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Haemostatic Alterations after Sildenafil and Tramadol Administration in Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Author RBJ designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author EME managed and supervised the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Haemostatic parameters constitute measurable indices in the haemostatic system used to assess the functionality of the coagulation system of an individual to establish a state of health or disorder. This study evaluated haemostatic parameter such as platelets count, mean platelet volume (MPV), platelets distribution width (PDW), prothrombin time (PT) and activated partial thromboplastin time (APTT) in 22 Male Albino Rats grouped and orally treated daily for three weeks with Sildenafil (4 mg/200 g.bwt), Tramadol(6 mg/200 g.bwt) and Sildenafil/Tramadol combination (4+6 mg/220 g.bwt). Rats were sacrificed by cardiac puncture and 5 mls of blood collected for the analysis of the parameters using Sysmex haematology analyser and Agape Diagnostic reagents kits. Results obtained shows a statistically significant increase in platelet count, PT and APTT compared with control across the various groups ($p<0.05$). A statistically significant decrease was observed in MPV, PDW in Sildenafil+tramadol group, significant decrease in platelets distribution width for Tramadol group when compared with control ($p<0.05$). No significant difference was observed in the mean platelets volume and platelet distribution width in Sildenafil group. A comparison of Sildenafil+tramadol and Sildenafil groups shows no statistically significant difference in all the parameters analysed. There was also no significant difference in the mean platelets count, PDW, PT and APTT when Sildenafil+tramadol and Tramadol groups were compared ($p<0.05$). However, a statistically significant increase was seen in platelets count when Sildenafil+tramadol and

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tramadol were compared ($p<0.05$). Sildenafil and tramadol causes significant increase in platelets count, prolonged PT and APTT following single/combined daily administration in rats. Further research on these parameters, assessment of liver function, and measurement of intrinsic and extrinsic pathway coagulation factors in human taking this medication is recommended.

Keywords: Haemostatic; sildenafil; tramadol; prothrombin; thromboplastin.

1. INTRODUCTION

The need for sexual satisfaction and fulfilment coupled with energy for artisan works has predisposed a lot of youth in Nigeria to the abuse of tramadol and Sildenafil which are drugs of choice for treatment of pains, premature ejaculation, erectile dysfunction and pulmonary hypertension. Haemostatic parameters constitute measurable indices in the haemostatic system used to assess the functionality of the coagulation system of an individual to establish a state of health or anomaly/disorder. They include bleeding time, clotting time, thrombin test (TT), prothrombin time (PT), fibrinogen level, Activated partial thromboplastin time (APTT) among others.

Tramadol are narcotic-like, centrally acting pain relieve drug having its action at μ-opioid adrenergic and 5- hydroxytryptamine (5-HT) receptors used for the management of acute and chronic pain, premature ejaculation due to its ability to inhibit weak re uptake of norepinephrine and serotonin [1,2]. They exhibit a multimodal sequence of action with one promoting the weak inhibition and re-uptake of norepinephrine and serotonin which constitutes its basis for use in treatment of premature ejaculation in men [3].

Phosphodiesterase type-5 inhibitor (PDE-5) inhibitors constitute a therapeutic class, and sildenafil is one of its members and it is a drug normally administered on patients for the treatment of erectile dysfunction and pulmonary hypertensions of various etiologies in men [4]. The mechanism of action of this drug is that they selectively act on the smooth muscles of the lungs and the penis due to the mammoth number of receptors primarily distributed within this areas causing their relaxation and allowing free flow of blood into blood vessels of the lungs and penis [4,5,6,7]. Sildenafil drug have been so named because it selectively obstruct the action of phosphodiesterase type 5, an enzyme which stimulates the degradation process for cyclic guanosine monophosphate (cGMP) thus enhancing prolong erection as long as the cyclic guanosine monophosphate is not degraded within smooth muscles in the penis [8].

These groups of drugs aretop in recreational useamongst youth in Nigeria who intends to achieve longer lasting erection and perform better during sexual escapade [9]. Several researches on the effects of these drugs on haematological parameters reveals derangement in haemoglobin concentration and Packed cell volume [9,7,10,11,12]. Most of the conclusion from these studies suggested that these drugs have no suppressive or inhibitory tendencies on the erythropoietic system and thus attributed the derangement to drug enhanced defective haem-biosynthesis during erythropoiesis which could have resulted in drug induced haemolysis (destruction of already produced and circulating red blood cells).

