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**Evaluation of Some Hemostatic Parameter in Albino Wistar Rats Administered Tramadol**Obasohan Lovely Osayuki\*<sup>1</sup> and Austin I. Aruomaren<sup>2</sup>Federal Neuro-Psychiatric Hospital, Uselu. P.M.B. 1108, Benin City, Edo State, Nigeria <sup>1</sup>, Department of Medical Laboratory Sciences, School of Basic Medical Sciences, University of Benin, Benin City, Nigeria <sup>2</sup>Author for Correspondence\*: osayukiigbinoghodua@gmail.com/+234-807-396-4289/+234-706-715-0722. <https://dx.doi.org/10.4314/sokjmls.v10i1.14>**Abstract**

Tramadol, a synthetic analogue of codeine, is an opioid with central effects, recommended for its lower abuse potential and reduced respiratory depression compared to other opioids. It is used to manage acute and chronic pain of moderate to severe intensity. However, tramadol abuse has increased due to its speculated role in enhancing male sexual activity, prompting further research. This study investigates the effects of tramadol on haemostasis and haematological parameters. Twenty adult Albino Wistar rats were divided into five groups, including a control group and four treatment groups. The Low Oral and High Oral dose groups received 25 mg/kg body weight/day orally for 21 days, while the Low Intramuscular and High Intramuscular dose groups received 15 mg/kg body weight/day intramuscularly for the same duration. Blood samples were collected from the abdominal aorta and heart using a 25G needle and 5 ml syringe. Haemostatic parameters, including Prothrombin Time (PT), Partial Thromboplastin Time with Kaolin (PTTK), and plasma fibrinogen concentration, as well as Full Blood Count (FBC) and body weights, were measured using standard manual and automated methods. Data analysis was done using GraphPad Prism (version 6.04). Results revealed a statistically significant increase in PT and PTTK in the test groups compared to the control ( $p < 0.05$ ), indicating prolonged clotting times. However, no significant difference was observed in plasma fibrinogen concentration ( $P > 0.05$ ) or FBC. Prolonged tramadol administration also reduced the animals' body weight. In conclusion,

tramadol demonstrated antithrombotic effects by prolonging PT and PTTK. These findings suggest a potential therapeutic role for tramadol in managing thrombotic disorders.

**Keywords:** Tramadol, Prothrombin Time, PTTK, Haemostasis

**Introduction**

Blood, a fluid tissue, travels throughout the body in healthy blood vessels including veins, arteries, and capillaries to carry out several essential tasks. Blood must flow freely and readily through blood vessels in order for them to function at their peak. Blood will flow out and be lost if a blood artery is damaged or punctured, which might be lethal. To seal a break in vascular endothelium and stop blood loss, a "plug," also known as a "blood clot," is formed by a variety of inherent physiological mechanisms. Together, these processes are referred to as "haemostasis." They include individual blood arteries, specialised blood cells called platelets, and certain blood proteins called clotting factors. Haemostasis aims to limit blood loss from damaged blood vessels and maintain blood fluidity within intact (uninjured) blood arteries (Hoffbrand *et al.*, 2001; Escobar *et al.*, 2002; Laffan & Manning, 2002). By halting excessive bleeding, haemostasis—the mechanism that keeps blood in a fluid condition inside the blood vessels—helps to minimise blood loss. Haemostatic processes maintain an undamaged circulatory system that is free of lesions or obstructions and allows blood to flow. Thus, it may be said that haemostasis preserves the circulatory system's integrity. Damage to a blood vessel

initiates haemostasis. Among the many processes that underlie haemostasis are platelets, coagulation factors, and the fibrinolytic system (Ochie and Kolhatkar, 2000). Thrombosis or bleeding may result from coagulation issues. Damage to a blood artery's endothelium triggers coagulation very immediately (Gene, 2011). Primary haemostasis is the term used to describe the instantaneous plug that platelets create at the site of damage. Concurrently, secondary haemostasis takes place, in which the components of the coagulation process create fibrin strands that, via a complicated cascade response, reinforce the platelet block (Gene, 2011). The haemostatic mechanism must be understood in order to comprehend the haemostatic alterations brought on by tramadol usage.

