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Full Length Research Paper

Health care providers' knowledge, attitude and experience of adverse drug reaction reporting

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Adverse drug reaction (ADR) is a noxious and undesirable reaction to drugs at dosage normally used in humans for diagnosis, treatment or prophylaxis of diseases or ailments. Spontaneous reporting is currently the major back bone for the detection of adverse drug reactions. The objective of the study was to assess the knowledge, attitude, and practice of health professionals towards ADR reporting in Boru Meda Hospital, North East Ethiopia. A quantitative cross-sectional study design was used. A self-administered questionnaire was used to collect data from the health professionals, and the collected data was analyzed using SPSS (version 16). A test of association for selected variables was done using Pearson chi–square. From a total of 57 respondents, 40 (70.1%) were able to differentiate ADR from side effects. Thirty six (63.2%) and 34 (59.6%) respondents knew the availability of national reporting system and ADR reporting form in Ethiopia, respectively. Majority, 46 (80.7%), of the respondents said that ADR should be reported only when they are serious and life threatening. Out of 12 respondents who encountered ADR in the past 12 months in their clinical activities, 10 reported to responsible body. Health professionals working in Boru Meda Hospital have good attitude towards ADR reporting and good reporting culture of encountered ADRs, but insufficient knowledge about ADRs. The unavailability of ADR reporting forms take the lions part in significantly discouraging them to detect and report ADRs.

Key words: Adverse drug reactions, knowledge, attitude, practices, health professionals, pharmacovigilance, Boru Meda Hospital.

INTRODUCTION

Nowadays drugs have changed the way in which diseases are treated. Despite all their advantages, adverse reactions to medicines are a common but preventable, cause of illness, disability and even death (WHO, 1972). An adverse drug reaction (ADR) is defined as noxious, unintended and which occurs at dosages normally used in human beings for prophylaxis, diagnosis or therapy of disease or for the modification of physiological function (Parker, 1983). Basically, ADRs are defined as type A, type B, type C and type D. Type A reaction (predictable) is related to dosage and is an extension to the normal pharmacology of the medication; type B reaction (unpredictable) is unrelated to normal pharmacology; type C reactions are associated with prolonged therapy; type D reactions are delayed reactions (Rawlings and Thompson,1977; Naranjo et al., 1981).

The history of pharmacovigilance (ADR monitoring)

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dates back as much as thirty years when the 20th world's gathering adopted a mechanism to start a task on the possibility of international system of monitoring adverse reactions of drugs. This was based on Thalidomide disaster that caused death of thousands of children, creating the basis of the World Health Organization (WHO) program on worldwide system for drug monitoring. As a result international system for monitoring ADR was proposed (WHO, 2000, 2001).

ADRs have been regarded as a major public health problem since they result in a measurable percentage of hospital admissions and an economic burden (Lundkvist and Jonsson, 2004). Hence, ADRs reduce patients' quality of life and impose a significant financial burden on the health care systems. The commitment of health care providers to ADRs databases is massively significant and has energized continuous ascertainment of the risk-benefit ratio of few drugs and in addition, added to signal detection of unsuspected and unusual ADRs (Wysowski and Swarlz, 2005). The part of healthcare providers is crucial in recording and reporting suspected ADRs in order to caution regulatory agencies about rising safety concerns and thereby calls for immediate and appropriate action. All health care providers ought to be urged to report all suspected adverse reactions resulting from medicines, especially when the reaction is not expected and potentially serious or clinically significant (WHO, 2006).

Once a drug is available to the public, making a determination about its safety is the shared responsibility of all healthcare providers (Zolezzi and Parsotam, 2005). Pharmacovigilance programs encourage ADR detection, enable ADR documentation, promote the reporting of ADRs and elucidate a mechanism for monitoring the safety of drug use in high risk patient population. A complete progressing ADR system ought to incorporate components for observing, distinguishing, assessing, recording, and reporting ADRs and in addition, mediating and giving instructive criticism to prescribers, other health care providers and patients (Kohn et al., 2000). So far, there is no study conducted in Boru Meda Hospital to assess ADR reporting. This study investigated the knowledge, attitudes and practices of healthcare professionals towards ADR reporting in Boru Meda Hospital.

MATERIALS AND METHODS

A cross sectional study was conducted among the healthcare providers (Nurses, Doctors, Pharmacy Personnel, Midwifery and Health Officers) working at Boru Meda Hospital, North East Ethiopia from March 2014 to June 2014. The hospital is located 411 km North East of Addis Ababa, Ethiopia. It serves Dessie town and the surrounding population, which is about 2.1 million. The hospital has different units: internal medicine, pediatrics, gynecology/obstetrics, surgery, emergency, psychiatry, ophthalmology, hospital pharmacy, dermatology, orthopedics and multidrug resistant tuberculosis treatment unit.

The dependent variables were knowledge, attitude and practice of ADR reporting whereas the independent variables were age, sex, profession, and years of experience. A structured questionnaire, adapted from other similar studies (Kamtane and Jayawardhin, 2015; Santosh et al., 2013) on knowledge, attitude, and practice of the health professionals on ADR reporting with a little modification to suit the hospital setting, was used to collect data. The questionnaire was distributed to the healthcare professionals who were willing to participate in the study. The completed questionnaires were collected from the participants within one week. Questionnaires included socio-demographic variables, variables used to measure knowledge, attitude and practice about ADR reporting. Knowledge about ADR reporting was determined using fourteen multiple choice questions. Participants who scored seven and above out of fourteen were considered to have adequate knowledge, otherwise they were considered as having inadequate knowledge. Attitude was evaluated utilizing twelve questions rated on a three point Likert scale. Based on the cumulated score, respondents who scored greater than or equal to 50% and less than 50% of the total were considered as having favorable attitude and unfavorable attitude, respectively. Regarding practice about ADR reporting, those who record and report at least one of the encountered ADRs are considered to have good practice while those who are unable to do this are regarded as having poor practice.

The collected data was checked for completeness, categorized and the results were analyzed using SPSS version 16, interpreted and presented using tables and charts. The chi-square test was used to find out the association between the outcome and independent variables and statistical significance was set at $p<0.05$.

Ethical clearance was obtained from College of Medicine and Health Sciences, Wollo University, and permission was sought from Boru Meda Hospital.

RESULTS

Demographics

Out of 62 questionnaires distributed, only 57 were filled and returned within the stipulated time frame giving a response rate of about 92%. From 57 health professionals, majority (57.9%) were nurses. Most of the participants 45 (78.9%) were in the age range of 20-29 years and 34 (59.6%) were males as shown in Table 1.

Knowledge

Forty (70.2%) of respondents could differentiate ADR from side effects. Only 21 (36.8%) respondents knew the term pharmacovigilance. Likewise, 36 (63.2%) and 34 (59.6%) respondents were aware of the availability of national reporting system and ADR reporting form in Ethiopia, respectively. Out of the total study participants, 46 (80.7%) and 48 (84.2%) health professionals said that ADRs should be reported only when they are life threatening and cause disability, respectively as shown in Table 2.

