of extracts of *Hibiscus sabdariffa* in acetaminophen – induced toxicity in rabbits. J. Anim. Plant Sci. 22:273-277.

academicJournals

Vol. 11(20), pp. 260-265, 29 May, 2017
DOI: 10.5897/AJPP2017.4791
Article Number: 71692F564567
ISSN 1996-0816

http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Perception, knowledge and use of antibiotic among communities in Chad

Ndoutamia G.[1*], Fissou H. Yandaï[2], Nadjilem D.[3] and Gatsing D.[4]

[1]University of Doba, Chad.
[2]Laboratory of Hospital of Mother and Child in Ndjamena, Chad.
[3]University of N'Djamena, Chad.
[4]University of Dschang, Cameroon.

Received 27 April, 2017 ; Accepted 17 May, 2017

Chad is a country where self-medication and use of antibiotic without medical prescriptions are still observed. These practices lead to the apparent emergence of resistance to antibiotics for bacteria. The aim of this study was to assess the abusive and inappropriate use of antibiotics among the communities. The surveys were conducted in N'Djamena and Moundou, two major cities of Chad. All the respondents were aged more than 14. They had different social status and some were married. All were interviewed through questionnaire sheets. The collected data were cleaned and validated using the Statistical Software Package for the Social analysis. Data analysis was performed using the Pearson chi-square test. Of the 500, 472 returned sheets contained complete information. Therefore, the response rate was 94.4%. 296 (62.7%) were male and 176 (37.3%) women. The majority of the participants were between 25-34 years (36.9%). Their levels of training vary: primary (40.8%), secondary (33.1%) and higher (26.1). Their professional and marital status also varied. The marital status revealed 37.7% single and 46.8% married. The rate of antibiotic use was significant among the young people, from 25 to 34 years old (61.49%; p = 0.002). As far as the antibiotics acquisition is concerned, 33.2% of the people interviewed affirmed that they had received probabilistic medical prescription of drugs of which 2.5% is on customers' demand. It was also established that 6.8% of medicines were purchased without medical prescriptions and 4.2%, on the advice of unqualified street vendors. With regards to the sources of drugs, 20.6% of the antibiotics were purchased from pharmacies and 11.0% from street vendors. Bad practices in the use of antibiotics could be related to knowledge gaps on the subject. Young people, in particular, those with low incomes and lower level of education were the most concerned. Educational, informational, communicative initiatives and application of regulations on sales of antibiotics are needed to control the misuse of antibiotic in Chad.

Key words: Antibiotic resistance, socio-demographic characteristics, bacteries, virus, education, Chad.

INTRODUCTION

Antibiotic resistance has become a global concern and more so for Africa which is almost entirely absent from

*Corresponding author. E-mail: ndoutamia@gmail.com. Tel: 00235 66 32 46 67.

the statistical data. The deplorable hygiene conditions facilitate the transmission of bacterial infections at both inter-human, animal, and environmental level as well. The inadequate prescriptions, self-medication and free sale of antibiotics are factors that increase the irrational consumption of these molecules (Chauvin, 2009). The use of antibiotics of last generation in the animal industry has more aggravated the situation (Bywater, 2004; Gillani et al., 2010). Various studies, in particular those on the group antibiotic resistance (RIIP), confirm the magnitude of the problem of bacterial resistance to antibiotics in the developing countries, in particular, the resistance by the production of bêta-lactamases in broadened spectrum in *E. coli* and *Salmonella*, and the resistance to Quinolones among Gram-negative bacteria (RIIP, 2014; Iroha et al., 2009; Ibrahim *et al*., 2013; Aruna and Mobashshera, 2012). Similarly, the resistance of the Gram-positive bacteria such as *Streptococcus pneumoniae* to the penicillin G has been reported elsewhere (Jacobs, 1999). Moreover, in many African countries, it was noticed that the pharmaceutical products can be easily obtained in the markets, by the roadside, managed by the sellers; thus, favoring the anarchic use of these products. While information on antibiotic in developed countries is readily available, similar knowledge relating to the resistance to antibiotic is rare in Chad. Therefore, the present study proposes to assess the demographic and socio-economic factors associated with the improper use of antibiotics.