People often experience pressures or injuries in their everyday lives that may seem to cause pain. Research into the use of medications known as analgesics to manage pain is crucial. Nonsteroidal anti-inflammatory medicines (NSAIDs) and opioids are the two categories of analgesic medications. The earliest analgesics that people took were opioids (Cuauhtémoc *et al.*, 2005). The first synthetic version of the opioid, tramadol, was created in 1962 by the German company Grünenthal GmbH. It functions centrally as a synthetic form of codeine (Afshari, 2011), although compared to other opiates, it is less prone to be misused and results in less respiratory depression (Grond and Sablotzki, 2004). It is used to treat both acute and chronic pain that is mild to (moderately) severe. In addition to having opioid-like actions, tramadol is a potent yet quick-acting serotonin releaser and norepinephrine reuptake inhibitor (Smith *et al.*, 2014). Compared to other opioids, Tramadol has a superior safety record and a considerably reduced risk of addiction, which is why it is often recommended. It may be given orally, intravenously (IV), subcutaneously (SC), intramuscularly (IM), rectally, and spinally (Husslein *et al.*, 1987; Kupers *et al.*, 1995). Tramadol has depressive properties with minimal immunosuppressive effect (Liu *et al.*, 2008).

Tramadol may disrupt the equilibrium between the coagulation and fibrinolytic systems, which is required to maintain the integrity of the blood vessel, by raising the international normalised

ratio (INR) and lowering the number of red blood cells and platelets (Harshem, 2014).

## Materials and Method

### Animal Experimentation

Twenty (20) male Albino Wistar rats weighing 153g-194g were used for this study. Rats were left in the animal's house for one week before experimentation so that they can adapt and acclimatize to laboratory conditions under the conditions of natural light and dark cycle and given free access to commercial balanced diet (Growers feed) and tap water *ad libitum* all over the period of experimentation. The animals were placed in well ventilated cages which were cleaned and disinfected daily throughout the period of this study.

### Drug Dosing

The drug tramadol hydrochloride injection (Ampoule) 100mg/2ml made by Ancalima Life Sciences Limited, India, and the drug Tramadol capsule B.P. 100mg (Jontral Tramadol) made by Globela Pharma Limited, India, were administered intramuscularly and orally. Insulin was administered orally using a Gavage tube and intramuscularly using an insulin syringe. The High Intramuscular Dose (HID) and Low Intramuscular Dose (LID) groups got an inoculation of 15 mg/kg body weight/day for 14 days, whereas the High Oral Dose (HOD) and Low Oral Dose (LOD) groups received 25 mg/kg body weight/day. The animals were handled in accordance with established experimental standards that have been authorised by the Ministry of Health. Doses were administered early in the morning while the animals were continuously observed.

### Experimentation Design

The animals were divided into five groups consisting of a control group (CG), high dose intramuscular test group (HDI), low dose intramuscular test group (LDI), high oral dose test group (HOD), and low oral dose test group (LOD) comprising four animals each.

**Group 1:** This is the control group. Animals in this group received only food and water throughout the period of experimentation. Tramadol was not given to this group.

**Group 2:** This is the High Intramuscular Dose group. This group received a high dose of Ampoule Tramadol (Tramadol injection) intramuscularly for fourteen days

**Group 3:** This is the Low Intramuscular Dose group. This group received low dose of Ampoule Tramadol (Tramadol injection) intramuscularly for fourteen days.

**Group 4:** This is the High Oral Dose group. This group received high dose of Tramadol capsule B.P for fourteen days.

**Group 5:** This is the Low Oral Dose group. This group received low dose of Tramadol capsule B.P for fourteen days.

### **Ethical Approval**

Ethical approval was obtained from the Ministry of Health, Benin City, Edo State, Nigeria.