Attitudes

The study showed that 53 (93.0%) respondents

Table 1. Socio-demographic characteristics of health professionals in Boru Meda Hospital, North East Ethiopia, March – June, 2014.

Variables	Number (n = 57)	Percent
Age		
20-29	45	78.9
30-39	9	15.7
40-49	3	5.4
Sex		
Male	34	59.6
Female	23	40.4
Profession		
Physician	5	8.8
Pharmacy personnel	8	14.0
Nurse	33	57.9
Health officer	3	5.3
Midwifery	8	14.0
Year of experience		
0-3	21	36.8
4-6	20	35.0
7-9	5	8.7
>10	11	19.5

established that ADR reporting should be part of their duty and 40 (70.1%) supported that ADR reporting should be mandatory. Six (10.5%) of health professionals believed that ADR reporting is time consuming activity with no outcome. The results also showed that, 38 (66.7%) health professionals agreed that they were not adequately trained in ADR reporting as shown in Table 3.

Practices

From these 57 study participants, only 12 (21.1%) met patients with ADR in their clinical practice in the past 12 months, among which 10 (83.3%) recorded the encountered ADR in patient follow up card and also reported to responsible body as shown in Table 4.

Association between the respondents' profession and the outcome variables

From a total of 57 health care providers, 24 (42.1%) have adequate knowledge (more than 50% right response), the rest have inadequate knowledge (less than or equal to 50% right response) of the total 14 questions used to assess their knowledge as shown in Tables 2 and 5. With respect to overall level of attitude towards ADR reporting, the majority 49 (86.0%) have favorable attitude towards ADR reporting based on a three point Likert scale as shown in Tables 3 and 5. Table 5 also, shows the association between respondents' profession and knowledge, attitude and practice of ADR reporting in the study area. There is a significant ($X^2 = 11.348$; $p = 0.023$) association between respondents' profession and their practice on ADR reporting. On the contrary, there is no significant association between respondents' profession and knowledge and attitude towards ADR reporting.

DISCUSSION

Adverse drug reaction monitoring, is an area of pharmaceutical consideration which bargains principally with the recognition, management and reporting of ADRs which may result from drugs that are taken in normal dose for prophylaxis or treatment of diseases. These ADRs may vary from simple reactions to permanent disability and death (Lazarou et al., 1998; Wiffen et al., 2002). The knowledge, attitude and practice of health professionals on ADR reporting is closely associated with their professional roles and can alleviate problems associated with under reporting ADRs.

In the present study out of the total 57 respondents, 15 (26.3%) of them are unable to differentiate ADRs from side effects. This might be due to lack of sufficient information regarding ADR in the courses and/or

Table 2. Knowledge on ADR reporting and monitoring in Boru Meda Hospital, North East, Ethiopia, March – June, 2014.

Variables	Yes (%)	No (%)	Neutral (%)
Do you believe all the drugs available in the market are safe?	6 (10.5)	51 (89.5)	0
Do you think that ADR is the same as with side effects?	15 (26.3)	40 (70.2)	2 (3.5)
Do you know the term pharmacovigilance?	21 (36.8)	33 (57.9)	3 (5.3)
Do you know the national ADR reporting system?	34 (59.7)	21 (36.8)	2 (3.5)
Do you know the availability of ADR reporting form?	36 (63.2)	21 (36.8)	0
Do you know how to report ADR?	37 (64.9)	19 (33.3)	1 (1.8)
ADRs should be reported only when they are:			
Serious and life threatening	46 (80.7)	11 (19.3)	0
Sever and cause disabilities	48 (84.2)	9 (15.8)	0
Mild and cause less inconveniences	24 (42.1)	33 (57.9)	0

Table 3. Attitudes towards ADR reporting among health professionals in Boru Meda Hospital, North East, Ethiopia, March – June, 2014.

Variables	Yes (%)	No (%)	Neutral (%)
Do you feel that ADR reporting can benefit the public health?	52 (91.2)	3 (5.3)	2 (3.5)
Do you feel that ADR reporting improves quality of patient care?	52 (91.2)	5 (8.8)	0
Do you feel that one report can make a difference?	38 (66.7)	17 (29.8)	2 (3.5)
Do you feel that ADR reporting is part of duty of health professionals	53 (93.0)	2 (3.5)	2 (3.5)
Do you feel that reporting ADR should be compulsory?	40 (70.1)	12 (21.1)	5 (8.8)
Do you feel that only ADR that cause persistent disability should be reported?	20 (35.0)	36 (63.2)	1 (1.8)
Do you feel that ADR reporting is time consuming activity with no outcome?	6 (10.5)	50 (87.7)	1 (1.8)
Do you feel that proper training should be provided to the health professionals for ADR reporting?	36 (63.2)	17 (29.8)	4 (7.0)
Do you feel that you are adequately trained in ADR reporting?	17 (29.8)	38 (66.7)	2 (3.5)
Do you feel that confidentiality should be maintained while ADR reporting?	35 (61.4)	17 (29.8)	5 (8.8)
Do you worry about legal problems while you think of ADR reporting	21 (36.8)	28 (49.1)	8 (14.1)
Do you feel that ADRs should be reported at regular bases?	40 (70.1)	15 (26.4)	2 (3.5)

Table 4. General practices regarding ADR reporting in the past twelve months in Boru Meda Hospital, North East, Ethiopia, March – June, 2014.

Variables	Yes (%)	No (%)
Have you ever encountered patients with ADR in your clinical practice, in the last 12 months?	12 (21.1)	45 (78.9)
Have you noted the ADR you encountered on the patients clinical record?	10 (83.3)	2 (16.7)
Have you ever reported the encountered ADRs?	10 (83.3)	2 (16.7)
To whom did you report the encountered reaction?		
Hospital	2 (20)	
Manufacturer	0	
MOH[a]	0	
FMHACA[b]	4 (40)	
Pharmacy personnel	4 (40)	

[a]Federal ministry of health of Ethiopia, [b]Food, Medicine and Health care administration and control authority of Ethiopia.

trainings. However, WHO recommend that in order to avoid increasing the figures of drug induced problems; it is helpful to hold the term side effect for minor effects which are related to the pharmacological properties of the

Table 5. Association of respondent' profession with knowledge, attitude and practice of ADR reporting in Boru Meda Hospital, North East, Ethiopia, March – June, 2014.

Variables	Profession					Chi-square (*p-value*)
	Physician (%)	Pharmacy personnel (%)	Nurse (%)	Health officer (%)	Midwifery (%)	
Knowledge level						
Adequate	2 (40)	7 (87.5)	10 (30.3)	1 (33.3)	4 (50)	8.957
Inadequate	3 (60)	1 (12.5)	23 (69.7)	2 (66.7)	4 (50)	(0.062)
Attitudes						
Favorable	5 (100)	8 (100)	27 (81.8)	2 (66.7)	7 (87.5)	3.534
Unfavorable	0	0	6 (18.2)	1 (33.3)	1 (12.5)	(0.473)
Practice level						
Good	0	5 (62.5)	20 (60.6)	1 (33.3)	2 (25)	11.348
Poor	5 (100)	3 (37.5)	13 (39.4)	2 (66.7)	6 (75)	(0.023)*

*Statistical significance was set at $p<0.05$.

drug (Ernst and Grizzle, 2001). Among the 57 participants, 34 (59.7%) and 36 (63.2%) health care providers were aware of the availability of national ADR reporting system and reporting form in Ethiopia. But a similar study in Jimma Zone showed that 23.17% and 25.61% health professionals knew the availability of national ADR reporting system and reporting form in Ethiopia (Angamo and Wabe, 2012). Knowledge on the availability of ADR reporting system and reporting forms can foster the practice of ADR reporting and hence reduce the risk associated with ADRs.