Fewer studies of the effect of these drugs on haemostatic parameters have been reported by Shatha& Adnan, [13] who concluded that there was a mild decrease in prothrombin time (PT) and a marked decrease in activated partial thromboplastin time (APTT) following administration of these drugs and attributed the decrease to increase platelet activity that activated their aggregation. Bol'shakov et al. [14] had also concluded that the combination of tramadol and droperidol enhance the coagulation of platelets plasma proteins and suppresses the thrombocytes disaggregation process. Ayten et al. [15] in their studies with tramadol administration in patients with gynaecologic malignancies concluded that tramadol affects the coagulation system in vivo and causes impairment/ suppression in a concentration dependent manner.

Therefore, with regards to the increase rate in recreational use of these drugs and recourse to possible side effects, paucity of information on the effects of this drugs singly or in combination on haemostatic parameter, it is of necessity to generate research proven information and knowledge in an attempt to explain the effect of these drugs singly or in combination on haemostatic parameters and thus this research work.

2. MATERIALS AND METHODS

2.1 Animal Preparation

A total of 22 male albino rats weighing between 200-220 kg were purchased and given normal rat chows *ad libitum,* allowed free access to waterduring the experimental period after one week of acclimatization to its new environment.

2.2 Experimental Design and Drug Administration

The rats were randomly assigned into 3 groups (n=6), Group I (Sildenafil+tramadol- (4+6 mg/220 g. body weight), Group II (Sildenafil-(4 mg/200 g. body weight) and Group III (Tramadol-(6 mg/200 g. body weight) treated daily by oral gavage method as treatment groups and control group (n=4) fed with normal rats chow and water for 3 weeks.

2.3 Collection of Blood Sample

On completion of the experimental period the Male albino rats were sacrificed by cardiac puncture using 3.8% chloroform as anaesthesia and 5 ml of blood collected from subjects and control. Blood samples were dispensed into pre-labelled EDTA anticoagulated sample bottle, Sodium citrate anticoagulated sample bottle with gentle mixing to ensure proper distribution of anticoagulant and avoidance of clot formation.

2.4 Measurement of Haemostatic Parameters

Determination of parameters such as Platelets count, Platelet distribution width (PDW) and Mean platelet volume (MPV) were analysed using Sysmex KX-2IN Autoanalyser, Kobe, Japan, following standard calibration as prescribed by the manufacturer. Prothrombin time (PT), activated partial thromboplastin time (APTT) were determined using Agape Diagnostic reagents kits (Switzerland).

2.5 Statistical Analysis

GraphPad Prism 5.03 software was used to perform post hoc (Turkey's) multiple comparison tests on data generated. Other Statistical measures used were one way analysis of variance (ANOVA). Results were presented as mean ± standard Deviation (SD) and displayed in Tables. Values of $p<0.05$ was the criterion for statistical significance.

3. RESULTS

3.1 Haemostatic Parameters in Male Abino Rats after 3 Week of Treatment for Various Drug Regimens

Table 1 shows a comparison of haemostatic parameters in the different experimental groups following 3 weeks of treatment with various drug regimens. A statistically significant increase in platelet count, prothrombin time (PT) and activated partial thromboplastin time (APTT) was observed in the mean value when compared with control across the various groups ($p<0.05$). A statistically significant decrease was observed in mean platelet volume (MPV), Platelets distribution width (PDW) in Group I (Sildenafil+ Tramadol group), significant decrease in platelets distribution width (PDW) for Tramadol group when compared with control ($p<0.05$). No significant difference was observed in the mean platelets volume (MPV) and platelet distribution width (PDW) in Sildenafil group. A comparison of Sildenafil+Tramadol and Sildenafil groups shows no statistically significant difference in all the parameters analysed. There was also no significant difference in the MPV, PDW, PT and APTT when Sildenafil+Tramadol and Tramadol groups, Sildenafil and Tramadol are compared $p<0.05$. However, a statistically significant increase was seen in platelets count when Sildenafil+Tramadol and Tramadol were compared, also when Sildenafil and Tramadol was also compared ($p<0.05$).

