Original Research Article

Does Single/Combined Administration of Tramadol/Viagra has Reversal Effects on Haematological and proinflammatory Cytokines in Male Albino Rats

Abstract

Aim: This study evaluated reversal effects of single/combined doses of tramadol/sildenafil drugs on haematological and pro-inflamatory cytokines in male albino rats.
Study design: Case control study
Methodology: Forty (40) male albino rats (180-220g body weight) were grouped into 4 (n=12) administered orally with tramadol+sildenafil (6 and 4mg/220g.bwt), sildenafil (4mg/180g.bwt), tramadol (6mg/180g.bwt) and control (n=4) for 3weeks and allow for additional 3weeks without treatment. Rats were sacrificed by cardiac puncture at the end of week 3 and 6 with 4ml of blood collected for analysis of haemoglobin concentration (Hb), Packed cell volume (PCV), Red blood cell (RBC) count, White blood cell (WBC) count, platelet count, interleukin 8 (IL-8) and interleukin 10 (IL-10) using Elabscience ELISA-kits. GraphPad Prism 5.03 software was used to analyze data generated.
Results: There was no statistically significant difference in PCV , Hb, RBC and WBC count in sildenafil+tramadol, sildenafil only group when both phases are compared ($p>0.05$). There was however a reversal of effect in platelets count (630±48.9 vs 370±44.3) for sildenafil+tramadol group but not sildenafil only group when both phases are compared ($p<0.05$). There was significant reversal effect in HB (13.8±1.68 vs 15.4±0.42), PCV (40.5±0.53 vs 47.8±1.09) and plaetelets count (813±34.8 vs 506±104) in tramadol only group when both phases were compared ($p<0.05$). No statistically significant difference was observed in RBC and WBC count in tramadol group ($p>0.05$). Cytokine parameters in sildenafil group showed no significant difference in IL-8, IL-10. There was reversal effect in IL-8(282.1±7.65 vs 556.8±8.42), IL-10 (7.98±0.29 vs 12.88±0.83) in sildenafil+tramadol, IL-8 (341.7±5.63 vs 507.2±6.23), IL-10 (14.33±2.36 vs 23.8 ±2.18) in tramadol group.
Conclusion: Routine use of sildenafil+tramadol, Sildenafil, Tramadol causes derangement in haematological proinflammatory cytokine resulting in negligibly reversibility in haematological parameters, marked reversibility in proinflammatory cytokine following their withdrawal. This effect can be applicable in humans who abuse these drugs. Thus, evaluation of the effect of these drugs on the haemostatic and pro-inflammatory cytokines in humans is necessary.

KEY WORDS: Tramadol, Sildenafil, haematological, pro-inflammatory, cytokine, interleukin

INTRODUCTION

Tramadol and Viagra are drugs used for the treatment to acute and chronic pain, premature ejaculation and erectile dysfunction in men respectively (Ayoub *et al*., 2014; Senay *et al.,* 2003). They are ranked amongst top commonly abused drugs amongst youths in Nigeria (Baribefii *et al*, 2018, Nna *et al.,* 2015). Following its association with seizure and death, several cases of instant death has been recorded when tramadol is taken together with alcohol (Daubin *et al*., 2007). Tramadol (commonly called Tramal) is a narcotic-like pain

reliever drug which is a centrally acting analgesic agent with activity at μ-opioid, adrenergic and 5- hydroxytryptamine (5-HT) receptors used for the treatment of both acute and chronic pain of moderate to (moderately) severe intensity, treatment of premature ejaculation due to its ability to inhibit weak re-uptake of norepinephrine and serotonin (Ayoub *et al*., 2014; Senay *et al.,* 2003). Its multimodal action of inhibiting weak re-uptake of norepinephrine and serotonin constitute the basis for its use in treatment of premature ejaculation in men (Salam *et al*., 2008). Tramadol and Sildenafil have been shown to have pronounced effects on the haematological parameters both in rat model and human studies (Ayoub *et al*., 2014; Shatha & Adnan, 2015). Nna *et al*, (2016) reported a significant decreased in haematological parameters such as packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell (RBC) count following the administration of tramadol and Viagra singly and in combination. Akhtardanesh *et al*, (2014) observed that there was no significant difference in red blood cell (RBC) count in test group compared with control when these drugs were administered.