MATERIALS AND METHODS

Type, period and area of study

This cross sectional study was performed by surveys carried out from June to July 2016 in two major cities of Chad, which were Moundou, the economic city, located at 437 km to the south of the capital, and N'Djamena, the capital city. These two communities were chosen on the basis of the heterogeneity of the population, the technical plateau of hospitals, the densities of the populations and the relatively high literacy rate that is likely to facilitate the filling of the questionnaire sheets without interpreters.

Target population

The target population was composed of men and women of varying age above 14 years. Due to the absence of data on the knowledge of antibiotics by the community of Chad, it was assumed that 50% of the population practice auto medication. Thus, on the basis of this estimation, the following formula was applied: $n = \frac{\varepsilon^2 p(1-p)}{i^2}$ (Mandhouj et al., 2004). This gave a sample size of 384 individuals, by opting for a confidence level of 95%, and a margin of error of 5%.

The study tool (questionnaire)

The questionnaire is composed of 54 questions divided into three sections. The first part focused on the sociodemographic characteristics of respondents such as sex, age, the level of study, occupation and marital status. The second section involved the origin of antibiotics (doctor and street vendor) and the mode of access to medicines (pharmacy, market). The third part focused on the assessment of the knowledge of respondents on the subject of antibiotics and the reasons for which these antibiotics were used (bacterial infection, viral infection, Influenza and fever), the duration of the activity of an antibiotic in the body and its side effects.

Nature of the investigation

Formal surveys have helped to collect data on the basis of the questionnaires, by direct interview of people in their home, the streets and the markets. These items of information were confidential; therefore, precautions had been taken to avoid the presence of a third person in accordance with the ethical principles of statistics.

Data analysis

The quantitative data were entered and processed using the Excel software and Statistical Package for the Social Sciences (SPSS). The analysis of the different parameters was made using the SPSS software (version 18) and the results were presented as the percentages. The graphs were obtained using the excel software (version 2010). The comparison of parameters such as the sociodemographic characteristics and the consumption of antibiotics were performed according to the test of Chi-square of Pearson. The differences were considered significant for values of $p < 0.05$.

RESULTS

In total, 500 people responded to the survey. Out of the 500, 472 returned sheets contained complete information whereas 28 incomplete sheets were removed. Therefore, the response rate was 94.4% (472/500).

Socio-demographic characteristics of respondents

Most of the participants were men (62. 7%) and 37.3% were women as shown in Table 1. The majority of the participants were between 25 and 34 years old (36.9%). Their levels of training varied: primary (40.8%), secondary (33.1%) and higher (26.1%). Their professional and marital status also varied. The marital status revealed 37.7% of single and 46.8% of married.

Respondents' attitudes and behaviors regarding antibiotic use

The study indicated (Table 2) that 50.4% of individuals surveyed had used the antibiotics in the six months that preceded the investigation against 49.6% who had not consumed. Among the 50.4% consumers of antibiotics, 2.5% had obtained it on their request, 2.7% on medical requirements after the laboratory examinations. 33.2% of medical prescriptions were systematic and probabilistic. In contrast, 6.8% of people had used the antibiotics

Table 1. Demographic and socio-economic characteristics of respondents.

Variables	Number	Percentage
Sex		
Male	296	62.7
Female	176	37.3
Age		
15-24	169	35.8
25-34	174	36.9
35-44	70	14.8
45-54	20	4.2
55-64	27	5.7
65 and more	12	2.5
Level of education		
Primary	193	40.8
Secondary	123	26.1
University	156	33.1
Professional status		
Without defined profession	40	8.5
Civil servant	43	9.1
Workers	59	12.5
Unemployed	33	7.0
House wife	39	8.3
Retired	24	5.1
Person of private means	29	6.1
Others (farmer, businessman, shoe-maker)	80	16.9
Marital status		
Single	178	37.7
Married	221	46.8
Divorced	54	11.4
Widow (Widower)	19	4.0

without medical prescription and 4.2% were advised by the street vendors. The modes of access to antibiotics varied. 3.4% of respondents consumed antibiotics available in their home; 20.6% had bought it in pharmacies; 11.0% bought it from street vendors; 2.3% took the remains of previous treatments, 6.4% purchased in the shelves by the roadside and 6.8% consumed drugs from the clinics and the physician offices. Among consumers of antibiotics, 23.09% were able to specify the nature of the antibiotics used, while 27.33% of them experienced difficulties.