Knowledge of the term pharmacovigilance and its roles is one of the components used to assess the overall knowledge of the study participants on ADR reporting. Accordingly, among the total of 57 respondents, only 21(36.8%) of the respondents (6 pharmacy personnels, 3 physicians, 10 nurses and 2 health officers) knew pharmacovigilance and its roles. According to a study done in Nigeria on attitude of doctors to ADR reporting showed that 40.4% of the respondent were aware of the existence of National Pharmacovigilance Center in their country (Rehabs and Vasudeuk, 2002; Kazeam and Jacob, 2009) and this shows that they have more awareness towards ADR monitoring than professionals in this study. On the contrary, a study in Jimma Zone (Angamo and Wabe, 2012) showed that 19.5% of the participants were aware of the term pharmacovigilance and its roles, showing that they have less awareness towards ADR monitoring than professionals in this study.

Attitudes are potentially modifiable variables exerting a strong influence on ADRs reporting, the greater the patient attitude the more positive influence on the overall ADRs reporting rate (Herdeiro et al., 2006). In this study, fifty three (93.0%) respondents felt that ADR reporting should be part of their duty and 40 (70.1%) supported that ADR reporting should be mandatory. On the contrary, 17 (29.8%) did not believe that one report of ADR makes a difference. Besides, most respondents 52 (91.2%), agreed that reporting ADR is important for the public and improves quality of patient care respectively. Educational program can significantly modify health professionals' reporting-related attitudes and influence the ADRs reporting behavior in a positive manner.

Reporting the occurrence of ADRs is important to prevent morbidity and mortality associated with the specific drug that caused the adverse effect. From a total 57 respondents, only 12 (21.1%) met patients with ADRs and ten of them recorded and reported it to the concerned body. A study done in Turkish showed that 65% of the health care providers met patients with ADRs and 7% of them reported ADR to their National Pharmacovigilance Center (Toklu and Uysal, 2008). ADR detection and reporting requires an appropriate knowledge regarding the outcome of ADRs. So there should be awareness raising programmes and trainings regarding ADRs in order to encourage health professionals to detect and report ADRs.

The overall knowledge level of health professionals in this study is below the average, and hence, this might affect their experience in recording and reporting of ADR, despite their higher level of perceptions towards ADR reporting. It is important to note the lack of association between respondents profession with the knowledge and attitude level in this study. Notwithstanding, one needs to look into consideration several confounding factors that affect the validity of such comparisons, such as the use of different instrument to measure the outcome variable.

Limitations

The self-reporting nature of the study depends on the

exactness and trustworthiness of the respondents. So results may stray from what really happens in practice. The small sample size may make it hard to extrapolate conclusions from this study; however, it can provide an indication of perspectives and experiences of these groups of health care providers.

Conclusion

This study showed that health professionals working in Boru Meda Hospital have favorable attitude about ADR reporting, and there is a good reporting culture of encountered ADRs, but insufficient knowledge about ADRs and unavailability of ADR reporting forms take the lions part in significantly discouraging them to detect and report ADRs. However, encouraging all health professionals to report and also provide trainings that would significantly improve ADR reporting. Our study emphatically proposes that there is an awesome need to make awareness and to advance the reporting of ADRs amongst health care providers, which will establish a strong framework for health care providers to be steadily included in quality pharmacovigilance and unconstrained reporting in their future practices.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Detoxification potentials of an alcoholic bitter on carbon tetrachloride-induced oxidative damage in wistar albino rats

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The increased demand for herbal remedies and natural quest for alcohol consumption has positioned alcoholic herbal preparations (bitters) as ideal drink. Bitters are acclaimed to have blood detoxifying and liver cleansing potentials. This study investigated the acclaimed detoxifying potentials of an alcoholic bitter (AB) on carbon tetrachloride (CCl_4) induced toxicity. Twenty five male Wistar albino rats were grouped and treated, thus: group I served as normal control, groups II, IV and V were given single dose of 1.2 ml CCl_4/kg body weight (bwt). Groups IV and V were administered 1.4 and 2.8 ml AB/kg bwt, respectively, while group III animals were administered 1.4 ml AB/kg bwt. Results obtained showed significant ($p<0.05$) increase in lipid peroxidation and in activities of liver function enzymes, reductions in glutathione concentration and activities of catalase, glutathione peroxidase and reductase in groups administered AB and CCl_4 only as well as in groups treated with AB after CCl_4 exposure. These observations indicate manifestation of oxidative stress induced by excessive consumption of high percentage alcoholic content of the bitter. Similarly, the result trends of other antioxidant parameters studied indicated significant oxidative damage and thus the inability of the alcoholic bitter to ameliorate xenobiotics induced damage.

Key words: Alcohol, bitters, toxicants, oxidative stress, hepatotoxicity, xenobiotics.

INTRODUCTION

Bitters are botanical ingredients prepared by alcoholic extraction of aromatic herbs, barks, roots and/or fruits for their flavour and medicinal properties, such that the end result is characterized by a bitter or bitter sweet flavour. Bitters have been reported to meet both health and other needs of its users (Hadley, 2005). In recent times, bitters prepared and sold to consumers tend to be highly alcoholic (≥ 42%) and because of the natural quest for alcohol consumption many tend to abuse alcoholic bitters despite the obvious consequences of acute and chronic

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intoxication of alcohol (Nwodo, 1999; Ramond et al., 2011). The long term health effects of consuming more than the required amount of bitters may be basically the same as taking excess vodka, whisky or rum. The possible adverse health effects of this habit include stroke, anaemia, liver cirrhosis, cancer and reduced fertility (Expert Care, 2013). Numerous pathways act in concert, reflecting the spectrum of an organism's response to a myriad of direct or indirect action of alcohol (Cederbaum, 2001; Wu and Cederbaum, 2003). Excessive generation of reactive oxygen species (ROS) is one of such mechanism, and this has been the focus of much research (Adachi and Ishii, 2002).

The liver is the major metabolizing organ that ingested toxicants encounter, and has very high metabolic activity due to the high content of cytochrome P-450. The liver is a major target organ of CCl_4 toxicity (Södergren et al., 2001) as well as alcohol toxicity (Wu and Cederbaum, 2003). In most developing countries, toxicity and therapeutic information of herbal concoctions are scarce or none existence. This study was designed to determine the therapeutic or adverse effect of an alcoholic bitter with herbal ingredients. The ability of the alcoholic bitter to ameliorate carbon tetrachloride induced oxidative damage was used to evaluate the therapeutic potentials. This was predicated on the alleged use of the alcoholic bitter as a detoxifying (detoxicant) and/or systemic cleansing agent.