Sildenafil commonly called Viagra is a class of drugs known as the phosphodiesterase type-5 inhibitor (PDE5i) used for the treatment of erectile dysfunction of various etiologies in men and pulmonary hypertension (Nna *et al*. 2016). Its works by the mechanism of selective action on the smooth muscles of the lungs and the penis as a result of the large number of receptors primarily distributed within these areas (Supuran *et al.,* 2006; Aversa *et al.,* 2006; Oka *et al.,* 2015; Nna *et al.,* 2016) and are named phosphodiester type 5 drugs due to their ability to selectively inhibit the action of phosphodiesterase type 5, an enzyme which promotes the degradation of cyclic Guanosine monophosphate (cGMP) in the smooth muscles of the penis causing them to dilate and allowing free flow of blood for perfect and sustained erection during sexual intercourse thus enhancing prolonged erection as long as the cyclic guanosine monophosphate is not degraded (Salam *et al*., 2015).

Cytokines are generic name for a category of loosed or broad proteins, peptides and/or glycoproteins molecules produced at various immunologic sites by variety of cells that play important role in cell signaling to aid cell to cell communication in immune response to stimulus by foreign antigens (Venugopal, 2007). Cytokine play a pivotal role in coordination and regulation of immune responses (Gaspani *et al*., 2002; Niemand *et al*., 2003). There are pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α produced majorly by T-helper 1 cells and anti-inflammatory cytokines IL-1 receptor antagonist, IL-4, and IL-10, IL-8, IL-13 produced by T helper 2 cells (Zhang and Jianxiong, 2007). Studies on interleukin 10 (IL-10) has revealed that it has predominant inhibitory abilities on lipopolysaccharides and bacterial products mediated induction of pro-inflammatory cytokines (TNF-α, IL-1β, and IFN-γ secretion from toll like receptors (Varma et al, 2001). Interleukin 8 (IL-8) function to induce

chemokine in target cells primarily neutrophils and other granulocytes causing their migration to the site of infection and inducing phagocytosis on arrival with corresponding physiological response required for the migration of cell for phagocytosis to occur. This response produces increase in intracellular Ca^{2+}, exocytosis (classically, histamine release and respiratory burst (Dixit & Simon, 2012; Itoh *et al*, 2005; Brat *et al*, 2005). Studies have correlated the increased plasma levels of cytokines, pain, and disease severity with osteoarticular pain (Geiss *et al*, 1997). Uceyle *et al.,* (2006) in their research has also found a decrease in IL-10 and attributed this to an altered immune response, with a reduction in the production of IL-10 and IL-4 demonstrated in the CSF or plasma of patients with chronic pain treated with tramadol (Uceyle *et al.,* 2007)

Following the high incidence of abuse of tramadol and sildenafil drugs amongst youths who want to get high and last longer during sexual escapade with cause to paucity of concrete information regarding reversal effect of this drugs on haematological and proinflammatory cytokine, it has become expedient to synthesize available information and knowledge in an attempt to explain or decipher the effects of this drugs singly and/or in combination on haematological and pro-inflammatory cytokine.

METHODOLOGY

2.1 Animal Preparation

A total of 40 male albino rats weighing between 180-220kg were purchased and house in the animal house Pharmacology Department University of Port Harcourt. The rats were allowed for one week to acclimatize to its new environment prior to treatment, they were given normal rat chows *ad libitum* and allowed free access to water during the experimental period.

2.2 Experimental Design and Drug Administration

The 40 Male albino rats were randomly assigned into 4 groups (n=6) thus: control, Tramadol, Sildenafil, Tramadol+Sildenafil groups respectively. 50mg pfizer branded Sildenafil drug and 50mg of Embassy branded Tramadol for the experiment were purchase from commercial Pharmacies and prepared into syrup form of 6mg/ml of Tramadol and 4mg/ml of sildenafil respectively. Control group (feed with normal rat chows and water), Tramadol group (orally treated with 6mg/ml/180g body weight once daily), Sildenafil group (orally treated with 4mg/ml/180g body weight once daily) Tramadol/Sildenafil group (orally treated with 4&6mg/ml/220g body weight once daily) for a period of three (3) weeks and another 3 weeks without treatment to assess reversal of effects of drugs.

2.3 Collection of Blood Sample

At the completion two phases of the experiments, The Male albino rats was sacrificed by cardiac puncture using chloroform as anaesthesia and 4millilitre of blood collected from subjects and control. 2millilitres dispensed into pre-labelled EDTA anticoagulated sample bottle and 2millilitres into plain tube bottle with gentle mixing to ensure proper distribution of anticoagulant and avoidance of clot formation.