Level of knowledge of respondents on the subject of antibiotics

The level of knowledge of the respondents on antibiotics is presented in the Figure 1. 42.9% of respondents believed that antibiotics can kill all microbes. 23. 72% asserted that antibiotics treated viral infections. Only 34.7% of the participants knew that antibiotics treat bacterial infections. For the symptoms of fever and flu, 40.8% argued that antibiotics can lower a fever and 54.4% thought that antibiotics cure the flu. 63.2% of the respondents knew that the duration of the activity of an antibiotic in the body was a few hours and 37.7% of them testified that its use could be harmful.

Demographic and socio-economic variables and use of antibiotics

The bivariate analysis of the data of the consumptions of antibiotics and the sociodemographic variables showed a use of antibiotics quite frequent among men than women ($p = 0.0060$). However, the most affected individuals in

Table 2. Respondents' attitudes and behaviors regarding antibiotic use.

Variables	Number	Percentage
Consumption of antibiotic the last six months		
Yes	238	50.4
No	234	49.6
Respondent-clinician interaction		
Consumption of antibiotic on respondent's request	16	2.5
medical prescription on the basis of antibiogramme	13	2.7
medical systematic prescription	157	33.2
without prescription	32	6.8
On mobile vendor's prescription	20	4.2
others	2	0.4
Knowledge of the nature of the antibiotic used		
Yes	113	23.09
No	129	27.33
Sources of antibiotic		
At home	16	3.4
In pharmaies	97	20.6
Through mobile vendors	52	11.0
Left over antibiotics	11	2.3
By the roadside of the streets	30	6.4
The physician offices and clinics	32	6.8

