



ASN- PH-020919
ISSN: 2315-537X

International Journal of Herbs and Pharmacological Research

IJHPR, 2015, 4(3): 40 - 45

www.arpjournals.com; www.antrescentpub.com

E- ISSN: 2384-6836

RESEARCH PAPER

THE EFFECT OF TOBACCO SNUFF ON BLOOD COAGULATION PARAMETERS IN ADULT WISTAR RATS

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Received: 22nd June, 2015

Accepted: 29th July, 2015

Published: 31st July, 2015

Endorsed By: Innovative Science Research Foundation (ISREF). **Indexed By:** African Journal Online (AJOL); Texila American University; Genamics; Scholarsteer; EIJASR; CAS-American Chemical Society; and IRMS Informatics India (J-Gate)

ABSTRACT

This study evaluates the effect of tobacco snuff on platelet count, prothrombin time (PT), and partial thromboplastin time (aPTT) in adult Wistar rats weighing between 150-300g and divided into four group (A - D; n=12 each). Group A served as control, while B, C and D served as test groups. The test groups were further divided into four subgroups (B1 - D1; B2 - D2; B3 - D3; and B4 - D4); representing experimental durations of 2, 4, 6 and 8 weeks respectively. Groups B1-D1; B2-D2; B3-D3; and B4-D4, received varying doses of tobacco snuff and at the end of 2, 4, 6 and 8 weeks respectively, 3 rats from the sub groups were prepared for blood sample collection into EDTA and plain containers via cardiac puncture. Platelet-poor-plasma for PT and aPTT determination was obtained by spinning the blood at 2000rpm for 15 minutes, while sample analysis was performed using Diammonium ethanedioate and the prothrombin time/activated partial thromboplastin test kits. The results obtained showed that tobacco snuff has capacity to induce platelet count reduction, prolong aPTT and delay PT. Thus, as more public enlightenment in this regard is required, specific molecular studies are necessary to fully explain the mechanisms.

Key words: Blood, coagulation, coagulation factors, Tobacco, Yaji,

INTRODUCTION

Across the globe, the use of smokeless tobacco is quite popular (Bates *et al.*, 2003) and despite several anti-tobacco campaigns against its pathological effects, tobacco consumption remains a growing phenomena (Changrani and Gany, 2005; Okonkwo *et al.*, 2013). According to Blomfield (1992), Nicotine is the primary constituent of smokeless tobacco. However, several other phytochemicals have been identified in tobacco including heavy metals with carcinogenic potentials (Hoffmann and Hecht, 1985; Hecht and Hoffman, 1988; Chiba and Mansironi, 1992; Hecht *et al.*, 2007; Addo *et al.*, 2008; Stepanov *et al.*, 2010; Addo *et al.*, 2011; Garg *et al.* 1995).

Of particular interest, is the influence of tobacco on coagulation as available scientific literature does show that tobacco can induce severe liver damage with the attendant inhibition of clotting factor's production, and in turn,

resulting in prolonged bleeding (Mammen, 1994; Kerr, 2003; Kerr *et al.*, 2003). This was further highlighted by Peyvandi, (2006) that bleeding abnormalities are attributable to coagulation factors inhibitors, which of course, is a serious source of concern among pregnant women with pre-pregnancy addiction to tobacco and especially in this era of concerted efforts to stem the tide of maternal mortality and morbidity. More so, only a few studies have been conducted to address the effect of smoking on platelets and such studies however, did not compare the findings with those from nonsmoking control groups (Pasupathi *et al.*, 2009; Wolfram *et al.*, 2008; Gremmel *et al.*, 2009).

Nevertheless, tobacco has been implicated in the acute increase of platelet aggregations (Joseph *et al.*, 1995), recurrent miscarriages, excessive bleeding in pregnancy and infertility due to blood coagulation, and platelet defects (Homan *et al.*, 2007; Hassan and Killick, 2004; Augood and Duckitt *et al.*, 1998). This controlled study therefore, assesses the effect of tobacco snuff on platelet count, prothrombin time, and partial thromboplastin time in adult Wistar rats.

MATERIALS AND METHODS

Experimental Animals: 48 adult Wistar rats of comparable sizes and weighing 150-300g was purchased from the animal farm of Anthonio Research Center, Ekpoma, Edo State, Nigeria and transferred to the experimental site where they were allowed 2-week acclimatization in a wooden wire mesh cages under standard laboratory procedure.

Substance of Study/ Substance Preparation: Dry leaves of tobacco and potash were purchased from Ogbete main market, Enugu State, Nigeria. The dried tobacco leaves and potash were blended into powder using a mortar and iron pestle and then stored appropriately prior to the commencement of study. The blended tobacco leaves with potash and weighed using an electronic balance (Denver Company, USA, 200398. IREV. CXP-3000) to obtain the various required doses. For the purpose of this study, feed pellets were prepared as described by Nwaopara *et al.* (2011).

