EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 9
102-107
Year: 2018

HAEMATOLOGICAL AND CYTOKINE PARAMETERS IN MALE ALBINO RATS ADMINISTERED ORALLY WITH SINGLE/COMBINED DOSES OF TRAMADOL/VIAGRA

Jacob, Ransom Baribefii,*[1] Eze, Evelyn Mgbeoma.[2] and Jeremiah, Zaccheaus Awortu[3]

[1]Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria.
[2]Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria.
[3]Department of Medical Laboratory Science, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

***Corresponding Author: Jacob Ransom Baribefii**
Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria.

Article Received on 16/07/2018 **Article Revised on 06/08/2018** **Article Accepted on 26/08/2018**

ABSTRACT
This study evaluated haematological and cytokine parameters in male albino rats administered orally with single/combined doses of tramadol/viagra. Twenty-two male albino rats (180-220g body weight) were grouped into 4 (n=6) administered orally with tramadol+viagra (6&4mg/220g.bwt), viagra (4mg/180g.bwt), tramadol (6mg/180g.bwt) and control (n=4) for 3weeks. Rats were sacrificed by cardiac puncture and 9ml 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. Haematological parameter analysed showed no statistically significant difference in the values for RBC count of the various treatment groups compared with control group ($p>0.05$). There was however, a statistically significant decrease Hb and PCV values across the various treatment groups compared with control group ($p<0.05$). A statistically significant increase was seen in WBC count in viagra, tramadol group, whereas a statistically significant decrease WBC was observed in tramadol+viagra group ($P<0.05$). Cytokine parameters showed no statistically significant difference in IL-8 in sildenafil, tramadol groups ($p>0.05$), significant decrease in IL-8 value of tramadol+viagra group compared with control ($p<0.05$). There was no statistically significant difference in IL-10 for tramadol+viagra, sildenafil groups compared with control ($p>0.05$) but a statistically significant increase was seen in the value of IL-10 for tramadol group ($p<0.05$). Daily use of single/combine doses of Tramadol, Viagra causes significant alterations in haematological, cytokine parameters. Thus, anaemic, cancer patients should be properly monitored to avoid the risk of aggravating their condition. There is need to evaluate the effects of these drugs on the haematological and cytokines parameters in humans.

KEYWORD: Tramadol, Viagra, Haematological, Cytokine, interleukin.

INTRODUCTION
It is currently on record that Tramadol and Viagra drugs ranked amongst top commonly abused drugs amongst youths in Nigeria (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). The basis for its use in treatment of premature ejaculation in men is based on its multimodal action of inhibiting weak re-uptake of norepinephrine and serotonin (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 following the administration of tramadol and Viagra singly and in combination.

Sildenafil (commonly called Viagra) belong to a grouped of drugs known as the phosphodiesterase type-5 inhibitor (PDE5i) that is used for the treatment of erectile dysfunction of various etiologies in men and pulmonary hypertension (Nna *et al.* 2016). The mechanism or pharmacodynamics of the drugs is that they selectively act 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).

They 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 inflow 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 is a 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) and are named depending on the site from which they are produced, those made by lymphocytes are named as lymphokine, those made by monocytes are called monokines, chemokine (cytokines with chemotactic activities), and interleukin (cytokines made by one leukocyte and acting on other leukocytes) (Venugopal, 2007). 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).

This research work evaluated haematological parameters such as Haemoglobin concentration (Hb), packed cell volume (PCV), Red blood cell (RBC) count, White blood cell (WBC), platelets count and cytokine parameters such as interleukin 8 (IL-8) and Interleukin 10 (IL-10) in Male Albino Rats administered orally with single/combine doses of Tramadol/Viagra.

2. METHODS

2.1 Animal Preparation

A total of 22 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 22 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 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.

2.3 Collection of Blood Sample

At the completion of the experiments, The Male albino rats was sacrificed by cardiac puncture using chloroform as anaesthesia and 9ml blood collected from subjects and control and dispensed into pre-labelled EDTA anticoagulated sample bottle and 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 Cytokine profiles

Cytokine profile 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

4.1 Haematological Parameters in Male Abino Rats After 3 Week Of Treatment for Various Drug Regimens

Table 4.1 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 4.1 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.

4.2 Cytokine (Interleukin 8 and 10) In Male Abino Rats After 3 Week of Treatment for Various Drug Regimens

Table 4.2 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 4.3a 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= Tramadol+ Viagra treated group, B= Viagra treated group, C= Tramadol treated group.