MATERIALS AND METHODS

Procurement of samples

Five bottles of an alcoholic bitter (200 ml per bottle) were bought from Ekeonuwa market in Owerri Municipal Local Government Area, Imo State. The bitter is an alcoholic preparation containing seven herbal ingredients which include *Khaya invorensis, Capparis erythrocarpus, Mondia whitei, Lecaniodis cuscupancides, Dialium guineense, Treculia africana* and *Crytolepsis sanguinolenta.* The alcoholic bitter is characterized by a bittersweet flavour. The animals used in this study were 25 male Wistar albino rats with an average weight of 150±20 g. The rats were obtained from the small animal holding unit of the Department of Veterinary Medicine, University of Nigeria Nsukka. The rats were housed in laboratory cages kept in a well-ventilated animal house in the Department of Biochemistry, Federal University of Technology, Owerri (FUTO). The animals were allowed to acclimatize to laboratory conditions for two weeks and were allowed free access to feed and water. This study adhered to the guideline for the handling of laboratory animals (NIH, 1985) after the approval by the Department of Biochemistry Research Ethics Committee.

Experimental design

The amount of alcoholic bitter used in this study was calculated based on how people consume the mixture. Our preliminary survey showed that most consumers take the whole content of 200 ml per bottle while others take 50% content of the 200 ml bottle (100 ml) at a time. Assuming that the average weight of adult human male is 70 kg, the volume of alcoholic bitter administered to the animals were calculated using the corresponding body weight per rat. Also using the consumption pattern of the alcoholic bitter by adult males, two groups which received 200 ml (100%) and 100 ml (50%) of the alcoholic bitter were created.

Twenty five male Wistar albino rats were divided into five groups with each group having 5 rats. Groups I and III served as normal and alcoholic-bitter control, respectively and were not exposed to carbon tetrachloride (CCl_4). Groups II, IV and V were administered intraperitoneally a single dose of 1.2 ml/kg body weight (bwt) CCl_4 on day one. Carbon tetrachloride used was dissolved in olive oil at 2:1 ratio. Twenty four hours after this induction, the animals were treated every day for seven days as follows: Group I received rat pellets only (Normal control); Group II received rat pellets + 1.2 ml/kg bwt of CCl_4 (CCl_4 control); Group III received rat pellets + 1.4 ml/kg bwt of alcoholic bitters (Alcoholic-bitter control); Group IV received rat pellets + 1.2 ml/kg bwt CCl_4 + 1.4 ml/kg bwt alcoholic bitters; Group V received rat pellets + 1.2 ml/kg bwt CCl_4 + 2.8 ml/kg bwt alcoholic bitters.

Collection of blood and liver samples

On the 8th day after 24 h fast, the animals were euthanized and blood samples were drawn from the heart by cardiac puncture into anticoagulant free bottles. Afterwards, the blood was centrifuged at 3000 rpm for 10 min to obtain serum for biochemical studies. Rat livers were excised and washed in 1.15% KCl buffered solution and then liver homogenate was prepared in 10 mMKCl/phosphate buffer with ethylene diaminetetra acetic acid (EDTA; pH 7.4). This was centrifuged at 12,000 × g for 60 min to obtain the supernatant (liver sample).

Biochemical analyses

Assay of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity were done by the method of Reitman and Frankel (1957) for the quantitative *in-vitro* determination of alanine aminotransferase in serum using commercial test kit purchased from Randox Randox Laboratories Ltd, Crumlin Co Antrim, UK. Also, activity of alkaline phosphatase (ALP) was assayed by the method described by Klein et al. (1960) and Babson et al. (1966) as described in commercial test kit purchased from Randox Laboratories Ltd, Crumlin Co Antrim, UK. Serum albumin (ALB) concentration was determined by the method of Doumas et al. (1971) as described in commercial test kit purchased from Biosystems USA. Concentration of serum total protein (TP) was determined as described by Tietz (1995). Briefly, into tubes labelled reagent blank, standard, sample and sample blank were added 0.02 ml distilled water, standard protein preparation, sample and sample, respectively. Afterward, 1.0 ml of total protein reagent 1 was added to all the tubes, but sample blank in which reagent 2 was added. The content of these test tubes were mixed appropriately and incubated at 25°C for 30 min. The absorbance was taken at 546 nm, and the concentration determined. Serum globulin concentration was calculated using the formula:

Total Protein (TP) – Albumin (ALB).

Malondialdehyde (MDA) concentration was determined by the method described by Wallin et al. (1993). Briefly, test tubes were prepared and labelled sample and blank. To the sample tube, 0.1 ml liver sample and 0.45 ml normal saline were added appropriately mixed before 0.5 ml, 25% trichloroacetic acid (TCA) and 0.5 ml of 17% thiobarbituric acid (TBA) in 0.3% NaOH were added. To the Blank tubes, 0.1 ml dH_2O and same quantity of TCA, TBA and normal saline were added. The mixture was incubated at 95°C for

40 min, cooled and 0.1 ml 20% sodium dodecyl sulphate was added and absorbance read spectrophotometrically at 532 and 600 nm against blank.

Glutathione concentration was determined as described by King and Wootton (1959). Briefly, 0.1 ml liver sample and 0.1 ml dH_2O were, respectively added to test tubes labelled test and blank. Also, added to both tubes were 0.9 ml dH_2O and 0.02 ml 20% sodium sulphite. The setup was mixed properly and allowed to stand at 25°C for 2 min. Afterwards, 0.02 ml of lithium sulphate and 0.02 ml of 20% Na_2C0_3 were added to all test tubes and mixed. Then, 0.2 ml phosphor-18-tungstic acid was added, mixed and allowed to stand for another 4 min for maximum colour development. Finally, 2.5 ml 2% sodium sulphite was added and absorbance taken at 680 nm within 10 min.

The activity of glutathione peroxidase (GPx) was assayed by the method described by Paglia and Valentine (1967). Briefly, in a test tube containing 0.1 ml liver sample, 3 ml phosphate buffer, 0.55 ml guaiacol, 0.03 ml H_2O_2 were added and appropriately mixed. The absorbance of the mixture was taken at 436 nm for 2 min at 30 s intervals. Glutathione reductase activity was determined by the Mavis and Stellwagen (1968) continuous spectrophotometric rate determination as described in commercial test kit purchased from Sigma-Aldrich, MO USA.

Superoxide dismutase (SOD) activity in liver homogenate was assayed by the method described by Xin et al. (1991). Briefly, a stock solution was prepared with 0.1 ml liver sample and 0.9 ml dH_2O in a test tube. Afterwards, from the stock, 0.1 ml was taken and mixed appropriately with 0.9 ml carbonate buffer, and 75 µl xanthine oxidase. Then absorbance was read at 500 nm for 3 min at 20 seconds intervals. Rate of absorbance change indicated activity of SOD.

