Original Research Article

Antipuritive Effect of aqueous root extracts of *Moringa Oliefera* on alcohol induced Haematoxicity in wistar rats.

ABSTRACT

The Aim of the study: The study is aimed at investigating the antipuritive effects of aqueous root extract of *Moringa oleifera* on hematological indices following sub-chronic consumption of alcohol in Wistar rats.**Introduction**: The consumption of alcohol causes a number of health challenges including; kidney and liver diseases as well as other social costs. **Materials and Methods:** Thirty (30) experimental rats weighing 100-120g were randomly divided into 6 groups of 5 rats each. Group 1, the normal control group was given just rat feed and water, group 2, negative control was administered alcohol only (1.5ml/kg body weight), rats in group 3, 4 and 5 received combined administration of alcohol 1.5ml/kg body weight and 200, 400 and 600 mg/kg body weight of aqueous root extract of *Moringa oleifera* respectively, group 6 rats were administered 250mg/body weight of extract only. At the end of fourteen days (14) the experimental rats were then sacrificed, and blood was collected for hematological analysis. **Results:** The result obtained shows a dose-dependent response in almost all the parameters, white blood cell (WBC) count increases significantly($P<0.05$) with 600mg/kg while dose 200mg/kg and 400mg/g shows no significant change. Red blood cells (RBC) decreases non-significantly($P<0.05$) with increased amount of aqueous Moringa root extract. Likewise, there was also a dose-dependent decrease in haematological count and more noticeable with 200mg/kg dose. It was also observed that mean corpuscular haemoglobin concentration(MCHC) and mean corpuscular volume(MCV) increases in all the treatment groups with significant observed in 400mg/kg and 600mg/kg. **Discussion:** The increase in alcohol can bring about suppression of the blood cells production, enhance if there

is reduction in the red blood cells, it therefore implies that the will be reduction in the oxygen carrying capacity of the red blood cells, that would be carried to the tissues as well as the level of carbon dioxide returned to the lungs. Also the PCV is involved in the transport of oxygen and absorption of nutrient. Therefore, increases in PCV, shows a better transportation and thus results in increase in both primary and secondary polycythemias. **Conclusion:** The root extract of *moringa oleifera* plant is widely recognized as a medicinal plant and sometimes consumed concomitantly with alcohol. This research work shows that the plant extract contains some bioactive component or phytochemical constituents that are capable of ameliorating the toxicity effect of alcohol on animal models.

Key: Packed cell volume; White Blood Cells; Red Blood Cells; Mean Volume Corpuscular; Mean Corpuscular Haemoglobin Concentration; Moringa oliefera.

Background Information

Medicinal plants are plants that are used in attempt to maintain health, to be administered for a specific condition or both, whether in modern medicine or in traditional medicine [1] (Smith-Hall *et al.*, 2012; Ahn, 2017). They are widely used in both developing and developed societies as sources of drugs or herbal extracts for various chemotherapeutic purposes mainly because they are readily available and cheaper than modern medicines. On a scientific ground, these plants are used because of their active phytochemical constituents. The medicinal and pharmacological activities of medicinal plants are often attributed to the presence of secondary plants metabolites known as phytochemicals. Unlike the ubiquitous macromolecules of primary metabolites like monosaccharide's, polysaccharides, amino acids, proteins, nucleic acids and lipids which are present in all plants, secondary metabolites with medicinal properties are found only in few plants species and they have more medicinal value. Secondary plants metabolites with reported medicinal properties include alkaloids, phenolics, saponins, glycosides and terpenoids [2] (Ebong, 2015a). They offer the anti-microbial, anti-oxidative, anti-inflammatory properties of plants. Natural constituents based on plant can be derived from every plant part like roots, bark, seeds, flowers, leaves etc. [3] (Gordon and David, 2001).

Phytochemicals are chemical compounds produced by plants through primary or secondary metabolism [4] (Harborne *et al.*, 1999; Molyneux *et al.*, 2007). They are non-nutritive plant chemicals that have disease preventive or protective properties, they offer plants natural defense

system providing protection against such thing as attack from insects, grazing animals and microbes. Examples include flavonoids, phenols, terpenes, alkaloids etc. They contribute to plant colour, aroma and flavour [5] (Ebong, 2015b). *M. oleifera*, which is also known as the "Miracle Tree" and "Mother's Best Friend," has been named the most nutrient-rich plant. Other than having a high concentration of vitamin A, vitamin C, potassium, and calcium, the plant contains all the essential amino acids [6] (Mahmood *et al*., 2010). Different parts of the *Moringa oleifera* tree have been established as being a good source of unique glucosinolates, flavonoids and phenolic acids [7] (Amaglo *et al*., 2010; Coppin *et al*., 2013), carotenoids (Sani *et al*., 2014a), tocopherols, (Sani *et al*., 2014b), polyunsaturated fatty acids (Sani *et al*., 2014c), highly bio-available minerals and folate [8] (Sani *et al*., 2014d).

Worldwide adults consume about average 5liters of pure alcohol from beer, wine and spirits per year. The average alcohol consumption is highest in Europe following America then Africa. It tends to increase with economic development. Alcohol consumption is the leading risk factor for disease burden in low mortality developed countries [9] (Rehm and Eschmann, 2002). Alcohol causes 1.8 million deaths (3.2% of total) and a loss of 58.3 billion (4% total of disability adjusted life years [10] (WHO, 2002; 2004). Excessive alcohol consumption is widely associated with liver damage [11] (Heathcole, 2000). Just along the streets of Nigeria today, one is sure to find at least one or two herbal concoction sellers either hawking or situated somewhere in the vicinity. This herbal brew known popularly as agbo has drawn quite a significant attention from the nation's health sector with lots of debates trailing it. In recent times however, this trend has taken on another dimension in the country as consumption of the locally brewed gin mixed with some roots and herbs has reached an all-time high. While some people are quick to attribute this trend to poverty level in the country which has financially incapacitated many citizens, others feel it is as a result of lack of adequate provision of medical care. However, for whatever reason and with the recent indiscriminate sell of agbo that is attracting more subscribers from other classes in the society and despite fears that the consumption of agbo would be detrimental to health, the number of people patronizing it keeps soaring. The notion at the back of an average of agbo consumer's mind is to keep healthy in the most natural way without any negative side effect from chemical reactions.

