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Journal Name:	European Journal of Medicinal Plants
Manuscript Number:	Ms_EJMP_33046
Title of the Manuscript:	EFFECTS OF ETHANOLIC EXTRACT OF <i>Moringa oleifera</i> (Lam) LEAVES ON NICOTINE-INDUCED CHANGES IN HAEMATOLOGICAL PARAMETERS IN MALE WISTAR RATS (<i>Rattus norvegicus</i>)
Type of the Article	Original Research Article

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This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound.

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(<http://www.sciencedomain.org/page.php?id=sdi-general-editorial-policy#Peer-Review-Guideline>)



SDI Review Form 1.6

PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Compulsory REVISION comments		
Minor REVISION comments	EFFECTS OF ETHANOLIC EXTRACT OF <i>Moringa oleifera</i> (Lam) LEAVES ON NICOTINE-INDUCED CHANGES IN HAEMATOLOGICAL PARAMETERS IN MALE WISTAR RATS (<i>Rattus norvegicus</i>) There is nothing like ethanolic instead it is "ethanol extract of <i>Moringa oleifera</i>...." Study design: Experimental animal study of what? This statement is not complete. Please clarify. Methodology: Thirty adult male Wistar rats were randomly divided into six groups of 5 each. Group A served as the untreated control. What do you mean by this please? Is it normal control animals or what? Please be clear on this. You can use normal control group instead Group F rats received only the moringa extract (150mg/kg bw). What do you mean by this please? Is it normal animals that received moringa extract or what? Please be clear on this. 2.0 MATERIALS AND METHOD 2.1 EXPERIMENTAL ANIMALS 30 male wistar rats weighing between 180 and 220g (State the age of the animals used in the study) were obtained from a private animal farm in Ibadan, Nigeria. 2.3 COLLECTION OF PLANT MATERIAL AND PREPARATION OF EXTRACT Fresh <i>Moringa oleifera</i> leaves were purchased at a market in Iwo, Osun State, Nigeria. The herbarium specimens were deposited in department of Botany, University of Ibadan (Voucher Specimen no. UIH-22387). The plant material was authenticated by Esimekhuai, D. The leaves were dried for about 12 days in an airy room, away from direct sunlight to avoid possible damage to their phyto-	The correction has been effected on the manuscript. Experimental animal study of ameliorative effect of <i>Moringa oleifera</i> on nicotine-induced changes in haematological parameters. The untreated control is the normal control group. I have corrected it in the manuscript. Group F rats is given Moringa extract only and it is not given nicotine at all. It comprises the normal animals given Moringa extract. They are about 4-5 months.



SDI Review Form 1.6

	constituents. The dried leaves were ground into powder (What is the weight of the ground powder of dried leaves?) and then soaked in 70% ethanol, shaken for 10minutes and allowed to stay in refrigerator for 72 hours at 4oC. 2.4 EXPERIMENTAL DESIGN A total of 30 male animals were used in this study and the administration was done for 24 days. These were divided randomly into 6 groups consisting of five rats each. The 6 experimental groups were as follows;	100g of the ground powder of dried Moringa oleifera leaves was soaked in 1000mls of 70% ethanol.
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SDI Review Form 1.6

Optional/General comments	Untreated control group (A) (it is preferable to you use normal control group than untreated control group A) 2.5 BLOOD COLLECTION Blood samples were obtained from the tail vein on the 12th day of administration. (Why was blood collected on the 12th day of administration before the animals were eventually sacrificed? Please state the reason here). You failed to include the methods used in the determination of differential white blood cell count in your study. This was absent in your methodology section. Please include them. Red blood cell (RBC) membrane lipids are rich in polyunsaturated fatty acids; therefore, the oxidative effects of oxygen on RBC membranes are greater than on other tissues. Moreover, RBCs contain haemoglobin, which is one of the most potent catalysts of lipid peroxidation. The invasion of the RBC membrane by peroxidants, which occurs with haemoglobinopathies, radioactive radiation, the consumption of oxidative drugs such as nicotine, and the decreased function of antioxidant systems, can lead to RBC haemolysis [3]. In addition to causing lipid peroxidation, peroxidants can cause the oxidation of -SH groups in proteins and RBC membranes. The -SH groups are highly reactive and can be a target during oxidative stress. Glutathione directly protects membrane proteins and preserves their stability. Decreased levels of glutathione lead to a decrease in -SH groups [4], and can result in the oxidization of membrane -SH groups and loss of membrane stability [22]. In a study by Lopez-Revuelta et al., (2005) [23], the ethanolic extract of moringa leaves, showed increased glutathione (GSH) content. GSH acts as an antioxidant both intracellularly and extracellularly, and it is a major nonprotein sulfhydryl compound, with many biological functions, including maintenance of membrane protein-SH groups in the reduced form, the oxidation of which can otherwise cause altered cellular structure and function [24]. The increased GSH may thus prevent the oxidative damage of the red blood cell membrane. Like GSH content, a similar pattern was observed for Malonyl-aldehyde Dehydrogenase (MDA) [23], the aqueous extract of Moringa leaves maintained the normal level of erythrocyte MDA, suggesting that the extract may have a mixture of biomolecules with hydroxyl groups that prevent the	Correction has been made. The blood samples were collected at the 12th day (about 2 weeks) of the experiment to see if the administration of the test extract would ameliorate the effect of nicotine within short time duration. This also collected at the expiration of the study(24th day). It has now been included. It was by omission error it was not included initially. Thanks for calling my attention to it. Ok, we have removed the speculations.
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	abstraction of hydrogen atom from the double bond of lipid bilayer thereby avoiding the damage of lipid membrane of red blood cell. (There were a lot of speculations here. Some of the parameters you discussed here were not assayed for in your study, therefore why are speculating so much about them? Please minimize your discussion on literature mentioned here and limit your result discussion on your major research findings please). Revise this section please. 5.0 CONCLUSION From the available findings in this study, it can be concluded that administration of <i>Moringa oleifera</i> to Wistar rats attenuated the nicotine-induced changes in the red blood cell count, haemoglobin concentration and other red blood cell indices; however, post-treatment with <i>moringa</i> produced a protective effect against these changes in haematological parameters, hence, this may be recommended to consumers of tobacco products as part of the management or rehabilitatory measures for such individuals.	This is a good idea. I have incorporated it. Thanks.
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