DISCUSSION

Tramadol and Viagra are drugs used in the treatment of severe to moderate pain, premature ejaculation and erectile dysfunction in men respectively. Both drugs have been administered in combination for the conditions mentioned above. This current research was aimed on evaluating Haematological and cytokine parameters in Male Albino Rats Administered Orally with Single/Combined Doses of Tramadol/Viagra**.** The research showed that haemoglobin concentration (Hb) and packed cell volume (PCV) were significantly decreased in all group compared with controls, this was in tandem with the findings of Nna *et al*., 2016, Aldalou *et al*., 2014; Udegbunam *et al*., 2015. Red blood cell (RBC) count showed no statistically significant difference in all groups compared with control and this finding is in line with the findings of Akhtardanesh *et al*, 2014 and not in line with the findings of Nna *et al*., 2016. The above results pattern in RBC count shows that the drugs regimens administered does not affect or have any inhibitory, impairment or suppressive tendencies on the erythropoietic system. However, the significant decrease observed in Hb and PCV can be attributed to possible invivo haemolysis (destruction of matured red blood cells) already in circulation in the study group as a result of drug administration. It could also be attributed to possible impairment of Haem biosynthesis during erythropoiesis (Nna *et al*., 2016).

A comparison of white blood cell (WBC) count with control shows no statistically significant difference in group A (Tramadol+Viagra) compared with control group indicating that administration of this drug in combination does not affect or suppress immune response. However, a statistically significant increase in white blood cell (WBC) count was observed in group B (Viagra) and C (Tramadol) groups compared with control. This finding was not in tandem with the Nna *et al*., 2016, Mohammed *et al*., 2015 whose results showed a significant decrease in white blood cell (WBC) count. This increase in the white blood cell (WBC) count can be associated to the non-suppression of the defense mechanism and immune system of the Rats with the likelihood that these drugs are seen as foreign and thus the elicitation of immune response resulting in production of more white blood cells (WBC). These could also explain the reason why the red blood cell (RBC) count was not affected.

A comparison of cytokine parameters in the various groups with control groups indicated that there was a significant decrease in the values of IL-8 obtained in Tramadol+Viagra group. This is an indication that the combine administration of these drugs hinders the production of IL-8 and can also be due to their massive recruitment in chemotactic response to inflammation. There was no significant difference in the values obtained in the Viagra and Tramadol group when compared with control, indicating that the use of Viagra and Tramadol does not affect IL-8.

There was no significant difference in the values of interleukin 8 (IL-8) obtained in the Viagra and Tramadol group when compared with control, indicating that the use of Viagra and Tramadol does not affect IL-8. However the IL-10 values obtained in the Tramadol group compared with control showed an increase following 3weeks of drugs administration, this findings is not in line with other studies done by Uceyle *et al*, (2006) who recorded 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, as has been demonstrated in the CSF or plasma of patients with chronic pain treated with tramadol (Uceyle *et al*, 2007). This increase observed in this study can be traceable to the increase seen in white blood cell (WBC) count as seen in the Tramadol treated group. Since IL-10 majorly acts to inhibit inflammatory and immune response, its production could be raise to handle the possible inflammatory response brought by the administration of tramadol.

CONCLUSION

Daily use of single/combine doses of Tramadol, Viagra causes significant alterations in Haematological and Cytokine parameters. Thus, anaemic, cancer patients should be properly monitored to avoid the risk of aggravating their condition.