MATERIALS AND METHODS

Apparatus

Sample (*M. oleifera* roots), grinding machine, electronic weighing balance, beakers, funnel, plastic buckets, filter paper (what man filter paper No 1), measuring cylinder, sieve basket, chess cloth, thermo-regulated water bath, palletized rat feed, alcoholic beverage (tombo), water bottles, saw dust, poly-carbonated cages, hand gloves, syringes, needles, Auto Analyzer, Department of Biochemistry, College of Basic Medical Science, University of Calabar, Calabar. Used for Hematological parameters.

Sample collection

Fresh sample of *Moringa oleifera* roots were harvested from the University of Calabar Staff Quarters. The roots were then chopped into smaller fragments, washed properly with tap water to remove debris and other physical contaminants and then air dried at room temperature for two weeks.

Preparation of extract (cold maceration method of extraction)

The mature root of *moringa oleifera* were obtained from the university of calabar, Botanical Garden and identified by Dr Francis Eko, of Botany Department university of calabar, calabar. Voucher specimen deposited in the Departments herbarium number 2435 was kept for future reference.The root was rinsed with water to remove debris and sand and then sun dried**.** The dried samples were pulverized to powder form using grinding machine. The grinded sample was then weighed using an electronic weighing balance and put in a plastic bucket. 2700ml of water was then added to the plastic bucket containing the sample. The soaked sample was allowed to stand for 24 hours, after which it was first filtered using a sieve basket and then chess cloth. The filtrate obtained was then filtered again using what man filter paper No 1. The filtrate obtained was collected in beakers and then placed into a thermo regulator water bath with temperature set at 50^{o}c and allowed to concentrate. The extract gotten was then collected into plastic vials and then stored in the refrigerator at 4^{o}c to be used for further experiment.

Toxicity study on *Moringa oleifera*

According to one acute toxicity study of various extracts of *Moringa oleifera* roots, results of that study showed a safe range. The LD50 for the aqeous extract was 15.9/kg. The results were supported by the work done by [12] Adedapo et al., (2009) (Kasolo *et al*., 2010).

Experimental procedure

Thirty (30) *albino wistar* rats weighing 100- 120kg from the animal house of the Department of Biochemistry, University of Calabar were used. The animals were acclimatized for a week in the animal house, they were fed with standard rodent pellets and water was provided *ad libitum*. The animals were maintained there at standard conditions of temperature and relative humidity. The rats were then afterwards divided into 6 groups of 5 rats each.

Table 1.0 Experimental design

Group	**Designation**	**Treatment**	**Dose**
1	NC	Water	*Ad libitum*
2	NgC	Alcohol	1.5ml of alcohol/bw
3	AMR1	Alcohol and rMO	1.5ml of alcohol/bw + 1.5ml of 200mg of Rmo
4	AMR2	Alcohol and rMO	1.5ml of alcohol/bw + 1.5ml of 400mg of Rmo
5	AMR3	Alcohol rMO	1.5ml of alcohol/bw + 1.5ml of 600mg of Rmo
6	EC	Rmo	1.5ml of 250mg of Rmo

Keys;

NC- Normal control; **bw – body weight**

NgC- Negative control

AMRn – groups administered with alcohol and extract (n = 1, 2, 3)

rMO - root extract of *Moringa oleifera*

EC – Extract control

Administration of alcohol and root extract.

Alcohol and root extract of *M. oleifera were* administered orally with the aid of a studded needle and syringe. During the first 3 days, rats in group 2, 3, 4 and 5 were induced with sub-chronic liver damage orally using a commonly available alcoholic beverage, Tombo (4.82%) at a dose of 1.5ml per body weight only after which on the 4^{th} day to the last day (14^{th}), they were given aqueous root extract of MO shortly after alcohol administration.At the end of the treatment period the rats were reweighed and then were sacrificed.

Collection and preparation of blood for analysis.

At the end of the 14^{th} day treatment period, the animals were anesthetized with chloroform. They were then dissected and their blood collected with sterile syringes by cardiac puncture into sterile labeled plain vials and was allowed to clot for about 2 hours, they were then centrifuged at 3000g for 15 minutes to allow for separation of serum from blood cells. The serum was then precipitated into a plain and well labeled vial for liver function test.

Haematological Estimation:

The blood samples collected into heparized tubes were immediately used for determination of heamatological parameters. Total red blood cells and white blood cells count were estimated according to the visual methods of Dacic and Lewis[13] (1975). The percentage packed cell volume was determined according to the hematocrits methods of Alexander and Griffith [14] (1993a). while the blood hemoglobin concentration in all the samples were estimated according to the cyanomethaemolobin method of Alexander and Griffiths [15] (1993b).

Differentials white blood cells count:

These was estimated using the methods of Osim et al [16] (2004). Adry micropipette was used to suck in blood from the blood sample bottle a small drop of the blood was applied to one end of a slide and quickly placed on the bench holding it in position, the end of the second slide was placed in the drop and held there until the blood had spread across its, it was then drawnslowly over the whole length of the first slide being held at an angle of $45^{0c.}$ After the blood had spread, it was dried before staining with Leishman'sstain. The film which was washed off in a gentile stream was dried with filter paper and examined under low and high power microscope and the different kinds of cells counted.

Ethical approval:

Ethical approval was also sought from the committee, faculty of basic medical science, university of calabar, calabar.