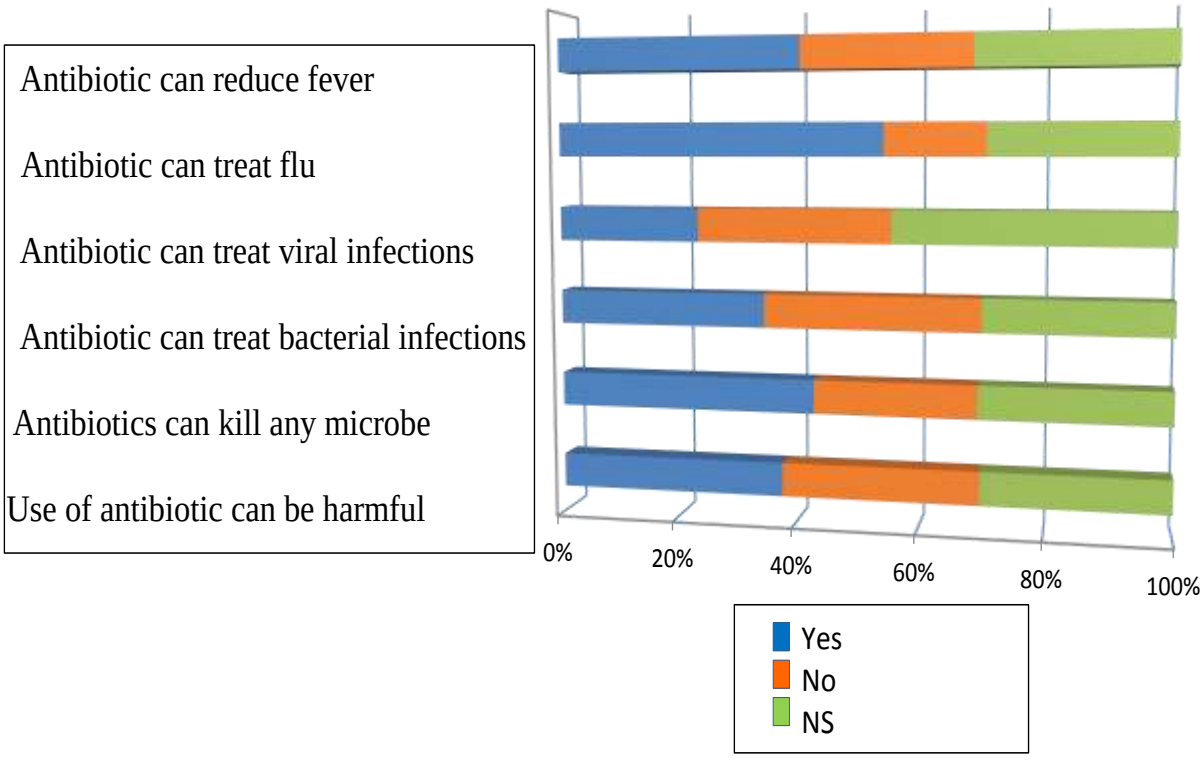

Figure 1. Respondents' knowledge on the use of antibiotic. NS: non specified.

the two sexes were within the age bracket of 25-34 years (107/174 = 61.49%) as compared to the other age groups: 15 to 24 years (79/168 = 46.74%), 35 to 44 years (32/70 = 45.71%), 45 to 54 years (4/20 = 20%), 55 to 64 (40.74%), 65 and more (5/12 = 41.66%). This difference was statistically significant (p = 0.002). The result of analysis showed that the consumption of antibiotics was quite high (40.8%) among individuals who had a relatively low level of training as compared to those of the secondary level (26.1%) or higher (33.1%). As regards the marital status, the married were the most concerned (116/221 = 52. 48%), as compared to the single (88/178 = 49, 43%), divorced (25/54 = 46. 29%) and widows/widowers (9/19 = 47. 36%).

DISCUSSION

The present study revealed a considerable rate of use of antibiotics and self-medication among the community during the six months preceding the survey (50.4%). This rate could be explained in part by the fact that antibiotics were considered as magical products and the high desire of the Community to have a fast relief of the disease (Widayati et al., 2012). However, it is interesting to mention that this percentage of the use of antibiotics observed in our study was much greater than that reported in other studies in Jordan which was 28% (Ghadeer et al., 2012). It is quite possible that this large gap between Chad and Jordan in the matter of self-medication and the use of antibiotics is linked to the difference in the human development index (HDI) of the two countries. It may be recalled that the development index of Chad (0.340) was lower than that of Jordan (0.700) (IDH, 2012). However, this rate is consistent with the results of Ivory Coast in which 59.7% of the community had used antibiotics during the 12 months preceding the survey of Hounsa (Hounsa et al., 2009). Other investigations had shown that approximately, 60% of prescribed antibiotics in Nigeria in 2000 were not justified (Raynaud, 2008). In Nepal, more than 50% of the prescribed antibiotics in 1996 were not necessary, and 40% of prescriptions were inadequate (Raynaud, 2008). On the whole, the proportion of prescriptions of antibiotics that was not justified reached an average of 50% (Raynaud, 2008). This misuse of antimicrobials that is widespread in many countries could be linked to the gaps in the application of regulations relating to the purchase of antibiotics (Al-Bakri et al., 2005; Sawair et al., 2009)

Moreover, the study showed that the consumption of drugs can vary depending on the sex and age. The frequency obtained was higher among men (p = 0.00601). Similarly, age was a factor that was associated with the consumption of antibiotics (25-34 years, p = 0.002). Similar studies carried out in France have also reported a significantly high use of antibiotics among young people, and which decreased with age for both men and women (Raynaud, 2008).

As far as the level of training is concerned, the data showed a link between the consumption of antibiotics and the level of training. It appeared that people with low level of education were more concerned than those with higher level of education and especially university (p = 0.002). This gap could be explained by the difference in the level of knowledge in the two categories of persons with respect to antibiotics. In contrast, it seemed that there was no correlation between the consumption of antibiotics and the profession of respondents (p = 0.079). The present study also showed a high percentage of consumption among the married than among the single (p = 0.0082). This could be explained by the fact that certain infections of the genital apparatus remained silent among women. The husband may be treated, but can be re-infected during each sexual intercourse. The persistence of the infection among the married was more apparent in polygamous families because a genital infection of one of the partners finally reached all the members of the family (women and the man). In such cases, the administration of antibiotic therapy to all the partners at the same time can stop the reinfection.