### **Measurement of Body Weight**

The body weight of the animals was measured six times during the course of the study: at the onset, Mondays and Fridays and at the end just before sacrificing. A scale was used to determine the individual weight of all animals. Weighing was done by removing the animals from the cage and placing them on the scale. Reading and recording of the animal's body weight was done when the animal was still resting on the scale. The average weight of each animal was calculated at the end of each weighing.

### **Collection of Blood Samples**

Following two weeks of tramadol administration and chloroform anaesthesia, the rats were mercifully killed. Rats were housed in cotton wool soaked in chloroform in a covered dish until the chloroform made them weaker. Like people, the rats were taken out and killed one by one.

The weaker rat was placed on its back on a level surface, and a "V-shaped" cut was made in its belly to draw blood. Blood samples were taken directly from the heart (cardiac puncture) and the abdominal aorta using a 25G needle and a 5ml syringe. Aliquot samples were put in a simple tube with sodium citrate anticoagulant for the coagulation profile and an ethylene-

diaminetetraacid (EDTA) acid container for the platelet count.

### **Methods of Analysis**

The samples collected will be analyzed for Full Blood Count (FBC) and haemostatic parameters such as prothrombin time (PT), partial thromboplastin time with kaolin (PTTK), and plasma fibrinogen concentration.

### **Full Blood Count Estimation**

The Full Blood Count test was done on all samples, both controls and tramadol treated groups using Erma Haematological analyzer PCE-210N.

### **Prothrombin Time**

Prothrombin time measure the clotting time of plasma in the presence of optimal concentration of thromoplastin and it indicate the overall efficiency of the extrinsic clotting pathway. If done appropriately it was originally known to measure only prothrombin (Factor, but it is now known to also measure Factors V, V11, and X (Dacie and Lewis, 2012). This test has become the basic coagulation screening test for the diagnosis of congenital and acquired deficiencies of factors II, V, VII, and X (Monica, 2006).

### **Partial Thromboplastin Time With Kaolin (PTTK)**

This screening test is also called Activated Partial Thromboplastin Time (APTT) or Kaolin Cephalin Clotting Time (KCCT). It has developed from the Partial Thrombolplastin Time (PTT), the recalcification time and the whole blood clotting time. It is a measure of the clotting factors of both the intrinsic and common coagulation pathways (Monica, 2006). It will detect the inhibition or deficiency of one or more of the following factors: II, V, VIII, IX, X, XI, XII and fibrinogen (Dacie and Lewis, 2012).

### **Fibrinogen Concentration by Dry Weight**

When calcium chloride is added to citrated plasma, a fibrin clot is formed. The resulting clot is weighed and the weight of the fibrin clot is directly proportional to the plasma fibrinogen concentration. (Ingram, 1961).

### Statistical Analysis

GRAPH Pad PRISM (VERSION 6.04) was used for statistical analysis. Error bars were used to report as mean  $\pm$  SEM. Statistical significance were determined using a one-way analysis of variance (ANOVA) to compare treated groups and control groups & oral administered groups and intramuscular administered groups and the difference between groups were analyzed using independent student T-test. Statistical significant levels of  $p < 0.05$  was used.