Statistical Analysis

Quantitative data were analyzed using one-way analysis of variance (ANOVA), followed with a post hoc (Duncan) test for significant values. P-value < 0.005 was considered statistically significant. Statistical package for the social sciences application software, SPSS version 20 was used for statistical analysis and the charts were plotted using Microsoft-Excel application software 08. Data are expressed as mean ± standard error of mean (SEM).

Results:

The table below shows the statistical representation of the outcome of the results in (table **2**).

Table 2

	WBC 10^3 cells/µL		RBC 10^6 cells/µL	HGB g/dL	HCT	MCV fl	MCH pg	MCHC g/dL	PLT 10^9 cells/µL
Control	8.47±1.00		8.02±0.79	13.57±1.37	53.95±5.85	67.00±1.53	16.93±0.03	25.23±0.70	641.33±66.46
Negative Control	8.04±0.72		8.64±0.93	14.05±1.55	52.30±5.44	60.50±0.50*	16.25±0.05	26.75±0.15	784.50±44.50
200mg Treatment	6.90±0.52		8.36±0.28	13.57±0.67	50.91±2.29	60.67±0.88*	16.23±0.32	26.70±0.20	750.67±39.35
400mg Treatment	7.20±0.20		6.63±0.44	11.50±0.60	44.43±3.34	67.33±2.40^{a,b}	35.53±18.24	26.00±0.78	792.33±28.83
600mg Treatment	11.62±0.47*^{,a,b,c}		6.33±0.12	10.70±0.20	45.48±0.39	72.00±2.00^{a,b}	16.90±0.00	23.45±0.65	623.50±10.50
Extract treatment	4.70±1.19*^{,a,c,d}		4.93±1.62*^{,a,b}	13.97±2.99	31.85±10.7*^{,b}	64.00±0.58^{d}	29.55±4.82	46.55±7.94*^{,a,b,c,d}	619.50±139.4

Concentrations of haematological parameters in the different experimental groups.
Values are expressed as mean ± SEM, n = 5.
*significantly different from Control at p<0.05
a=significantly different from Negative Control at p<0.05
b=significantly different from 200mg treatment group at p<0.05
c=significantly different from 400mg treatment group at p<0.05
d=significantly different from 600mg treatment group at p<0.05.

From the result of the White blood cell count of the experimental animals following alcohol toxicity as shown in figure below did not show significant ($p < 0.5$) change in count, though there was decreased in count relative to Normal control. According to the data obtained the WBC count of Control and Negative control was (8.47±1.00) and (8.04±0.72) respectively. Upon the administration of different doses of aqueous extract of *Moringa oleifera*, the groups treated with 200mg/b.wt. and 400mg/b.wt. Shows no significant ($p < 0.5$) decreased (6.90±0.52) and (7.20±0.20) respectively relative to the negative control notwithstanding the decrease in the white blood cell count. But, the group treated with 600mg/b.wt of *Moring oleifera* extract shows significant ($p < 0.5$) increase (11.62±0.47) in WBC count compared to the control groups as well as other treatment groups. Also, the group treated with only extract shows significant decrease (4.70±1.19) in WBC count relative to control and other treatment group

