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Does Single/Combined Administration of Tramadol/Viagra Have Reversal Effects on Haematological and Anti-Inflammatory Cytokines in Male Albino Rats?

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Authors' contributions

This work was carried out in collaboration between all authors. Author JRB designed the study, performed the statistical analysis, wrote the protocol and first draft of the manuscript. Author NI managed the literature searches. Authors EEM and JZA managed/supervised the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study evaluated reversal effects of single/combined doses of tramadol/sildenafil drugs on haematological and anti-inflamatory cytokines in male albino rats.
Study Design: Case control study.
Methodology: Forty (40) male albino rats (180-220 g body weight) were grouped into 3 (n=12) administered orally with tramadol+sildenafil (6 and 4 mg/220 g.bwt), sildenafil (4 mg/180 g.bwt), tramadol (6 mg/180 g.bwt) and control group (n=4) for 3weeks and allowed 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.

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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 were 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 platelets 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 (p>0.05). 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, anti-inflammatory cytokine resulting in negligibly reversibility in haematological parameters, marked reversibility in anti-inflammatory cytokine following their withdrawal. This effect can be applicable in humans who abuse these drugs. Thus, evaluation of the effect of these drugs on haemostatic and anti-inflammatory cytokines in humans is necessary.

Keywords: Tramadol; Sildenafil; haematological; anti-inflammatory; cytokine; interleukin.

1. INTRODUCTION

Tramadol and Viagra are drugs used for the treatment of acute and chronic pain, premature ejaculation and erectile dysfunction respectively in men [1,2]. They are ranked amongst top commonly abused drugs amongst youths in Nigeria [3,4] Following its association with seizure and death, several cases of instant deaths have been recorded when tramadol is taken together with alcohol [5].

Tramadol (commonly called Tramal) is a narcotic-like, centrally acting pain relieve drug 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 severe intensity; treatment of premature ejaculation due to its ability to inhibit weak re-uptake of norepinephrine and serotonin [1,2]. Its multimodal action of inhibiting weak re-uptake of norepinephrine and serotonin constitutes the basis for use in treatment of premature ejaculation in men [6]. Tramadol and Sildenafil have been shown to have pronounced effects on the haematological parameters both in rat model and human studies [1,7]. Nna et al. 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 [8]. Akhartardanesh et al. observed that there was no significant difference in red blood cell (RBC) count in test group compared with control when these drugs were administered [9].

Sildenafil are phosphodiesterase type-5 inhibitor (PDE5i) drugs used for the treatment of erectile dysfunction and pulmonary hypertensions of various etiologies in men [8]. It works by 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 [8,10,11,12] 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 [13].

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 [14]. Cytokine play a pivotal role in coordination and regulation of immune responses [15,16]. 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 [17]. 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 [18]. 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 [19,20,21]. Studies have correlated the increased plasma levels of cytokines, pain, and disease severity with osteoarticular pain [22]. Uceyle et al. 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 [23,24].

Following the high incidence of abuse of tramadol and sildenafil drugs amongst youths who desire to get high, achieve stronger and longer lasting erection during sexual escapade with cause to paucity of concrete information regarding reversal effect of this drugs on haematological and anti-inflammatory 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 anti-inflammatory cytokine.

2. METHODOLOGY

2.1 Animal Preparation

A total of 40 male albino rats weighing between 180-220 kg 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 3 groups (n=12) thus: tramadol, sildenafil, tramadol+sildenafil groups and control (n=4) respectively. 50 mg pfizer branded sildenafil drug and 50 mg of Embassy branded tramadol for the experiment were purchase from commercial pharmacies and prepared into syrup form of 6 mg/ml of tramadol and 4 mg/ml of sildenafil respectively. Control group (feed with normal rat chows and water), tramadol group (orally treated with 6 mg/ml/180 g body weight once daily), sildenafil group (orally treated with 4 mg/ml/180 g body weight once daily) tramadol/sildenafil group (orally treated with 4&6 mg/ml/220 g 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 of the first and second phases of experiment, animals were sacrificed under 3.8% chloroform anaesthesia. Collection of blood samples was done using 5 ml syringes with 21 G needles. The samples were collected from the animals through cardiac puncture and 2 millilitres each of the blood collected dispensed into pre-labelled ethylene diamine tetracetate (EDTA) bottles and plain vials with gentle agitation to ensure EDTA is spread uniformly after which the samples were immediately used for measurement of haematological parameters. The blood sample in plain vial were spun at 2000 g for 10minutes and separated to obtain serum in accordance with Nna et al., (2016).