4. DISCUSSION

Findings in this research contradicts Nna et al. [7] who recorded a statistically significant decrease in platelets count, platelets distribution width and mean platelet volume as our results shows a statistically significant increase ($p<0.05$) in platelet count, prothrombin time (PT) and activated partial thromboplastin time (APTT) and agrees partly with the research of Shatha & Adnan, [13] who concluded that there was a mild decrease in prothrombin time (PT) and a marked decrease in activated partial thromboplastin time (APTT) following administration of these drugs and attributed the decrease to increase platelet activity that activated their aggregation. The prolong PT and APTT time found in this study

Table 1. Comparison of Mean ± SD of Haemostatic Values after 3 Weeks of Administration of Drug Regimens in Study Groups and Control

Groups	Platelet(x10^3 cell/µL)	MPV (fL)	PDW (fL)	PT (seconds)	APTT (seconds)
Control (n=4)	292±71.98	8.8±0.18	14.6±0.37	26.4±3.19	56.3±2.20
Group I (n=6)	630±48.90	7.6±0.26	10.5±1.02	37.1±1.25	66.0±4.31
Group II (n=6)	650±155.3	8.3±0.59	12.5±2.63	39.4±5.14	68.5±6.89
Group III (n=6)	813±34.84	8.2±0.14	11.0±0.10	40.3±1.44	69.4±3.11
p-value	0.0002	0.0001	0.0021	0.0001	0.0022
Remark	S	S	S	S	S

Key: S= Significant, Group I = (Sildenafil+Tramadol), Group II = (Sildenafil Only), Group III = (Tramadol Only), MPV= Mean platelet volume, PDW= Platelet distribution width, PT= prothrombin time, APTT= Activated partial thromboplastin time

could be suggestive that the drug enhances possible haemostatic protein consumption *in vivo* and thus can result in possible aggravation of aggressive bleeding if not monitored during administration.

In an earlier research it was found that there was a statistically significant increase in Red blood cell count and white blood cell count [9] and this is suggestive of a possible effect of these drugs on the haemopoietic system leading to a corresponding increase production of cells from their various pluripotent cell lines including platelets count. The increase in platelet count could also be a pointer that these drugs does not hinder the production of platelets but fasten the consumption of haemostatic proteins drastically resulting in prolong prothrombin time (PT) and activated partial thromboplastin time (APTT) in study group when compared with control.

Since PT and APTT are drastically increased, it could also be inferred that these drugs singly or in combination have some level of effects on the liver. Haemostatic proteins are mainly produced in the liver and if there are deficiencies in the viability or functionality vis a vis quantity of this proteins then its means that there are some level of distortion in the liver where its production is domiciled. Furthermore, a prolonged PT is an indication that there is deficiency in one or more extrinsic factors of coagulation since PT measure the coagulation factors in the extrinsic pathway. From our results, it can be further inferred that the drugs both singly and in combination has effects on extrinsic factors of coagulation and thus the prolonged PT recorded in our result.

APTT are haemostatic test conducted to assess the functionality of the intrinsic factors in coagulation studies; a prolonged APTT thus serve as an indication to deficiencies in some coagulation factors found in the intrinsic pathway. Finding in this research shows that all groups showed prolonged APTT when compared with control values. This is a clear indication that factors found in the intrinsic pathway of coagulation are affected by these sets of drugs. Perhaps this drugs majorly affects intrinsic factor XII, then there will be only a prolonged APTT without bleeding disorder. Our findings have also established that there is prolong PT and APTT and this can be due to deficiency in the factor of the common pathway such as factor X,V,II and I.

Findings in this research is in tandem with the findings of Bol'shakov et al. [14] who concluded that the combination of tramadol and droperidol enhance the coagulation of platelets plasma proteins and suppresses the thrombocytes disaggregation process. This assertion/ findings could further explain the reason behind the prolong PT and APTT recorded in this research.

Very high values for platelets count was seen in group III (Tramadol treated) when compared to control from our result. This is an indication that Tramadol intake sponsors the production of platelets cells. This elevated platelet count seen in the study group could also be traceable to possible case of iron deficiency and reactive condition such as inflammation due to increased white blood cell count recorded in various study groups with these set of drugs as earlier reported in a previous research [9,16].

Furthermore, a higher value for APTT was noticed in group III (Tramadol treated) suggesting that tramadol has higher effect on intrinsic coagulation factors and this agrees with Ayten et al. [15] who in their studies with tramadol administration in patients with gynaecologic malignancies concluded that

tramadol affects the coagulation system in vivo and causes impairment/suppression in a concentration dependent manner.

5. CONCLUSION

Sildenafil and tramadol causes significant increase in platelets count, prolonged PT and APTT following single/combined daily administration in rats.

6. RECOMMENDATION

Further research on these parameters, assessment of liver function, and measurement of intrinsic and extrinsic pathway coagulation factors in human taking this medication is recommended.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical approval have been collected from the relevant authorities and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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