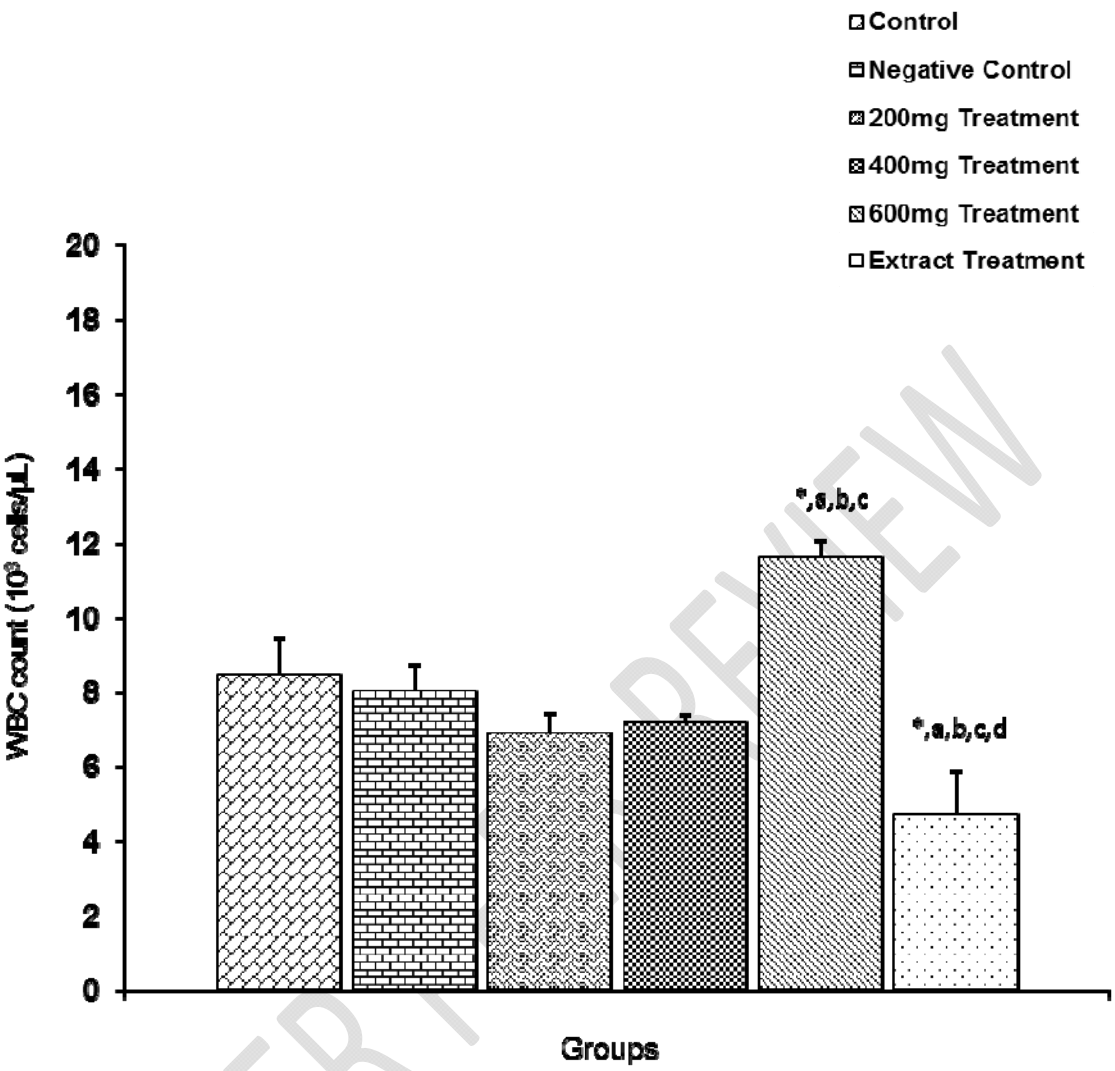

Figure : Comparison of white blood cell Count in the different experimental groups.
Values are expressed as mean ± SEM, n =5.
*significantly different from Control at p<0.05
a=significantly different from Negative Control at p<0.05
b=significantly different

From the data obtained, the negative control group shows no significant ($P<0.5$) increase (8.64 ± 0.93) compared to red blood cells (RBC) of animals in normal control group. But upon treatment with the different doses of *moringa oleifera* extract, there was decrease in the red blood cell count of 200mg, 400mg and 600mg showing a dose dependent response, though the decrease was insignificant compared to the negative control. Also, a significant decrease in red blood cell count was observed in the group treated with extract only. This result is demonstrated in the figure below.

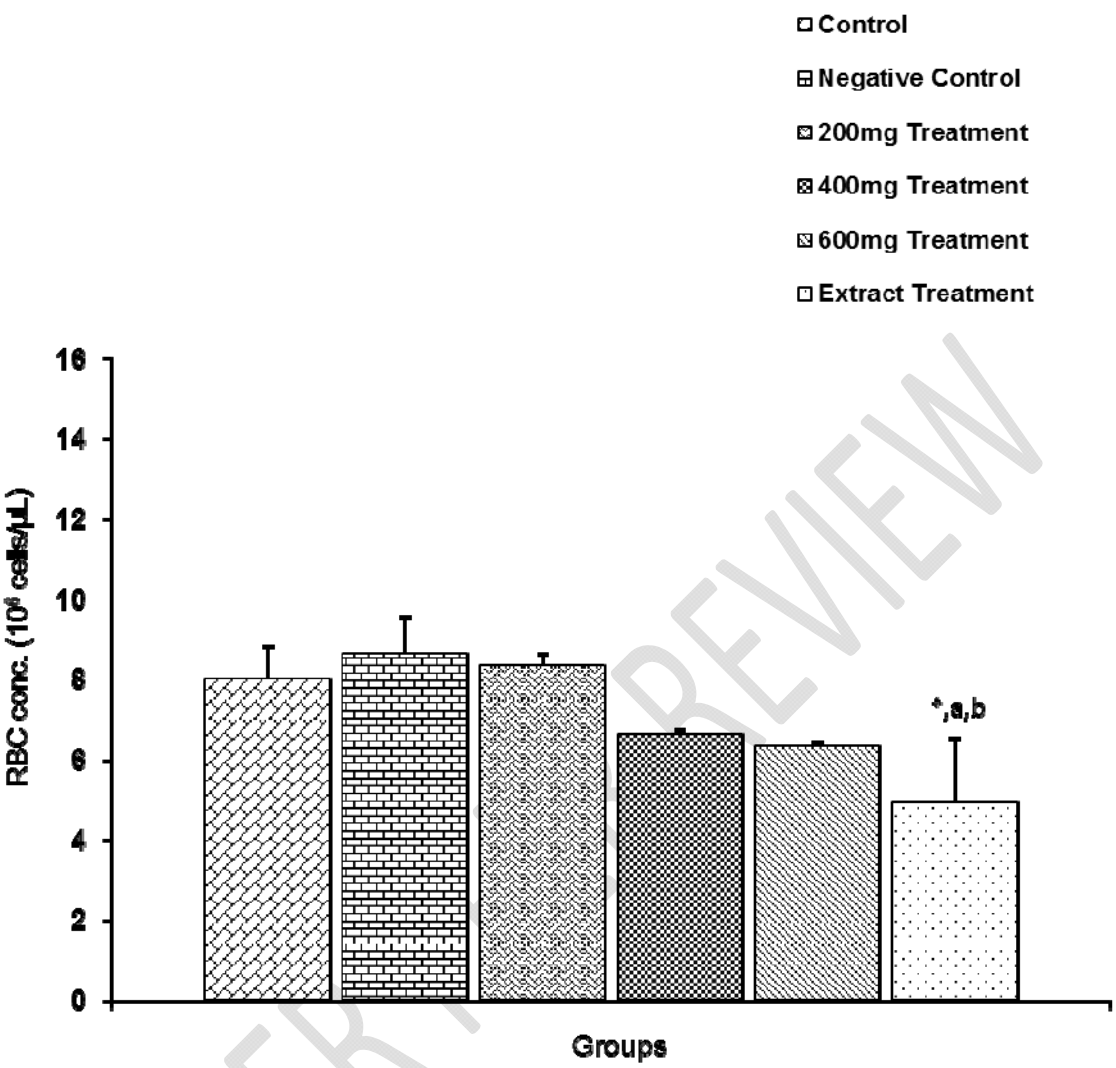

Figure Comparison of of RBC conc. in the different experimental groups.
Values are expressed as mean ± SEM, n = 5.
*significantly different from Control at p<0.05
a=significantly different from Negative Control at p<0.05
b=significantly different from 200mg

The data obtained as shown in the figure below indicated that the alcohol treated group (negative control) has an insignificant ($P>0.5$) decrease in packed cell volume (52.30 ± 5.44) compared to the normal control (53.95 ± 5.85). Upon treatment with different doses of *moringa oleifera* extract (200mg, 400mg and 600mg). there was further decrease in hematocrit values with the 400mg dose, though the decrease was insignificant ($P>0.5$)) with a mean value of (44.43 ± 3.34) compared to the negative control with a mean value of (50.51 ± 2.29 and 45.48 ± 0.39) of the doses of 200mg and 600mg respectively. But, a significant ($P<0.5$) decrease was also observed in the group treated with extract only without alcohol toxicity. The data shows that the extract treated group has packed cell volume (PCV) value of (31.85 ± 10.70).