2.4 Measurement of Haematological parameters

Determination of Haematological parameters such as Haemoglobin concentration (Hb), packed cell volume (PCV), Red blood cell (RBC) count, White blood cell (WBC), platelets count were analysed using Sysmex KX-2IN Autoanalyser, Kobe, Japan. The machine function based on the principle of counting blood cells using direct current detection. Whole non-clotted blood sample is aspirated and measured at predetermined volume after a further dilution at a specific ratio before feeding into a transducer. The transducer chamber has an aperture laden with electrodes at both sides of the aperture where direct current flow in. blood corpuscles in the sample on passing through these apertures create a resistance of direct current between the electrodes thus enabling the size of the blood to be detected as electrical impulses. The different blood cells count is calculated by counting the different pulses, and the size of the blood cell histogram is determined by the size of the pulse. Complete blood count parameters such as red blood cell (RBC) count, white blood cell (WBC) count, platelet count, packed cell volume (PCV) and haemoglobin concentration were analyzed.

2.5 Measurement of proinflammatory cytokine (IL-8 and IL-10)

Pro-inflammatory cytokine such as interleukin 8 and 10 were determined using ELISA Kits Elabscience Biotech Co., Ltd, China. This ELISA kit uses Sandwich-ELISA as the method. The micro ELISA plates provided with the kit are pre-coated with an antibody specific to Human IL-8 and IL-10 respectively. Standards or samples are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human IL-8 and IL-10 and Avidin-Horseradish Peroxidase (HRP) conjugate are added to each micro plate well successively and incubated. Free components are washed away. The substrate solution is added and Avidin-HRP conjugate will appear blue in colour. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450nm $\pm$2nm. The OD value is proportional to the concentration of Human IL-8 and IL-10 respectively. The concentration of IL-8 and IL-10 in each samples were calculated by comparing the OD of the samples to the standard curve.

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.

RESULTS

Haematological Parameters In Male Abino Rats After 3 Week Of Treatment For Various Drug Regimens

Table 4.1a shows a comparison of Haematological parameters in the different experimental groups following 3 weeks of treatment with the various drug regimens. There was no statistically significant difference in the mean value for red blood cell (RBC) count of the various treatment group when compared with control group ($p>0.05$). There was a statistically significant decrease in the mean value for Haemoglobin concentration (Hb) and packed cell volume (PCV) across the various treatment groups when compared to control group $p<0.05$. Also a statistically significant increase was seen in the mean value for white blood cell count (TWBC) in the Viagra group and Tramadol group, where as a statistically significant decrease in the mean value was observed in the Tramadol+ Viagra (T+V) group $P<0.05$. A comparison of Tramadol+ Viagra (T+V) group with Viagra group and Tramadol group shows no statistically significant difference in Haemoglobin concentration (Hb) and red blood cell (RBC) count ($p>0.05$), but a statistical significant increase in white blood cell (WBC) count when compared ($p<0.05$). There was a statistically significant increase in Packed cell volume (PCV) when Tramadol+ Viagra (T+V) group was compared with sildenafil group ($p<0.05$) but no statistically significant difference when compared with Tramadol group. A comparison of Viagra group with Tramadol group shows a statistically significant decrease in packed cell volume (PCV) ($p<0.05$)n but no statistically significant difference in Haemoglobin concentration (Hb), Red blood cell (RBC) count and white blood cell (WBC) count.

Table 4.1a Comparison of Mean ± SD of Haematological Values after 3 Weeks of Administration of Drug Regimens in Study Groups and Control

GROUPS/	PCV (%)	Hb (g/dl)	RBC($x10^6$cell/μL)	WBC($x10^3$ cell/μL)
D n=4)	46.4±0.17	14.9±0.76	7.9±0.88	18.9±3.01
A (n=6)	40.6±0.55	13.5±1.47	7.4±0.46	14.5±0.59
B (n=6)	43.2±0.53	13.7±1.68	7.8±0.45	28.5±7.29
C (n=6)	40.5±0.34	13.8±0.43	7.9±0.41	27.3±0.83
P-VALUE	0.0001	0.0004	0.41698	0.0002
REMARK	S	S	NS	S

KEY: NS= Not Significant, S= Significant, D= Control group, A= Tramadol+ Viagra treated group, B= Viagra treated group, C= Tramadol treated group.

Haematological Parameters in Male Abino Rats after 3 Week of Withdrawal from Treatment For Various Drug Regimens.