As regards the mode of access to medicines, the purchase of drugs in the illegal structures can be linked to economic factors and the easy access to drugs sold outside pharmacies. The majority of persons interviewed purchased antibiotics without medical prescriptions and on advice of street vendors. In addition, not all the prescribed antibiotics in a systematic way (33.2%) by clinicians were purchased in pharmacies (20.6%). This diversity of mode of access to antibiotics may be explained by the low economic power of the community associated with the low knowledge on the harmful effects of drugs sold in parallel. Furthermore, these drugs sold in pharmacies of Chad were usually two times more expensive than those sold in parallel markets, by the roadside or in mobile shops. This difference in the purchase prices may encourage the destitute people in the purchase of medicines outside pharmacies because they are less expensive and also sold without harassment.

The results showed that the perception of knowledge was disparate about the use of antibiotics. A minority of interviewees knew that antibiotics treated bacterial infections (34.7%). In contrast, a high percentage of respondents believed that antibiotics can kill viruses, heal flu or reduce fever. These false perceptions and confusions were reported in several surveys worldwide (Widayati et al., 2012). In Jordan, for example, a similar survey showed that the percentage of the population knowing that antibiotics treat bacterial infections was low (29.9%). Other investigations underscored medical prescriptions of antibiotics in the management of the symptoms such as colds, flu and fever which were often of viral origin (Stott, 1979; Rasamoelisoa et al., 1999; Mainous et al., 1996). This medical act may also

contribute to the maintenance of confusion within the community on the exact target of antibiotics. Another work specified that the prescription of antibiotic was also a way to give hope to the customers in order to keep patients 'confidence (Faber et al., 2010).

With regards to the duration of activity of an antibiotic in the human body, more than half of the respondents knew that the time of activity of any drug was of a few hours. This level of knowledge could be linked to the empirical knowledge because any medicine has limited activity in time. In contrast, very few people knew that antibiotics can be harmful and this could be explained by the low level of knowledge on the side effects of an antibiotic by the general public.

Conclusion

This study is the first of its kind conducted in Chad, focusing on the analysis of factors related to the use of antibiotics in the community. It revealed a large heterogeneity of socio-demographic factors involved in the inappropriate consumption of antibiotics from different sources. The medical requirements were often empirical. Thus, the risks of selection of multidrug-resistant strains within the commensal Flora was therefore quite high and constituted a great threat to human and animal health. Consequently, educational campaigns, training and increased awareness must be implemented in order to improve the knowledge on antibiotics so as to operate changes in behavior and attitude in the use of these products.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

ACKNOWLEDGEMENTS

This work is the result of a collaborative project between the Ministry of Health and the Ministry of Higher Education, and the authors thank them for their supports. The authors thank Dr. Hassan Mahamat Hassan (physician), Mr Dabsou Guidassou (statistician-demographer) and Mr Madjitoloum Moyangar (demographer) of the Ministry of Public Health of Chad for the collaboration in the achievement of this work. The authors thank the persons interviewed and who have sacrificed their time to participate in the investigation. Finally, the authors also thank Issa Djimet and Alladoum Roland from the University of Doba for their critical reading.

REFERENCES

Al-Bakri AG, Bustanji Y, Yousef AM (2005). Community consumption of antibacterial drugs within the Jordanian population: sources, patterns and appropriateness. Int. J. Antimicrob. Agents 26(5):389-395.

Aruna K, Mobashshera T (2012). Prevalence of extended spectrum beta-lactamase production among uropathogens in south mumbai and its antibiogram pattern. Excli J. 11:363-372.

Bywater RJ (2004). Veterinary use of antimicrobials and emergence of resistance in zoonotic and sentinel bacteria in the EU. J. Vet. Med. Series B, 51(8-9):361-363.