Catalase (CAT) activity was assayed as described by Aebi (1984). Test tube containing 0.5 ml liver sample, 2.5 ml phosphate buffer and 2.0 ml H_2O_2 were added and labelled stock. To 1.0 ml portion of the reaction aliquot from stock, 2 ml dichromate acetic acid reagent was added and mixed appropriately. The absorbance of the mixture was determined at 240 nm at a minute interval into 4 places.

Vitamin C was determined by adopting the method described by Omaye et al. (1979). To 0.5 ml of supernatant, 0.5 ml of water and 1 ml of TCA were added, appropriately mixed and centrifuged. Then, 1 ml of the supernatant and 0.2 ml of dinitrophenyl hydrazine thiourea copper sulphate (DTCS) reagent were added and incubated at 37°C for 3 h. Finally, 1.5 ml of sulphuric acid was added, mixed and absorbance taken at 520 nm.

Vitamin E was determined by Palan et al. (1973) method. To 0.5 ml of supernatant, 1.5 ml of ethanol was added, appropriately mixed and centrifuged. The supernatant was dried at 80°C for 3 h. To this, 0.2 ml of 2,2-dipyridyl solution and 0.2 ml of ferric chloride solution were added, appropriately mixed and 4 ml of butanol was added and absorbance taken at 520 nm.

Vitamin A was determined by adopting Dugan et al. (1964) method. Briefly, into 2 ml of liver sample in a test tubes, 2 ml of 95% ethanol and 3.0 ml of petroleum ether were added, with mixing, This was stopped and shaken vigorously for 2 min to extract vitamin A. Centrifuge slowly for three minutes, then take 2 ml of the petroleum ether (upper) layer and evaporate to dryness (40°C) in water bath. The residue was taken in 0.1 ml of chloroform and 0.1 ml of acetic anhydride. To this, 1.0 ml of trifluoroacetic acid (TFA) reagent was added and absorbance read at 620 nm (30 s after addition of reagent) against blank containing of 0.1 ml of chloroform and 1.0 ml of TFA reagent.

Selenium concentration was determined by the method established by Katamto and Al-Zehouri (2012) and reported by Mabeyo et al. (2015) with minor modification. Briefly, 2 ml of liver homogenate was digested with 6 ml 70% HNO_3 and 2 ml 30% H_2O_2 in 100 ml conical flask. The mixture was heated at 70 ± 5°C for 35 min on a hot plate. Then, filtered and diluted to 50 ml with deionized water. Selenium concentration was determined spectrophotometrically using 3,3′-diaminobenzidine hydrochloride (DABH) as chromogen. Aliquots of 5 ml sample solution were transferred into series of 30 ml heat resistant vials, 0.25 ml 3,3′-DABH was added, and the mixture was heated to 70°C for 20 min, cooled and the pH adjusted to 8.0 ± 1.0 with NH_3 solution. The coloured complex was extracted with 5 ml toluene and absorbance taken at 420 nm.

Statistical analysis

Data obtained were expressed as mean ± standard deviation. Statistical analysis was carried out using one way analysis of variance (ANOVA) and significance taken at $P<0.05$.

RESULTS

The activities of liver enzymes ALP (A), ALT (B) and AST (C), had significant ($p<0.05$) increases in all the groups when compared with normal control (Figure 1). Alkaline phosphate activity increased progressively from II to V, however, ALP activity in II and IV were not significant. Alanine aminotransferase activities were the highest in group V, whilst no significant changes in ALT activities were observed in groups I, III and IV. The activities of AST increased significantly in groups II to V when compared to group I. The groups showed an increasing order of AST activity in the order of groups V, II, III and IV. Between groups III and IV, no significant change in AST activity was observed.

Table 1 presents albumin, globulin and total protein concentrations in rats exposed to CCl_4 and treated with alcoholic bitter. Albumin concentrations varied significantly ($p<0.05$) in each treatment group. The concentrations of albumin reduced significantly in groups II to V when compared with the normal control (group I). Also, between groups IV and V albumin concentration varied non-significantly. Furthermore, the concentration of globulin varied significantly ($P<0.05$) amongst the groups. Groups II and III had a lower globulin concentration when compared with group I (normal control), but were higher in groups IV and V as shown in Table 1. Also, there were wide variations ($P < 0.05$) in total protein in samples collected from each group. Total protein concentration declined in groups II, III and IV when compared with the group I (normal control), whilst group V had the highest total protein concentration.

Figure 2 presents concentrations of GSH (A) and MDA (B) in rats exposed to CCl4 and treated with an alcoholic bitter. Glutathione concentration reduced significantly ($p < 0.05$) in all the treated groups (II to V) compared to group I. Amongst groups II, III, IV and V GSH concentrations varied non-significantly. Figure 2 showed that the concentration of MDA increased significantly ($p<0.05$) in all treated groups (II to V) when compared with normal control (I), with group II (treated with CCl_4 only) showing the highest MDA concentration.