2.4 Measurement of Haematological and anti-inflammatory Cytokine (IL-8 and IL-10)

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 while anti-inflammatory cytokine such as interleukin 8 and 10 were determined using ELISA Kits Elabscience Biotech Co., Ltd, China.

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

Table 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 (WBC) 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, platelets count should significant increase in all groups compared with control ($p<0.05$).

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

Table 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, platelets count should significant increase in all groups compared with control ($p<0.05$).

Table 1a. Comparison of mean ± sd of haematological values after 3 weeks of administration of drug regimens in study groups and control

Groups/ Phase 1	PCV (%)	Hb (g/dl)	RBC ($x10^6$cell/µL)	WBC ($x10^3$ cell/µL)	Platelets count (x103 cells/µL)
D n=4)	46.4±0.17	14.9±0.76	7.9±0.88	18.9±3.01	292±71.98
A (n=6)	40.6±0.55	13.5±1.47	7.4±0.46	14.5±0.59	630±48.90
B (n=6)	43.2±0.53	13.7±1.68	7.8±0.45	28.5±7.29	650±155.3
C (n=6)	40.5±0.34	13.8±0.43	7.9±0.41	27.3±0.83	813±34.84
P-VALUE	0.0001	0.0004	0.41698	0.0002	0.0002
REMARK	S	S	NS	S	S

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

Table 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)	Platelets count (x103 cells/µL)
D (n=4)	46.4±0.17	14.9±0.76	7.9±0.88	18.9±3.01	292±71.98
A2 (n=6)	40.9±1.80	13.2±0.55	7.2±0.63	14.2±0.87	370±44.3
B2 (n=6)	42.3±1.00	13.7±0.34	7.2±0.69	34.9±5.70	649±74.7
C2 (n=6)	47.8±1.09	15.4±0.42	8.7±0.31	25.0±1.84	506±104.0
p-value	0.0001	0.0021	0.0041	0.0001	0.0001
Remark	S	S	NS	S	S

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

3.3 Anti-inflammatory 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 ($p<0.05$).

3.4 Anti-inflammatory 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) ($p<0.05$).

4. DISCUSSION

Following the high incidence of abuse of tramadol and sildenafil drugs amongst youths who desire to get high, achieve stronger and longer lasting erection during sexual escapade with cause to paucity of concrete information regarding reversal effect of this drugs on haematological and anti-inflammatory 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 anti-inflammatory cytokine.

In this research, there was no statistically significant difference in PCV, Hb, RBC and WBC count in sildenafil+tramadol, sildenafil only group when both phases are compared. This finding is at variance with the findings of Nna et al, (2016) who discovered that RBC count, Hb

concentration and PCV were significantly higher in the recovery groups, compared with the treatment groups for sildenafil group, sildenafil + tramadol groups. This is suggestive of that fact that the derangements [8,9,25,26,27] in these parameters caused by the administration of this drug sildenafil, sildenafil+tramadol are very severe and thus withdrawal of treatment for 3 weeks cannot reverse completely the effects of these drugs.

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

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

There was however a reversal of effect in platelets counts for sildenafil+tramadol group when both phases are compared. This finding was in tandern with the findings of [8] who reported that Platelets count was also significantly higher in Sildenafil+tramadol recovery groups compared with their corresponding treatment groups. The significant increase in platelets count caused by sildenafil + tramadol is exclusively due to the effect of sildenafil since tramadol alone did not show any significant difference [8]. Findings in tramadol group revealed a significant reversal effect only in Hb and PCV when both phases were compared and this is in tandern with the findings of [8]. No statistically significant difference was observed in RBC and WBC count in tramadol group.