Animal Groupings: The rats were divided into four groups (A, B, C and D) with group A serving as control, while groups B, C and D served as the test groups. The test groups were further divided into four groups (B1, C1, D1; B2, C2, D2; B3, C3, D3; and B4, C4, D4) representing four experimental stages/duration of 2, 4, 6 and 8 weeks respectively and on varying doses of tobacco dust mixed with potash respectively. At the end of 2, 4, 6 and 8 weeks respectively, 3 randomly selected rats from the groups were prepared for blood sample collection via cardiac puncture.

Substance Administration: The concentrations of tobacco used in this study were adopted from the work of Bagchi *et al.* (1994) while that of potash was adopted from Ugbor *et al.* (2013). During the experiment, the animals received as follows:

1. Stage 1 (2 weeks; n=12): Group A (control) received 100g of feed and distilled water only whereas test group B1, C1 and D1 received 97.12g of feed, 2.4g of tobacco dust and 0.48g of potash; 94.24g of feed, 4.80g of tobacco dust and 0.96g of potash; and 91.36g of feed, 7.20g of tobacco dust and 1.44g of potash respectively. Each test group received distilled water *ad libitum*.
2. Stage 2 (4 weeks; n=9): Group A (control) received 75g of feed and distilled water only, whereas test group B2, C2 and D2 received 72.84g of feed, 1.8g of tobacco dust and 0.36g potash; 70.68g of feed, 3.6g of tobacco dust and 0.72g of potash; and 68.52g of feed, 5.4g of tobacco dust and 1.08g of potash respectively. Each test group received distilled water *ad libitum*.
3. Stage 3 (6 weeks; n=6): Group A (control) received 50g of feed and distilled water only, whereas test group B3, C3 and D3 received 48.56g of feed, 1.2g of tobacco dust and 0.24g potash; 47.12g of feed, 2.4g of tobacco dust and 0.48g of potash; and 45.68g of feed, 3.6g of tobacco dust and 0.72g of potash respectively. Each test group received distilled water *ad libitum*.
4. Stage 4 (8 weeks; n=3): Group A (control) received 25g of feed and distilled water only, whereas test group B4, C4 and D4 received 24.28g of feed, 0.6g of tobacco dust and 0.12g potash; 23.56g of feed, 1.2g of tobacco dust and 0.24g of potash; and 22.84g of feed, 1.8g of tobacco dust and 0.36g of potash respectively. Each test group received distilled water *ad libitum*.

Sample collection and sample analysis: At the end of each stage of the experiment, blood samples were collected from the rats via cardiac puncture into an EDTA container and a plain container with 3.2% trisodium citrate. The samples for PT and aPTT were spun at 2000rpm for 15 minutes to obtain platelet poor plasma. The tests were subsequently carried out using Diammonium ethanedioate and prothrombin time and activated partial thromboplastin test kits from Diagen Ltd (calcium rabbit brain thromboplastin and kaolin platelet substitute mixture were used in the study).

Data analysis: All the data collected were subjected to statistical analysis using SPSS (version 18). The test values were compared with the control using student's t-test and ANOVA (LSD) at 95% level of confidence.

Study Duration: The preliminary studies, animal acclimatization, substance procurement (tobacco leaves and potash), actual animal experiment and evaluation of results, lasted from July, 2013 to December, 2013. However, the actual administration of tobacco snuff to the test animals lasted for 8 weeks.

RESULTS

The effect of tobacco on platelet count: The effects of tobacco on platelets in the test groups and control are shown in table 1. The observed differences were not statistically significant ($P > 0.05$) compared to the control. Group D3 rats died before end of the experimental period (8 weeks) and as such, no values were recorded for D3.

Table 4: Platelet activity, activated partial thromboplastin time and prothrombin time in rats administered tobacco snuff and *Yaji* at the end of 8 weeks

GROUP	PLATELET COUNT	aPTT	PT
A (Control)	527500.00	17.19±7.57	19.70±3.85
B1	454000.00	2.34±0.20	7.01±10.32
C1	323666.67	2.8±0.57	17.79±27.90
D1	307666.67	3.35±0.83	17.81±27.89
B2	216666.67	20.59±9.00	15.74±4.86
C2	256000.00	25.03±2.81	12.68±11.67
D2	274000.00	20.37±10.49	10.04±3.60
B3	46000.00	17.03±4.40	9.82±3.29
C3	50333.33	18.83±6.23	12.07±2.10

The effect of tobacco on activated partial thromboplastin time (aPTT) and prothrombin time (PT): The effects of tobacco on aPTT in the test groups and control are shown in table 1. The aPTT was lower in the groups that received graded levels of tobacco snuff. There were differences in the aPTT of the control and tests groups, but the differences were not statistically significant ($P > 0.05$). Also, there were no values for group D3 because the rats died before the end of the last stage of the experiment (8 weeks).

On the other hand, the prothrombin time (PT) recorded at the end of the experiment for the control group was 19.70±3.85 as compared with the test values (Table 1). There were differences in the PT recorded for the control and test groups, but the observed differences were also not statistically significant ($P > 0.05$). However, the observed effect of Tobacco on PT was not dosage dependent but duration dependent. Similarly, no values were recorded for group D3 as the rats died before the end of the last stage of the experiment (8 weeks).