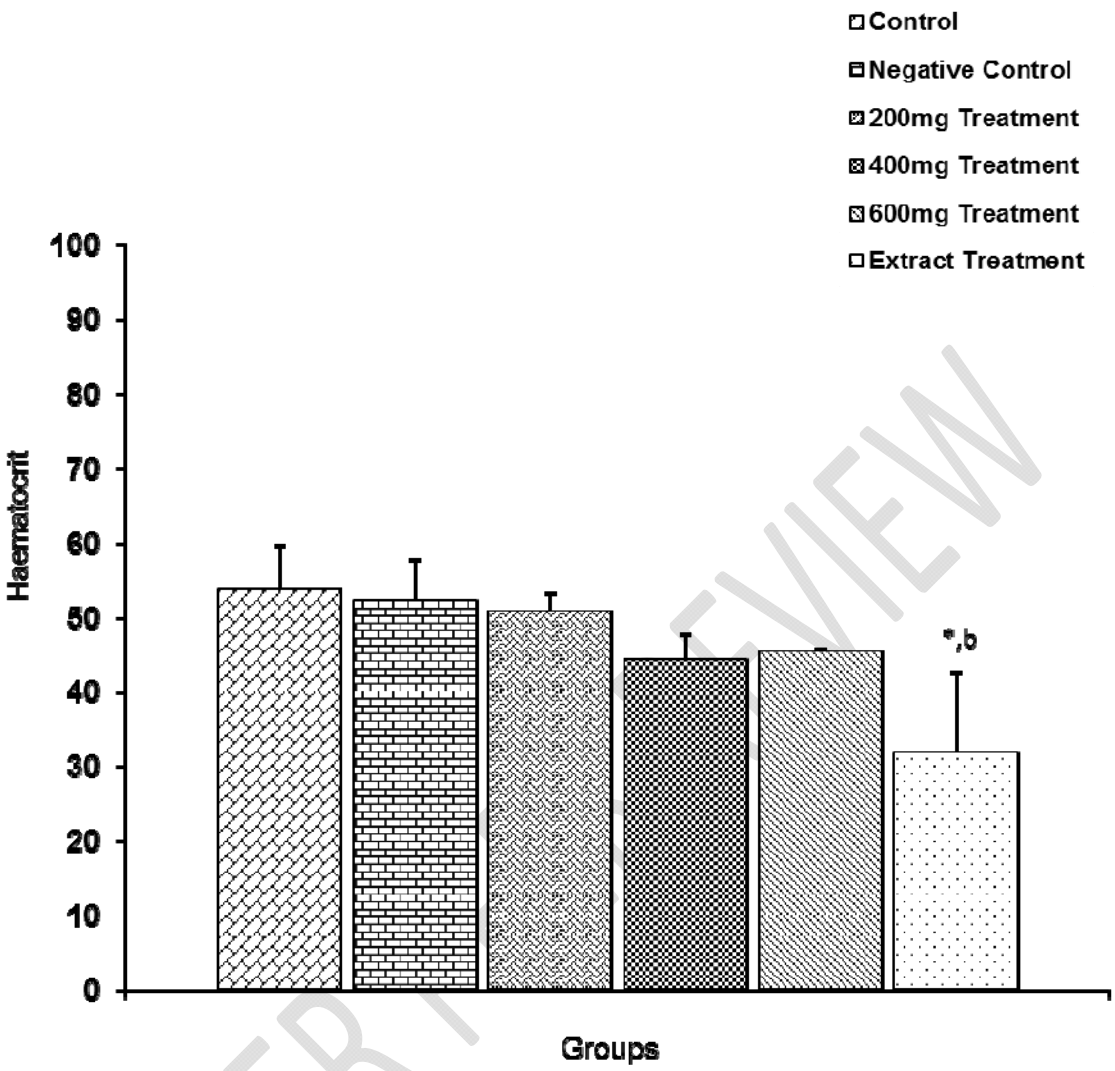

Figure : Comparison of haematocrit in the different experimental groups.
Values are expressed as mean ± SEM, n =5.
*significantly different from Control at p<0.05
b=significantly different from 200mg treatment group at p<0.05.

Following the induction of alcohol toxicity, the data obtained as shown in the figure below shows that there was a significant ($P<0.5$) decrease in mean corpuscular volume (MCV)value (60.50 ± 0.50) of the negative control and the normal control (67.00 ± 1.53). Upon treatment with 200mg dose of *moringa oliefera* extract there was no significant ($P<0.5$) change compared to the negative control, but the decrease was also significant ($P<0.5$) compared to the normal control.