Table 4.1b shows a comparison of the mean ± SD of Haematological values after 3 Weeks of withdrawal of drug regimens in the study groups and control. There was a statistically significant decrease in the packed cell volume (PCV), Haemoglobin concentration (Hb) in the sildenafil+Tramadol group, Sildenafil group when compared with control ($p<0.05$). No statistically significant difference was seen in the red blood cell (RBC) count, white blood cell (WBC) count in the Sildenafil+Tramadol group, Sildenafil group when compared with control group $p>0.05$. A statistically significant increase was observed in the white blood cell count of Sildenafil group when compared with control group $p<0.05$. Comparison of Tramadol Group with control group after 3 weeks of withdrawal of drug regimen shows no statistically significant difference in the packed cell volume (PCV), Haemoglobin concentration (Hb), red blood cell (RBC) count and white blood cell (WBC) count ($p>0.05$). A comparison of Sildenafil+Tramadol with Sildenafil group shows no statistically significant difference in mean value of packed cell volume (PCV), Haemoglobin concentration (Hb) and red blood cell (RBC) count ($p>0.05$). However, there is a statistically significant increase in WBC count ($p<0.05$). Comparison of Sildenafil+Tramadol is compared with Tramadol group, shows a statistically significant increase in PCV, Hb, RBC and WBC count ($p<0.05$). Finally, a comparison of Sildenafil group with Tramadol groups shows a statistically significant increase in PCV, Hb, RBC ($P<0.05$) and a statistically significant decrease in white blood cell (WBC) count.

Table 4.1b Comparison of the Mean ± SD of Haematological Values after 3 Weeks of Withdrawal of Drug Regimens in Study Groups and Control

GROUPS/ PHASE 2	PCV (%)	Hb (g/dl)	RBC (x106 cell/µL)	WBC (x103 cell/µL)
D (n=4)	46.4±0.17	14.9±0.76	7.9±0.88	18.9±3.01
A2 (n=6)	40.9±1.80	13.2±0.55	7.2±0.63	14.2±0.87
B2 (n=6)	42.3±1.00	13.7±0.34	7.2±0.69	34.9±5.70
C2 (n=6)	47.8±1.09	15.4±0.42	8.7±0.31	25.0±1.84
p-value	0.0001	0.0021	0.0041	0.0001
Remark	S	S	NS	S

KEY: NS= Not Significant, S= Significant, D= Control group, A2= Sildenafil+Tramadol withdrawal group, B2= Sildenafil withdrawal group, C2= Tramadol withdrawal group.

Proinflammatory cytokine (Interleukin 8 and 10) in Male Abino Rats after 3 Week of Treatment for Various Drug Regimens.

Table 2a: shows a comparison of the mean SD of cytokine (interleukin 8 and 10) values after 3 weeks of administration of various drug regimens to various study groups. There was no statistically significant difference in interleukin 8 (IL-8) in the Sildenafil only and Tramadol only group when compare with control group ($p>0.05$). there was also no statistically significant difference in interleukin 10 (IL-10) Values for Sildenafil+ Tramadol group and Sildenafil only group compared with control ($p>0.05$). However, there was a significant decrease in IL-8 value of Sildenafil+Tramadol group when compared to control ($p<0.05$). A statistically significant increase was also seen in the value of IL-10 for Tramadol only group compared with control group ($p<0.05$). Comparison between Sildenafil+Tramadol group with Sildenafil only group reveals a statistically significant increase in IL-8 $P<0.05$, and no statistically significant difference in IL-10 ($p>0.05$). Comparison of Sildenafil+Tramadol with Tramadol only group reveal a statistically significant increase in both IL-8 and IL-10 respectively ($p<0.05$). Tramadol and Sildenafil group comparison shows no statistically significant difference in the values obtained for IL-8, whereas there is a statistically significant increase in the value of IL-10 between the both groups ($p<0.05$).

Table 2a: Comparison of the Mean ± SD of Cytokine Values after 3 Weeks of Administration of Drug Regimens in Study Groups and Control

GROUP/PHASE 1	IL-8(pg/mL)	IL10(pg/mL)
D n=4)	377.3±26.60	8.00±0.62
A (n=6)	282.1±7.65	7.98±0.29
B (n=6)	378.0± 12.42	7.68 ±0.14
C (n=6)	341.7±5.63	14.33±2.36
p-value	0.0002	0.0006
Remark	S	S

KEY: NS= Not Significant, S= Significant, D= Control group, A= Sildenafil+Tramadol treated group, B= Sildenafil treated group, C= Tramadol treated grouP

Cytokine (Interleukin 8 and 10) in Male Abino Rats after 3 Week of Withdrawal of Drug Regimen for Various Study Groups.