Figure 3 presents the activities of antioxidant enzymes-

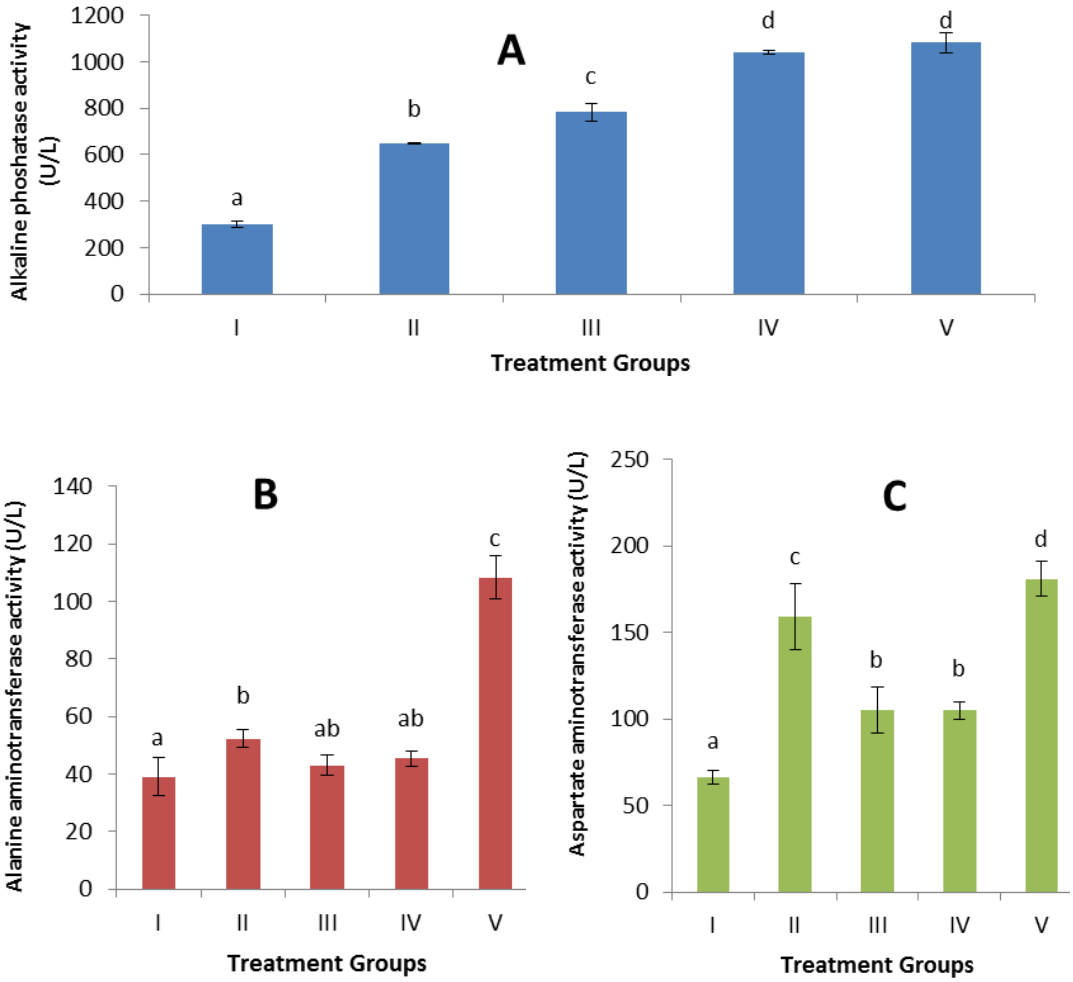

Figure 1. The activities of liver function enzymes (ALP (A), ALT (B) and AST (C) in rats exposed to CCl_4 and treated with an alcoholic bitter. Bars represent mean±standard deviation of five (n=5) determinations. Bars bearing different letters per graph are statistically significant ($p<0.05$).

glutathion peroxidase (GPx) (A), glutathion reductase (GRD) (B), superoxide dismutase (SOD) (C) and catalase (D). It shows significant decreases ($p<0.05$) in the activities of GPx, GRD and catalase in all the treated groups (II-V) when compared with the control group (I). However, the activity of SOD (C) increased significantly ($p<0.05$) in all the groups when compared with control, with the group unexposed to CCl_4 but administered 50% (1.4 ml) alcoholic bitter showing the highest SOD activity.

Table 2 presents concentrations of antioxidant vitamins and selenium determined from liver homogenate. It shows significant ($p<0.05$) decrease in vitamin A concentration in all groups (except group III) compared to normal control. On the other hand, the concentrations of vitamins C and E showed non-significant ($p>0.05$) decrease in groups II to V compared to normal control (I), while selenium concentration non-significantly ($p<0.05$) increased in groups II to V in comparison to normal control.

DISCUSSION

The smooth endoplasmic reticulum of liver is a metabolic clearing house for endogenous (e.g. proteins) and exogenous (e.g. drugs and alcohol) substances. The liver as a clearance and transformation centre for chemicals exposes it to toxic injury (Saukkonen et al., 2006). The increased activities of ALT, ALP and AST in rats exposed CCl_4 and treated with alcoholic bitters (II -V) indicate injury to hepatocytes. Damage or injury to hepatocytes in a form of toxic insult results to leakage of cell specific enzymes into serum and peak activities can be observed between 24 and 48 h (Mukherjae, 2002; Alisi et al., 2008; Ujowundu et al., 2011). Alanine aminotransferase and AST are considered as markers of hepatocyte parenchymal injury induced by xenobiotics (Amacher, 1998) as well as alcohol (Expert Care, 2013).

The significant increase of AST activity observed in groups II and V compared to other treated groups may be

Table 1. Concentrations of protein, albumin and globulin in rats exposed to CCl_4 and treated with alcoholic bitter.

Groups	Albumin (g/L)	Globulin (g/L)	Total Protein (g/L)
I	40.73±2.05^{d}	30.33±1.53^{c}	71.07±0.09^{d}
II	27.33±1.53^{b}	15.83±3.44^{a}	43.18±2.85^{a}
III	31.33±1.53^{c}	19.54±0.99^{b}	50.86±2.06^{b}
IV	24.33±1.53^{a}	42.83±0.25^{d}	67.17±1.40^{c}
V	24.00±1.00^{a}	55.96±1.76^{e}	79.96±1.90^{e}

Values are mean±standard deviation of five (n=5) determinations. Values per column with different superscript letters are statistically significant (p<0.05).

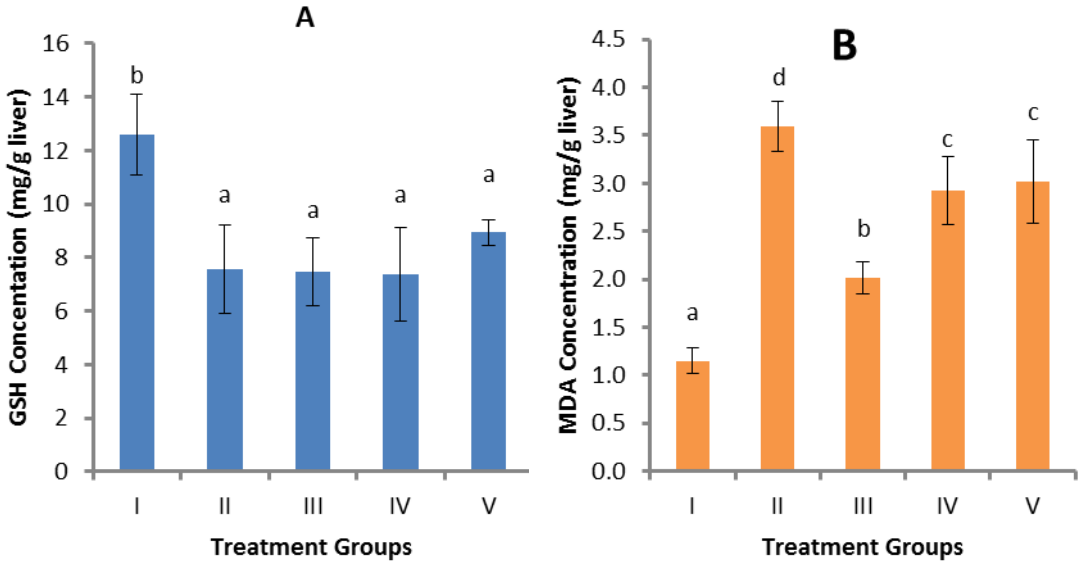

Figure 2. Concentrations of reduced glutathion (GSH) (A) and malondialdehyde (MDA) (B) in rats exposed to CCl_4 and treated with an alcoholic bitter. Bars represent mean±standard deviation of five (n=5) determinations. Bars bearing different letters per graph are statistically significant (p<0.05).

attributed to the unhindered toxic action of CCl_4, whereas that of group V may be a synergistic action of CCl_4 and alcohol. Carbon tetrachloride bioactivation to trichloromethyl radical is catalyzed by a nicotinamide adenine dinucleotide phosphate (NADPH)-dependent cytochrome-P450 (CYP2E1) that is inducible by alcohol or ethanol (Castillo et al., 1992). This finding as seen in the reduced AST activities in groups III and IV indicates that, the phytochemical content of the alcoholic bitter may ameliorate oxidation when taken in moderation (group III) or in the absence of xenobiotics (group IV). Phytochemicals have antioxidant properties (Aruoma et al., 2010), and can detoxify xenobiotics by neutralizing free radicals, inhibiting enzymes that activate xenobiotics and activate enzymes that detoxify xenobiotics (Narasniga, 2003).