Meanwhile, the acute alcohol toxicity and the subsequent treatment with different doses of *moringa oleifera* did not show any significant ($P<0.5$) effect on the blood MCH and MCHC as well as blood platelets relative to the controls. Nevertheless, the extract treated group shows significant ($P<0.5$) increase in MCHC relative to the controls and the other treatment groups

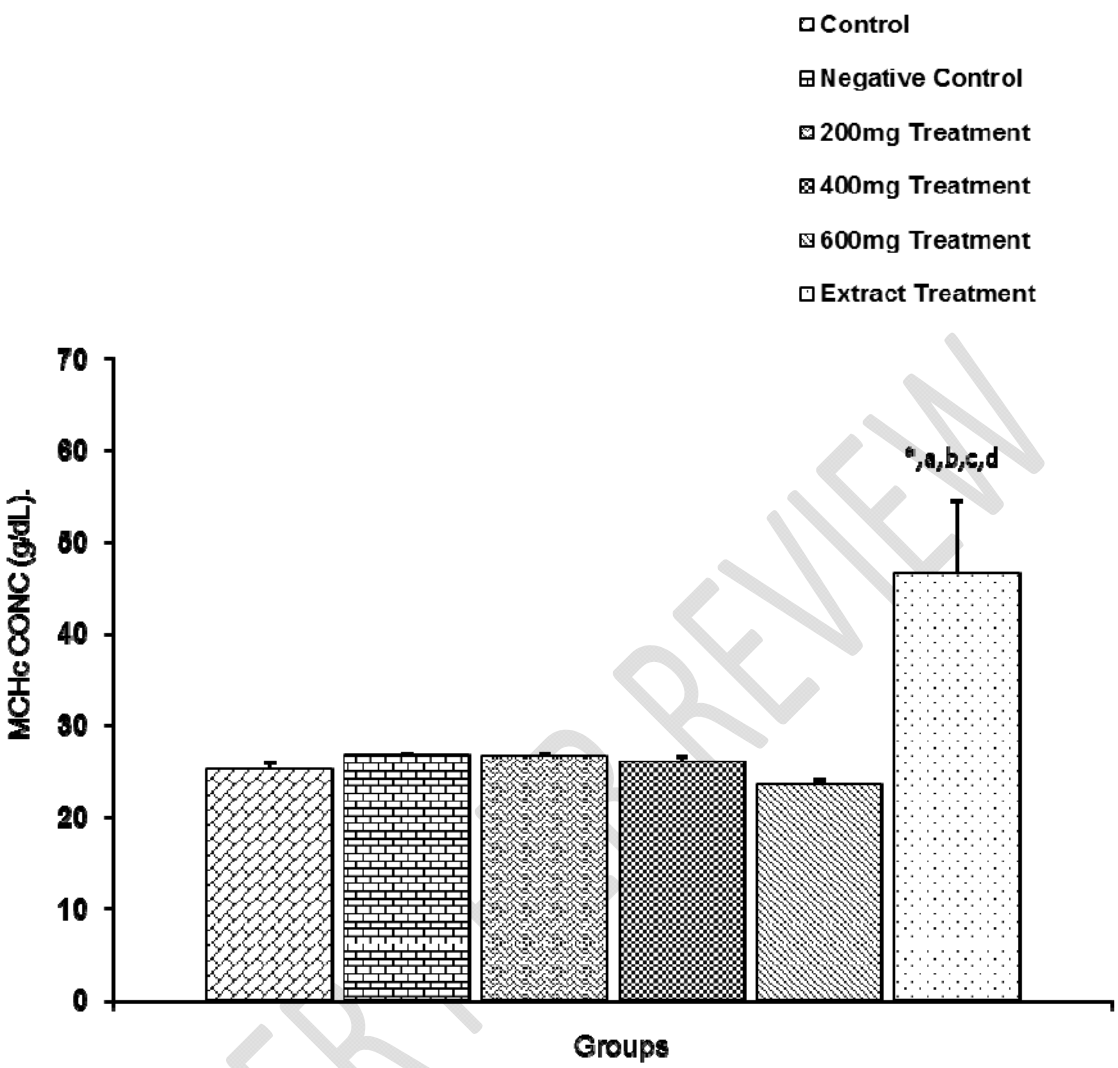

Figure Comparison of of MCHC in the different experimental groups.
Values are expressed as mean ± SEM, n = 5.
*significantly different from Control at p<0.05
a=significantly different from Negative Control at p<0.05
b=significantly different from 200mg treat

The figure below shows the effect of different doses of *moringa oleifera* extract following cute alcohol toxicity on haemoglobin count of rats. According to the data obtained, it was observed that haemoglobin volume increased insignificantly ($P<0.5$) following acute alcohol toxicity as shown in the negative group (14.05±1.55) compared to the control group (13.57±1.37). Further decrease was also observed following treatment of the induced animals with different doses (200mg, 400mg and 600mg) of *moringa oleifera* extract. While the decrease was more obvious in dose 400mg and 600mg (11.50±0.60 and 10.70±0.20) respectively, the 200mg dose and the non-induced group treated with the extract shows almost no change. Meanwhile, the overall effect of the treatment on haemoglobin count was insignificant ($P <0.5$) compared to the controls.