Chauvin C (2009). Usage des antibiotiques et résistance bactérienne en élevage de volailles (Thèse de doctorat), Université Rennes 1. 25 p.

Faber MS, Heckenbach K, Velasco E, Eckmanns T (2010). Antibiotics for the common cold: expectorations of germany's general population. Euro. Surveill. 15(35): 1-7.

Ghadeer ARY, Suaifan MS, Dana A, Darwish Hebah Al-Ijel, Al-Motassem M, Yousef RM, Darwish A (2012). Cross-sectional study on knowledge, attitude and behavior related to antibiotic use and resistance among medical and non-medical university students in Jordan. Afr. J. Pharm. Pharmacol. 6(10):763-770.

Gillani WS, Azhar SS, Ling AO (2010). Risk evaluation under various speculations of antibiotic usage; A cohort survey among outpatients of pinang, Malaysia. Euro. J. Gen. Med. 7(3):303-309.

Hounsa A, Kouadio L, De Mol P (2009). Automédication par les antibiotiques provenant des pharmacies privées de la ville d'Abidjan en Côte d'Ivoire. Med. Mal. Infect. 40(6):333-340.

Ibrahim ME, Bilal NE, Magzoub MA, Hamid ME (2013). Prevalence of Extended-spectrum β-Lactamases-producing Escherichia coli from Hospitals in Khartoum State, Sudan. Oman. Med. J. 28(2): 116-120.

Indice de Développement Humain (IDH) (2016). https://www.populationdata.net/palmares /idh/1. Accessed 24/4/2017.

Iroha IR, Ezeifeka ES, Amadi ES, Umewurike CR (2009). Occurrence of Extended Spectrum Beta Lactamase Producings Resistant *Escherichia coli* and *Klebsiella pneumoniae* in Clinical Isolates and Associated Risk Factors. Res. J. Biolog. Sci. 4(5):588-592.

Jacobs MR (1999). Drug-resistant Streptococcus pneumoniae: rational antibiotic choices. Am. J. Med. 106(5):19-25.

Mainous AG, Hueston WJ, Clark JR (1996). Antibiotics and upper respiratory infection: do some folks think there is a cure for the common cold. J. Fam. Pract. 42(4):357-361.

Mandhouj O, Ben Salem K, Ben Abdelazoz A, Letaif M, Soltani O (2004). Fiche technique pratique en epidemiologie - biostatique. Microbiol. Hyg. Alim. 46(16):76-77.

Rasamoelisoa JM, Tovone XG, Andriamady C, Rakotoarimanana DR (1999). Evaluation de l'utilisation des antibiotiques en milieu hospitalier pédiatrique. Arch. Inst. Pasteur Madagascar 65(2):124-126.

Raynaud D (2008). Les déterminants du recours à l'automédication. Rev. Franç. Aff. Soc. 1(1):81-94.

Réseau International de l'Institut Pasteur (RIIP, 2014). Paris, France. https://www.pasteur.fr/ fr /international

Sawair FA, Shayyab MH, Al-Rabab'ah MA, Saku T (2009). Prevalence and clinical characteristics of tori and jaw exostoses in a teaching hospital in Jordan. Saudi Med. J. 30(12):1557-1562.

Stott NCH (1979). Management and outcome of winter upper respiratory-tract infections in children aged 0-9 years. Br. Med. J. 1(6155):29-31.

Widayati A, Suryawati S, Crespigny C, Janet EH (2012). Knowledge and beliefs about antibiotics among people in Yogyakarta City Indonesia: a cross sectional population-based survey. Antimicrob. Resist. Infect. Control 1(38): 1-7.

African Journal of Pharmacy and Pharmacology

Related Journals Published by Academic Journals

- *Journal of Medicinal Plant Research*
- *African Journal of Pharmacy and Pharmacology*
- *Journal of Dentistry and Oral Hygiene*
- *International Journal of Nursing and Midwifery*
- *Journal of Parasitology and Vector Biology*
- *Journal of Pharmacognosy and Phytotherapy*
- *Journal of Toxicology and Environmental Health Sciences*

academicJournals