REFERENCES

1. Akhtardanesh, B., Sharifi, H., Rasodi, R. and Aghazamani, M. Evaluation of haematological and biochemical changes after short term tramadol usage in healthy dogs, *Iranian Journal of Veterinary Medicine*, 2014; 8(1): 41-45.
2. Aldalou, AR, Abdel-Aziz I. and Shahwan O. Impact of giving sildenafil (viagra) / tramadol (tramal) combination on the blood of domestic rabbits. *Journal of Science,* 2014; 4(3): 162-169.
3. Aversa A, Bruzziches R, Pili M and Spera G. Phosphodiesterase 5 inhibitors in the dysfunction, *Current Pharmaceutical Research*, 2006; 12: 3467–3484.
4. Ayoub R, Aldalou I, Abdel-Aziz R and Osama S. Impact of giving sildenafil (viagra) / tramadol (tramal) combination on the blood of domestic rabbits. *Journal of Science*, 2014; 4(3): 162-169.
5. Brat DJ, Bellail AC and Van-Meir EG. The role of interleukin-8 and its receptors in gliomagenesis and tumoral angiogenesis. *Neuro-oncology*, 2005; 7(2): 122–133.
6. Daubin C, Quentin C, Goullé JP, Guillotin D, Lehoux P, and Lepage O. Refractoryshock and asystole related to tramadol overdose. *Clinical Toxicology*, 2007; 45(8): 961-964.
7. Dixit N, and Simon SI. Chemokines, selectins and intracellular calcium flux: temporal and spatial cues for leukocyte arrest". Frontiers in Immunology, 2012; 3: 188.
8. Gaspani L, Bianchi M, Limiroli E, Panerai AE, Sacerdote P. The analgesic drug tramadol prevents the effect of surgery on natural killer cell activity and metastatic colonization in rats, *Journal of Neuroimmunology*, 2002; 129(1-2): 18-24.
9. Geiss A, Varadi E, Steinbach K et al. Psychoneuroimmunological correlates of persisting sciatic pain in patients who underwent discectomy. *Neuroscience Letter*, 1997; 237: 65-68.
10. Itoh Y, Joh T, Tanida S, Sasaki M, Kataoka H, Itoh K, Oshima T, Ogasawara N, Togawa S, Wada T, Kubota H, Mori Y, Ohara H, Nomura T, Higashiyama S, Itoh M. IL-8 promotes cell proliferation and migration through metalloproteinase-cleavage proHB-EGF in human colon carcinoma cells. *Cytokine*., 2005; 29(6): 275–282.
11. Niemand C, Nimmesgern A, Haan S, Fischer P, Schaper F, Rossaint R, Heinrich PC and Müller-Newen G. Activation of STAT3 by IL-6 and IL-10 in primary human macrophages is differentially modulated by suppressor of cytokine signaling 3. *Journal of Immunology*, 2003; 170(6): 3263-3272.
12. Nna VU, Akpan UP, and Osim EE. Separately administered phosphodiesterase-5 inhibitors (sildenafil and tadalafil) and opioid (tramadol), reversibly alter serum lipid profile in male albino wistar rats. *Asian Journal of Biochemistry*, 2015; 10: 132-144.
13. Nna VU, Victor O, Oka AL, Udefa EO, Ofutet OE and Ofem. High Doses of PDE5 inhibitors and tramadol reversibly alters haematological parameters in rats. *Journal of Applied Pharmaceutical Science,* 2016; 6(04): 086-092.
14. Oka, VO, Udefa AL, Nna VU. and Owu DU. Sildenafil citrate and tramadol administered separately and in combination affects basal metabolic rate, triiodothyronine (T3) and cortisol levels in albino wistar rats. *Trends in Medical Research*, 2015; 10(3): 51-62.
15. Salam AR, Rahman A, Sultana UN and Sultan Z. Study of *in vitro* interaction of sildenafil citrate with bovine serum albumin by fluorescence spectroscopy. *Pharmacology Journal*, 2015; 6: 94-101.
16. Salem EA, Wilson SK, Bissada NK, Delk JR, Hellstrom WJ. and Cleves, MA. Tramadol HCL has promise in on-demand use to treat premature ejaculation. *Journal of Sexual Medicine*, 2008; 5: 188-193.
17. Senay EC, Adams EH, Geller A, Inciardi JA, Muñoz A, and Schnoll SH. Physical de-pendence on Ultram (tramadol hydrochloride): both opioid-like and atypical with-drawal symptoms occur. *Drug and Alcohol Dependence*, 2003; 69(3): 233-41.
18. Shatha HA and Adnan M. The Effect of Pde-5 Inhibitors on blood homeostasis in relation to the type & duration of therapy. *International Journal of Security and Network*, 2015; 6(1): 28-35.
19. Supuran CT, Mastrolorenzo A, Barbaro G and Scozzafava A. Phosphodiesterase 5 inhibitors–drug design and differentiation based on selectivity, pharmacokinetic and efficacy profiles. *Current Pharmaceutical Description*, 2006; 12: 3459–3465.
20. Uceyler N, Rogausch JP, Toyka KV et al. Differential expression of cytokines in painful and painless neuropathies. *Neurology*, 2007; 69: 42-49.
21. Uceyler N, Valenza R, Stock M et al. Reduced levels of anti-inflammatory cytokines in patients with chronic widespread pain. *Arthritis Rheum*, 2006; 54: 2656-2664.
22. Udegbunam RI, Okereke HN. and Udegbunam SO. Single versus repeated tramadol injectionin laparotomized albino rats: comparison of effects on hematology, serum biochemical parameters, and bodyweight gain. *Journal of Advanced Veterinary and Animal Research*, 2015; 2(3): 316-320.
23. Varma TK, Toliver-Kinsky TE, Lin CY, Koutrouvelis AP, Nichols JE and Sherwood ER. Cellular mechanisms that cause suppressed gamma interferon secretion in endotoxin-tolerant mice". Infection and Immunity, 2001; 69(9): 5249–5263.

24. Venugopal J. Cytokine In: *Fundamentals of Immunology*. JayPee Brothers Medical Publishers Ltd New Delhi, 2007; 122-135.
25. Zhang J and Jianxiong A. Cytokine, Inflammation and pain, *International Anesthesiology Clinic.*, 2007; 45(2): 27–37.