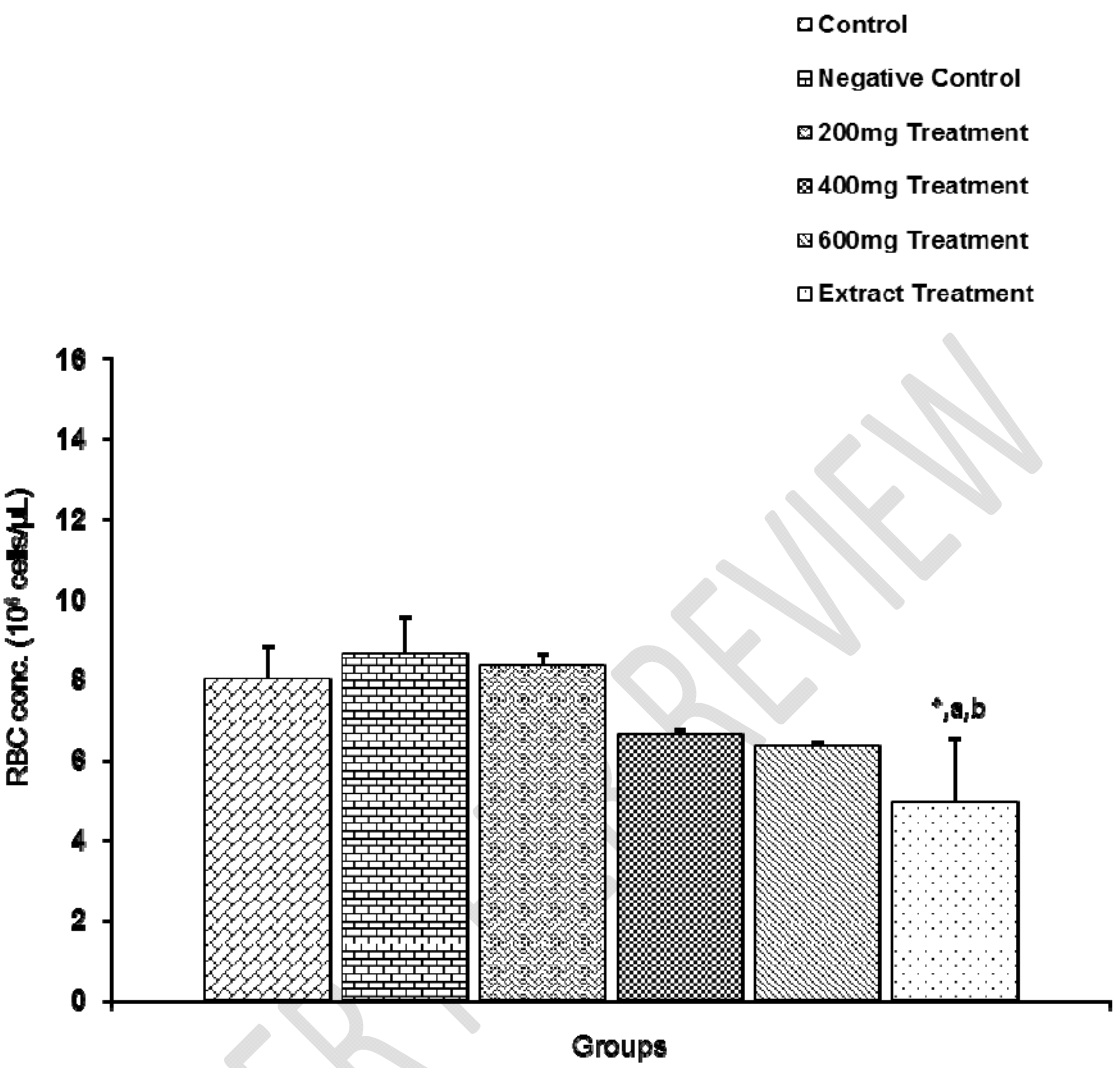

Figure Comparison of of RBC conc. in the different experimental groups.
Values are expressed as mean ± SEM, n = 5.
*significantly different from Control at $p<0.05$
a=significantly different from Negative Control at $p<0.05$
b=significantly different from 200mg

Discussion:

The administration of aqueous root extract of moringa oliefera for 14days produced a significant ($P<0.05$) decrease in RBC and WBC count comparing to that of the control group. The observed decrease in RBC count in moringa oliefera treated group may have been due to the suppressive effect of some of the active component of the plant extract on bone marrow. These bioactive components such as saponnins, alkaloids, flavonoids, and phenolic [17] (Muller 1980). They might also have suppressed the growth factors in the bone marrow. Another issues of the decreases in RBC may be due to hemolysis mediated via the phytochemical components of the plant extracts, or may be the extract could cause failure of erythropoietin production, which would lead to anemia. From the data obtained it was observed that white blood cells (WBC), packed cell volume (PCV) and mean corpuscular volume (MCV) insignificantly ($P<0.5$) decreases following acute alcohol toxicity, it is a well-known that PCV is otherwise called hematocrits which represent the percentage of bloodTherefore, increases in PCV, shows a better transportation and thus results in increase in both primary and secondary polycythemias.There is also a direct relationship between erythrocytes, PCVand haemoglobin concentration. [18] (Schalm *etal* 1975). This report is in accordance with the previous work reported by [19] (Elanchezhian et al.,2017) and (igboh et al.,2009). Also, the red blood cell (RBC) and haemoglobin (Hb) count were slightly increased following alcohol toxicity. This observation contradicts the previous report by [20] Isaacet al., (2013) which reported that there was a reduction in red blood cells and lymphocytes count relative to their control following treatment of rats with 10ml/kg of alcohol. Likewise, the same treatment causes no significant ($P<0.5$) change in total white blood cells (TWBC), Haemoglobin (Hb) and platelets relative to their controls this sharp changes in Hb concentration must have been a high dose of the extracts that

could results increase in hemolysis of the red blood cells. This also agrees with the outcome of this experiment as the alcohol toxicity did not show any significant ($P<0.5$) change in the blood indices. The same observation was noted as PCV, MCV and MCHC did not show any significant change.Upon treatment with different doses of *moringa oleifera* root extract, it was observed that there was a dose dependent response. Whereas, the 200mg and 400mg doses decreased insignificantly ($P<0.5$) relative to the negative control. The 600mg dose shows a significant ($P<0.5$) elevation in WBC count relative to both the negative control and the other treatment groups. This result indicates that with a dose of about 600mg aqueous root extract of *moringa oleifera*, the extract has the capacity to elevate WBC count which would equally impact the immunological integrity of the body by boosting the defense mechanism of the body. The elevation of WBC count is in tandem with the previous reports by [21] (Bamishaiye,2009) who reported that aqueous extract of *moringa oleifera* seed administered 100-400mg/kg weight significantly ($P<0.05$) increased neutrophil, eosinophil, basophil and monocytes counts at second and third week of administration relative to the control group. Also, from the result shown in the figure showing effects of extract on WBC count, it was observed that the group treated with extract without toxicity induction shows a significant decrease in WBC count which shows that aqueous extract of moringa extract with highly extremely doses can significantly ($P<0.05$) reduce WBC hence, compromising the body immune's system The decrease in the WBC count following the administration of moringa oleifera extracts is not in line with the normal physiological response following perception of a foreign attack by the body defense mechanism, this decrease may have resulted from the suppression of their production in the bone marrow. Also, it was observed that RBC counts were non-significantly elevated in the negative control relative to the normal control. While among the treatment group, it was observed that there was a

continuous non-significantly decreased RBC count as the extract doses increases from 200mg, 400mg and 600mg showing a dose-dependent response. While the group treated with extract alone shows a further decrease in RBC count which was significant ($P<0.05$). The result indicates that higher doses of aqueous root extract of moringa is toxic to RBC and such effect can lead to anemia.