Herbs or medicinal plants either as pure compound or extract offer unlimited medicinal and therapeutic purposes (Mahomodally, 2013). However, the activities of these assayed liver enzymes clearly indicate that the herbal content of this alcoholic bitter showed no hepatoprotective ability. Herbal phyto-content are known to render cell membranes less permeable to chemical injury (Asuzu and Onoh, 1988), stabilize and prevent hepatic tissue damage and enhance regeneration of hepatocytes (Thrabrew et al., 1987; Ujowundu et al., 2015). The none observable hepatoprotective effect or inability of the phyto-content of the alcoholic bitter studied to ameliorate the leakage of liver enzymes may be caused by the toxicity of the high percentage alcohol used as a base for bitters preparations.

Liver function was also evaluated by determining markers of liver biosynthetic capacity such as albumin and total protein concentration. The reduction in albumin concentration in groups II to V indicated negative impact of CCl_4 and CCl_4/alcoholic bitter exposure. Albumin is a blood protein synthesized in hepatocytes and transports various substances, including bilirubin, fatty acids,

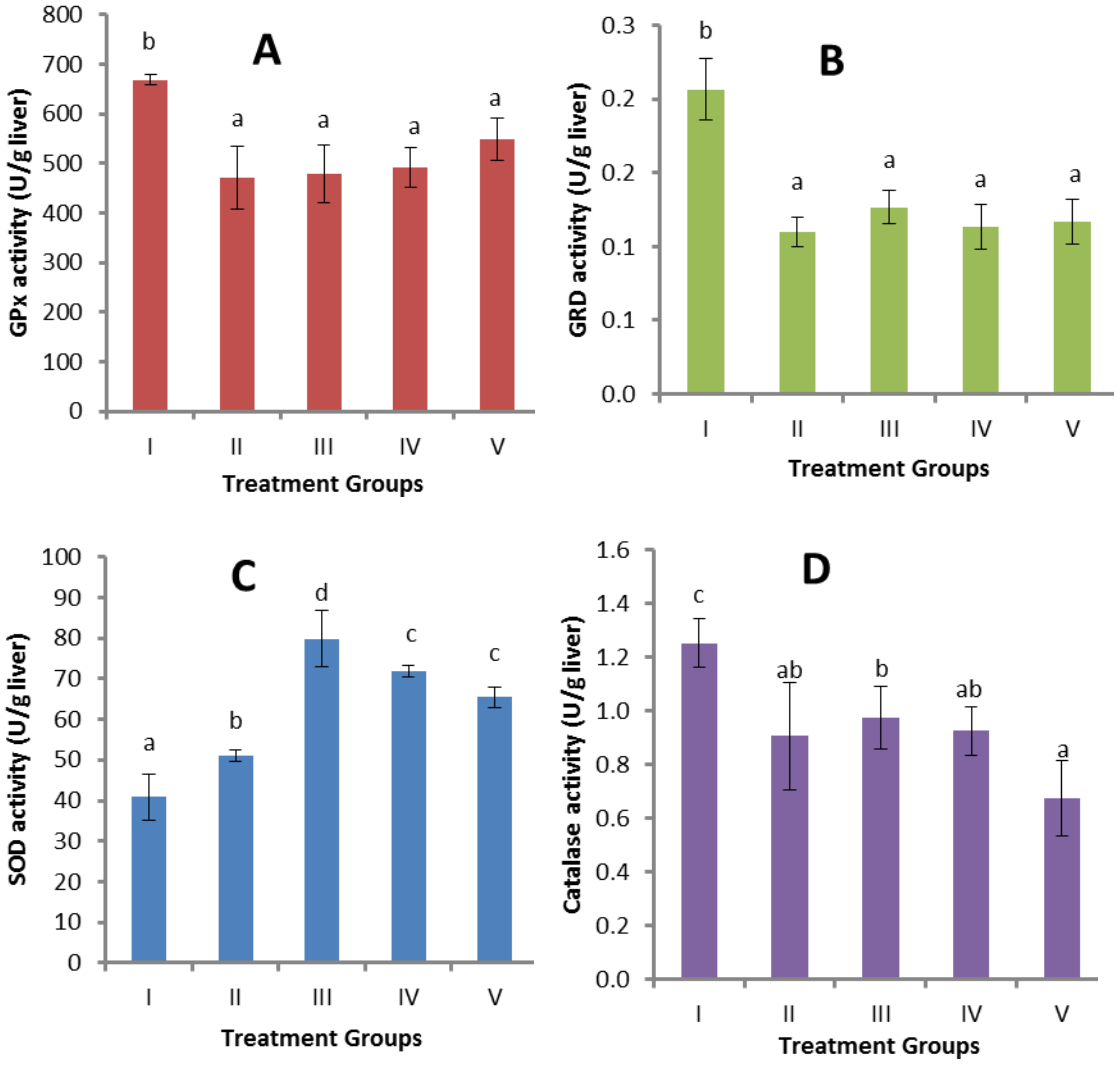

Figure 3. The activities of antioxidant enzymes GPx (A), GRD (B), SOD (C) and Catalase (D) in rats exposed to CCl_4 and treated with an alcoholic bitter. Bars represent mean±standard deviation of five (n=5) determinations. Bars bearing different letters per graph are statistically significant ($p<0.05$).

metals, ions, hormones, drugs and xenobiotics. Since the half-life of albumin is approximately 21 days, the decreased albumin concentration of the exposed rats is suggestive of increased degradation rate which is approximately 4% per day (Peralta and Pinsky, 2016). However, reduced albumin synthesis (Geuken et al., 2004) may also lead to decreased albumin concentration. The decreased concentration of globulin and total protein in some of the exposed group may be attributed to functional derangement. Serum total protein concentration indicates the functional capacity of the liver to synthesize albumins and globulins (Geuken et al., 2004). Total protein is often reduced slightly but the albumin to globulin ratio shows a sharp decline during hepatocellular injury (Singh et al., 2011).

Concentrations of MDA in groups II to IV indicate lipid peroxidation when compared with group I. This implies that CCl_4 and alcoholic bitter can induce lipid peroxidation at varying degrees with CCl_4 showing greater toxicity. Malondialdehyde concentrations observed in groups IV and V may indicate that excessive consumption of alcoholic bitter (of high % alcohol) in the presence of CCl_4 and probably any other toxicant may enhance lipid peroxidation at a dose-dependent manner. The significant increase in MDA concentration of group II animals intoxicated with CCl_4 only, compared to the normal control group animals corroborates the finding that CCl_4 metabolism was characterized by lipid peroxidation (Kamel et al., 2011). The observed decrease in MDA concentration of alcoholic bitter-treated groups (IV and V) compared to group II (CCl_4 only) could be attributed to the effect of phytochemicals in the bitter

Table 2. Concentrations of vitamins A, C and E and selenium (mg/dl)in rats exposed to CCl_4 and treated with alcoholic bitter.