Table 2b: shows a comparison of the Mean ± SD of Cytokine Values after 3 Weeks of Withdrawal of Drug Regimens in Study Groups and Control group. There was a statistically significant increase in the mean value of interleukin 8 and 10 in Sildenafil+Tramadol group compared with control group ($p<0.05$). There was no statistically significant difference in interleukin 8 and 10 for the Sildenafil group when compared with control group. A comparison of Tramadol group with control shows a statistically significant increase in the mean value of interleukin 8 and 10 ($p<0.05$). A statistically significant decrease ($p<0.05$) was seen in interleukin 8 and 10 when Sildenafil+Tramadol group was compared with Sildenafil group. Comparison of Sildenafil+Tramadol group with Tramadol group shows a statistically significant decrease in interleukin 8 (IL-8) and increase in interleukin 10 (IL-10) $p<0.05$. A comparison of Tramadol group with Sildenafil group shows a statistically significant decrease in interleukin 8 (IL-8) and an increase in interleukin 10 (IL-10) ($p<0.05$).

Table 2b: Comparison of the Mean ± SD of Cytokine Values after 3 Weeks of Withdrawal of Drug Regimens in Study Groups and Control

GROUP/PHASE 2	IL-8(pg/mL)	IL10(pg/mL)
D (n=4)	377.3±26.60	8.00±0.62
A2 (n=6)	556.8±8.42	12.88±0.83
B2 (n=6)	395± 2.89	8.10 ± 0.24
C2 (n=6)	507.2±6.23	23.8±2.18
p-value	0.0001	0.0028
Remark	S	S

KEY: NS= Not Significant, S= Significant, D= Control group, A2= Sildenafil+Tramadol withdrawal group, B2= Sildenafil withdrawal group, C2= Tramadol withdrawal group.

DISCUSSION

Following the high incidence of abuse of tramadol and sildenafil drugs amongst youths who want to get high and last longer during sexual escapade with cause to paucity of concrete information regarding reversal effect of this drugs on haematological and proinflammatory cytokine such as interleukin 8 (IL-8), interleukin 10 (IL-10), it has become expedient to synthesize available information and knowledge in an attempt to explain or decipher the effects of this drugs singly and/or in combination on haematological and pro-inflammatory cytokine.

There was no statistically significant difference in PCV, Hb, RBC and WBC count in sildenafil+tramadol , sildenafil only group when both phases are compared ($p>0.05$). This is

suggestive of that fact that the derangements in these parameters as a result of the administration of this drug are very severe and thus withdrawal of treatment for 3 weeks cannot reverse completely the effects ofthis drugs. There was however a reversal of effect in platelets count (630±48.9 vs 370±44.3) for sildenafil+tramadol group but not sildenafil only group when both phases are compared ($p<0.05$). There was significant reversal effect in HB (13.8±1.68 vs 15.4±0.42), PCV (40.5±0.53 vs 47.8±1.09) and plaetelets count (813±34.8 vs 506±104) in tramadol only group when both phases were compared ($p<0.05$). No statistically significant difference was observed in RBC and WBC count in tramadol group ($p>0.05$).

Comparison of Sildenafil+Tramadol treated group in both phases to ascertain reversal effect of drugs regimen revealed a highly significant increase in both interleukin 8 and 10 (IL-8, IL-10) showing that the effect of the drug is effectively reversed. The decrease interleukin value found in treatment group was significantly reversed following withdrawal of drug regimen in this treatment group. However in the sildenafil only group, there was no statistically significant difference the value of interleukin 8 and 10 when both phases of treatment were compared. This is a pointer that alteration in these parameters could not be reversed after Sildenafil drug is withdrawn. We could further that the reversal effect seen in the sildenafil+tramadol group is solely due to the tramadol and not Sildenafil drugs withdrawal.
A comparison of cytokine parameter (interleukin 8 and 10) in Tramadol treated group in both phases showed that there was a statistically significant increase in the values of interleukin 8 and 10 when compared with treatment group. This shows that withdrawal of tramadol administration have a better reversal of alterations in the values of interleukin 8 and 10 than Sildenafil drugs alone.

CONCLUSION

Routine use of sildenafil+tramadol, Sildenafil, Tramadol causes derangement in haematological proinflammatory cytokine resulting in negligibly reversibility in haematological parameters, marked reversibility in proinflammatory cytokine following their withdrawal. This effect can be applicable in humans who abuse these drugs. Thus, evaluation of the effect of these drugs on the haemostatic and pro-inflammatory cytokines in humans is necessary.

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