Meanwhile, the effect of the aqueous root extract of *moringa oleifera* shows a further decrease in hematocrits count relative to the negative control. Among the dose determined treated groups, the group treated with a 200mg dose shows a higher decrease compared to other groups, but all changes were insignificant ($P<0.05$) except the extract treated group which shows significant ($P<0.05$) decrease compared to the normal control.

It was also observed that they were a little increase in MCHC levels compared to the normal control across groups, a significant ($P<0.5$) increase was only observed in the last group (The group treated with only extract). The results above also show that alcohol group treated with 400mg and 600mgbody weight of the aqueous root extract of *moringa oleifera* shows a significant ($P<0.05$) increase in MCV which indicates that the extract is capable of reversing anemic condition.

Conclusion:

The root extract of *moringa oleifera*plant is widely recognized as a medicinal plant and sometimes consumed concomitantly with alcohol. This research work shows that the plant extract contains some bioactive component or phytochemical constituents that are capable of ameliorating the toxicity effect of alcohol on animal models.

Reference:

1 Ahn, K. (2017). The worldwide trend of using botanical drugs and strategies for developing global drugs. BMB Reports, 50 (3): 111–116

2 Ebong, P. (2015a). History of medicinal plants. In: *Phtyomedicine: Panacae for Primary Health Care Delivery in Nigeria*. University of Calabar Press. Pp 5-13

3 Gordon, M. &David, J. (2001). Natural product drug discovery in the next millennium. *Pharmaceutical Biology*, 39:8-17.

4 Harborne, J. Baxter, H. (1999). General introduction. In: *Phytochemical dictionary a handbook of bioactive compounds from plants*, 2nd edition. London: Taylor & Francis. p. vii.

5 Ebong, P. (2015a). History of medicinal plants. In: *Phtyomedicine: Panacae for Primary Health Care Delivery in Nigeria*. University of Calabar Press. Pp 5-13

6 Mahmood, K., Mugal, T., & Haq, I. (2010). *Moringa oleifera*: a natural gift - a review. *Journal of Pharmaceutical Sciences and Research.* 2:775–781.

7 Amaglo, N., Bennet, R. & Curto, L.(2010). Profiling selected phytochemicals and nutrients in different tissues of the multipurpose tree *Moringa oleifera* Lam grown in Ghana. *Journal of Food Chemistry*, 122:1047-1054

8 Saini, R., Shetty, N. &Giridhar, P. (2014a). Carotenoid content in vegetative and reproductive parts of commercially grown *Moringa oleifera* Lam. cultivars from India by LC–APCI–MS. *European Food Research and Technology*, 238:971–978.

9 Rehn, J., & Eschmann, S. (2002) Global monitoring of average volume of alchohol consumption. Cozialund Pruventrimedizin, 47:48-58.

10 WHO (2004). Management of substance abuse. Part 1, Global Status Report on Alcohol, Geneva

11 Heathcote, E. (2000). Management of Primary biliary cirrhosis. The American Association for the study of liver disease practice guidelines. *Hepatology* 31:1005-1013.

12 Adedapo, A., Mogbojuri, O. & Emikpe, B. (2009). Safety evaluation of the aqueous extract of the leaves of *Moringa oleifera* in rats. *Journal of Medicinal PlantsResearch*, 3(8):586-59

13 Dacie, J.V and Lewis, S.M (1975). Practical Haematology 5th ed. Churchill Livingstone, London, pp502-503.

14 Alexander., R.R and Grifiths., J.M.(1993a). Haematocrits determination by the cyanomethaemoglobin method in Biochemical methods., 2nd ed. John wiley and sons, Inc. Publication, New York, pp186-187.

15 Alexander., R.R and Grifiths., J.M.(1993b). Haematocrits determination by the cyanomethaemoglobin method in Biochemical methods., 2nd ed. John wiley and sons, Inc. Publication, New York, pp186-187

16 Osim E.E., Akpogomeh., B.A., Ibu.J.O., Eno, A.E (2004). Experimental physiology manual, Department of physiology, University of Calabar, Calabar, 3rd ed. Pp60-81.

17 Muller.H.Tobin., G .(1980). Nutrition and food processing Groom Helm Ltd., London.UK.

18 Schalm., O.W., Jaivi., N.C and Caroll E.I. (1975). Veterinary Heamatology, 3rd ed.Lea and Febiger London. Pp385-390

19 Elanchezhian, YoganandhT, Mayilsamy S, Radhakrishnan S. (2017). Comparison of haematological parameters between alcoholics and non-alcoholics. Int J Res Med Sci; 5:5041-7.

20 Isaac, L. J., Abah, G., Akpan, B., &Ekaette, I. U. (2013). Haematological properties of different breeds and sexes of rabbits. Proceedings of the 18th Annual Conference of Animal Science Association of Nigeria

21 Bamishaiye, E.I., Muhammad, N. O., &Bamishaiye, O. M. (2009). Haematological parameters of albino rats fed on tiger nuts (Cyperusesculentus) tuber oil meal-based diet. The International Journal of Nutrition and Wellness, 10(1). Retrieved from http://ispub.com/IJNW/10/1/9293