Groups	Vitamin A (mg/dl)	Vitamin C (mg/dl)	Vitamin E (mg/dl)	Selenium (mg/dl)
I	1.42±0.44[b]	2.01±0.91[a]	0.15±0.04[a]	0.41±0.15[a]
II	0.90±0.21[a]	1.73±0.50[a]	0.13±0.03[a]	0.46±0.24[ab]
III	1.04±.0.15[ab]	2.00±0.52[a]	0.15±0.03[a]	0.67±0.26[ab]
IV	0.85±0.10[a]	1.87±0.31[a]	0.13±0.01[a]	0.72±0.09[ab]
V	0.93±0.17[a]	1.67±0.25[a]	0.13±0.02[a]	0.78±0.11[b]

Values represent mean±standard deviation of five (n=5) determinations. Values per column with different superscript letters are statistically significant ($p<0.05$).

preventing CCl_4 activation required for lipid peroxidation to occur. Excessive consumption of alcoholic bitter upon redox imbalance as shown in groups IV to V could facilitate pro-oxidants induced toxicity (Bellomo et al., 1992). The cells of rats in groups II to V may be prone to membrane protein cross link and consequent damage to membrane fluidity. There could be further possibility of the formation of lipid-protein and lipid-DNA adducts which could adversely affect cell functions. In conditions of oxidative stress, oxidants may induce numerous pathophysiological events leading to depreciation of antioxidant concentrations (Kaplowitz, 2000; Videla et al., 2004). The significantly reduced GSH concentration of groups II to V when compare with group I, coupled with non-significant difference in GSH amongst treatment groups, may indicate consumption of glutathione in the presence of oxidant generating molecules (CCl_4 and alcohol) with their attendant adverse effect (Wu and Cederbaum, 2003).

The decreased activity of GPx as observed in groups II to V compared to group I, shows GSH as an important cofactor to GPx for the extra peroxisomal inactivation of ROS (Kaplowitz et al., 1996). The insignificant change of GPx activity in all alcoholic bitter treated groups (III to V) compared to the group administered CCl_4 only (group II), a known hepatotoxicant, supports the reported (Wu and Cederbaum, 2003) adverse effect of alcohol on hepatocytes. Glutathione reductase activity also reduced significantly in groups (II to V) compared to the normal control. Glutathione reductase (GRD) converts oxidized glutathione (GSSG) to reduced glutathione (GSH) (Reiter et al., 2005) supported by NADPH generated in pentose phosphate pathway. The depletion of GSH is expected to increase cellular GSSG concentration. Since GSSG is the substrate for GRD, it is expected that GRD activity should increase when GSSG increases. However, the reverse was observed, indicating extensive conjugation of GSH reactive electrophilic metabolites. Similarly, the insignificant fluctuations amongst the groups treated with CCl_4, alcoholic bitter and CCl_4/alcoholic bitter (II-V) revealed the tendency of the alcoholic bitter to negatively affect antioxidant enzymes and molecules (Wu and Cederbaum, 2003), used to scavenge radicals and protect organisms from oxidative damage (Ramond et al., 2011).

Furthermore, the elevated SOD activity of animals in groups II to V was indicative of induction of mitochondrial SOD due to oxidative stress (Wheeler et al., 2001) in the presence of CCl_4 andalcohol. Also, decreased catalase activity of CCl_4 group (group II) compared to control may be attributed to exhaustion of the antioxidant enzyme. Similarly, decreased catalase activity in the group intoxicated with CCl_4 and treated with 100% alcoholic bitter (group V) supports the report that alcohol induces oxidative stress (Adachi and Ishii, 2002).

Vitamin A could be available in diets either as preformed vitamin A (such as retinyl ester, retinol, and retinoic acid) or provitamin A (carotenoids). Most dietary vitamin A is internalized in hepatocytes, hydrolyzed to retinol and transferred to hepatic stellate cells for storage. In this study, hepatic vitamin A concentration decreased significantly in all treated groups (except group III) compared to control. This may be an indication that, CCl_4 and alcohol negatively affect hepatic vitamin A concentration. Hepatocytes and hepatic stellate cells contain retinyl ester hydrolases and in cellular retinol binding protein type 1, necessary to solubilize retinol in the aqueous environment of cells (Gottesman et al., 2001). These xenobiotics (CCl_4 and alcohol) may have impacted negatively on these proteins or inhibited vitamin A uptake from retinol binding protein (Folli et al., 2001; Kawaguchi et al., 2007). Cellular retinoic acid binding proteins may regulate the interactions between retinoic acids and their nuclear receptors by regulating the concentration of available retinoic acids (Donovan et al., 1995). Biochemically, vitamin A and its derivatives are involved in immune function, maintenance of epithelial tissue, and differentiation. Therefore, its deficiency may cause pathological derangements and immunodeficiency (Fields et al., 2007). However, it is important to note that excessive cellular vitamin A concentration can cause teratogenic effects including major alterations in organogenesis (Beeman and Kronmiller, 1994).

Vitamins such as α-tocopherol (vitamin E) and ascorbate (vitamin C) can prevent the propagation of lipid peroxidation. The non-significant decrease of ascorbate

and α-tocopherol concentration in groups exposed to CCl_4 and treated with alcoholic bitter compared to control may be due to the use of these antioxidant compounds in radical scavenging activities. Antioxidants protect cells against the consequent effect of radicals, as well as contribute to defence system and disease prevention (Pham-Huy et al., 2008). Antioxidant compounds such as vitamins E and C can increase significantly or be inhibited under chemical stress depending on the intensity and duration of the stress. Alpha-tocopherol is an important lipid soluble radical scavenging molecule (Buettner, 1993).

Low level of blood selenium (Se) is linked to increased risk of numerous diseases in man and other animals, despite its need in trace quantity. The non-significant increase in the Se concentrations of groups II to IV, and its significant increase in group V animals could be attributed to destruction of antioxidant enzymes used in radical scavenging which use selenium as a cofactor. Selenium is an important component of antioxidant enzymes, such as glutathione peroxidase (GPx), thioredoxin reductase (TrxR), iodothyronine deiodinases (IDD) and alcohol dehydrogenase (Tapiero et al., 2003; Ujowundu et al., 2010a). Ascorbic acid, iron, selenium, zinc and manganese improve the immune functions as antioxidants (Talwar et al., 1989; Ujowundu et al., 2010b).

Conclusion

Consumers are most times interested in quick access to products that were claimed could solve their problems, neglecting the potential adverse effect of the active substances used for its preparation. This study has shown that the integrity and synthetic function of the liver might be compromised under the influence of alcoholic content of bitters. The hepato-toxicity observed in this acute study may degenerate further, on chronic consumption of this highly alcoholic bitter or any other. We suggest that prolonged and increased consumption of these alcoholic bitters should be discouraged, considering the increased cases of liver damage and diseases.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest

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