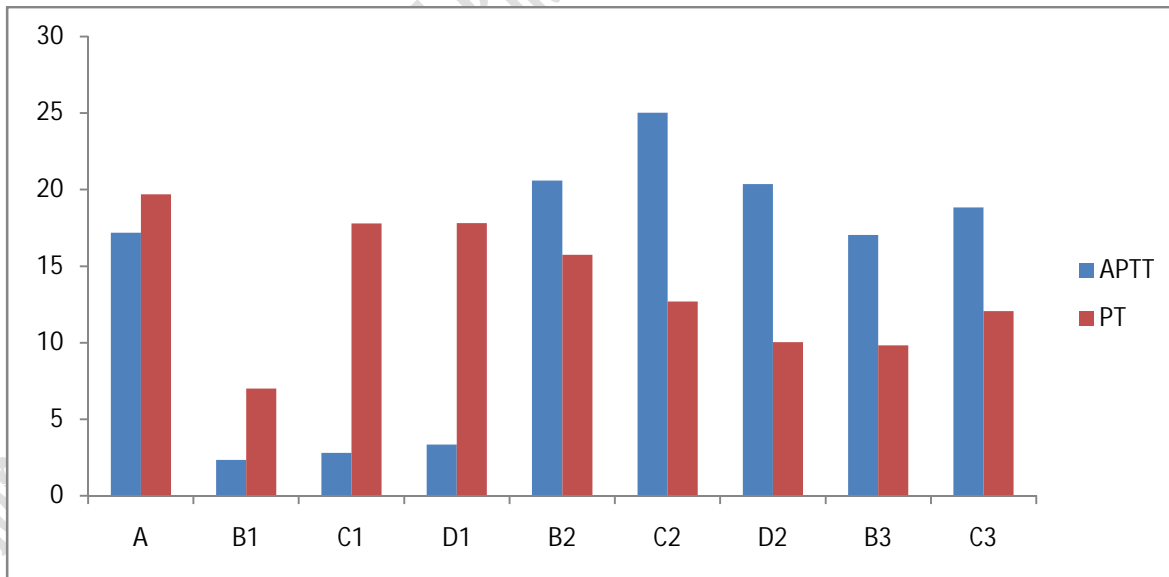


Figure 2: A bar chart showing activated partial thromboplastin time (aPTT) and prothrombin time (PT) in the test and control groups

DISCUSSION:

The observed changes in the platelet count of the test groups, as compared to the control values, can not be unconnected to the effect of tobacco on platelet aggregation. This is in line with the reports by Joseph *et al.* (1995) who stated that such changes in platelet aggregations is attributable to tobacco-induced arterial wall damage. Even Ochei and Kolhatkar (2000) had earlier reported that decreased platelet counts could be due to liver disease, which, according to Kuramitsu *et al.* (1985) and Ugbor *et al.* (2013b), can possibly be induced by tobacco snuff ingestion.

Of interest in these reports also, is the fact that renal disease has been implicated in platelet dysfunction and reduced aggregation (Hampton and Preston, 1997) which, by implication, could cause reduced platelet levels and bleeding defects. These earlier reports, including the observations of this study, may shed light on the possible mechanisms through which tobacco snuff could cause decreased platelet count as Okonkwo *et al.* (2013) had earlier asserted that tobacco snuff has the capacity to induce renal disease.

On the other hand, the observations that activated partial thromboplastin time was initially decreased but later became less definitive and comparatively prolonged in the test groups than the control, could possibly be due to liver damage that might consequently result in decreased clotting protein synthesis (Hampton and Preston, 1997). Even Mohammed (2010) had earlier asserted that activated partial thromboplastin time shows prolonged time among tobacco smokers. The finding on prothrombin time however, contradicts the report by Mohammed (2010) on comparative differences in Prothrombin time among smokers. Of course, the obvious difference in the nature of the subjects of study -human and rats respectively, may explain this contradiction with our findings; though the paucity of information in this regard remains a factor that can not be ignored.

Above all, the findings of this study suggest that tobacco snuff has the capacity to induce platelet count reduction, and prolonged and delayed aPTT and PT time respectively. It is our recommendation therefore, that in addition to the commonly advertised adverse effects of tobacco snuff consumption, information on the numerous secondary consequences of tobacco snuff consumption must be included in the public enlightenment campaigns against its tobacco abuse. More so, the public need to be educated that tobacco snuff consumption is not a safe alternative to cigarette smoking. We also advocate that the use of tobacco snuff as medication for long grief pain and aches should be discouraged, while specific molecular studies should be carried out to ascertain the actual mechanism through which tobacco influences coagulation.

ACKNOWLEDGMENT

The authors are thankful to our students who painstakingly participated in the animal studies that lasted several months.

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AUTHOR'S CONTRIBUTION

All the authors participated in the different stages of this study culminating with this publication.