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. However in the sildenafil only group, there was no statistically significant difference in the value of interleukin 8 and 10 when both phases were compared. This is a pointer that alteration in these parameters could not be reversed after Sildenafil drug is withdrawn.it could further be inferred 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 showed that there was a statistically significant increase in the values of interleukin 8 and 10 when compared with recovery 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. This finding is in line with [28] who observed that there was withdrawal of effect on cytokines following stoppage of drug administration.

5. CONCLUSION

Routine use of sildenafil+tramadol, sildenafil, tramadol causes derangement in haematological anti-inflammatory cytokine resulting in negligible reversibility in haematological parameters, marked reversibility in anti-inflammatory cytokine following their withdrawal. This effect can be applicable in humans abusing these drugs. Thus, evaluation of the effect of these drugs on the haemostatic and anti-inflammatory cytokines in humans is necessary.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1 Ayoub R, Aldalou I, Abdel-Aziz R, Osama S. Impact of giving sildenafil (viagra) / tramadol (tramal) combination on the blood of domestic rabbits. Journal of Science. 2014;4(3):162-169.

2 Senay EC, Adams EH, Geller A, Inciardi JA, Muñoz A, 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.

3 Baribefii RJ, Eze EM, Jeremiah ZA. Haematological and cytokine parameters in male albino rats administered orally with single/combined doses of tramadol/Viagra, European Journal of Biomedical and Pharmaceutical Sciences. 2018;5(9):102-107.

4 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.

5 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.

6 Salem EA, Wilson SK, Bissada NK, Delk JR, Hellstrom WJ, Cleves MA. Tramadol HCL has promise in on-demand use to treat premature ejaculation. Journal of Sexual Medicine. 2008;5:188-193.

7 Shatha HA, 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.

8 Nna VU, Victor O, Oka AL, Udefa EO, Ofutet OE, Ofem. High Doses of PDE5 inhibitors and tramadol reversibly alters haematological parameters in rats. Journal of Applied Pharmaceutical Science. 2016;6(04):086-092

9 Akhtardanesh B, Sharifi H, Rasodi R, 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.

10 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

11 Aversa A, Bruzziches R, Pili M, Spera G. Phosphodiesterase 5 inhibitors in the dysfunction, Current Pharmaceutical Research. 2006;12:3467–3484.

12 Oka, VO, Udefa AL, Nna VU, 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.

13 Salam AR, Rahman A, Sultana UN, Sultan Z. Study of *in vitro* interaction of sildenafil citrate with bovine serum albumin by fluorescence spectroscopy. Pharmacology Journal. 2015;6:94-101.

14 Venugopal J. Cytokine in: Fundamentals of immunology. JayPee Brothers Medical Publishers Ltd New Delhi. 2007;122-135

15 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.

16 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.

17 Zhang J, Jianxiong A. Cytokine, inflammation and pain. International Anesthesiology Clinic. 2007;45(2):27–37.

18 Varma TK, Toliver-Kinsky TE, Lin CY, Koutrouvelis AP, Nichols JE, Sherwood ER. Cellular mechanisms that cause suppressed gamma interferon secretion in endotoxin-tolerant mice, Infection and Immunity. 2001;69(9):5249–5263

19 Dixit N, Simon SI. Chemokines, selectins and intracellular calcium flux: Temporal and spatial cues for leukocyte arrest, Frontiers in Immunology. 2012;3:188.

20 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.

21 Brat DJ, Bellail AC, Van-Meir EG. The role of interleukin-8 and its receptors in gliomagenesis and tumoral angiogenesis. Neuro-oncology. 2005;7(2):122–133.

22 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

23 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.

24 Uceyler N, Rogausch JP, Toyka KV, et al. Differential expression of cytokines in painful and painless neuropathies. Neurology. 2007;69:42-49

25 Mohammed AA, Adel MHA, Mohammed AY. The effect of tramadol on some blood and biochemical parameters of male rats (*Rattus norvegicus*) Baghdad Science Journal. 2015;12(3):496-502.

26 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.

27 Udegbunam RI, Okereke HN, 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

28 Abd El-Hamid Mohamed Elwy, Ghada Tabl. Effects of chronic usage of tramadol, acetaminophen and tramacet on some biochemical and immunological changes in male rats. Egyptian Journal of Drug Research. 2014;35(1):63-71.

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