### Result

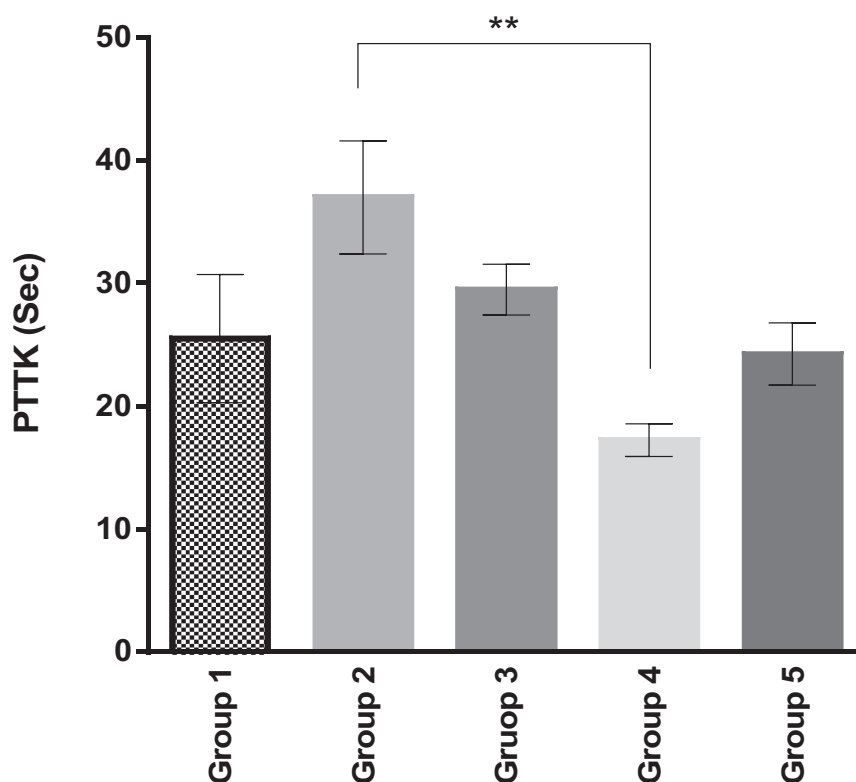
#### Physical Examination of Animals

Animals showed great compliance with tramadol from the onset of administration. There was no general increase in feed consumption before and during administration. The testes of all the treated groups increased in size and became reddish

(tender) as shown in slide 1. Animals were also noticed to become relatively violent and aggressive. On sacrificing animals, all internal organs appeared Normal. Tests for the assessment of the haemostatic status which include Prothrombin Time (PT), Partial Thromboplastin Time test with Kaolin (PTTK) and fibrinogen concentration were done and body weight of the experimental animals were taken. A Full Blood Count (FBC) test was also done. These parameters among Tramadol treated and control Groups were assayed and these two were compared.

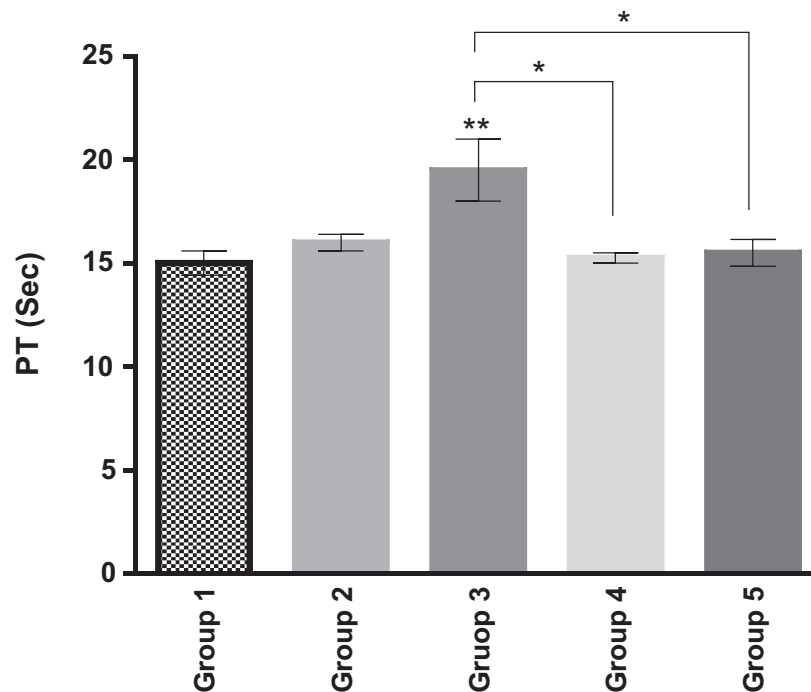
#### Estimation of Clotting Profile

To estimate the haemostatic effect of Tramadol treatment, clotting profile tests were used to detect haemostatic changes by carrying out a Prothrombin Time test, a Partial Thromboplastin Time with Kaolin and fibrinogen concentration.



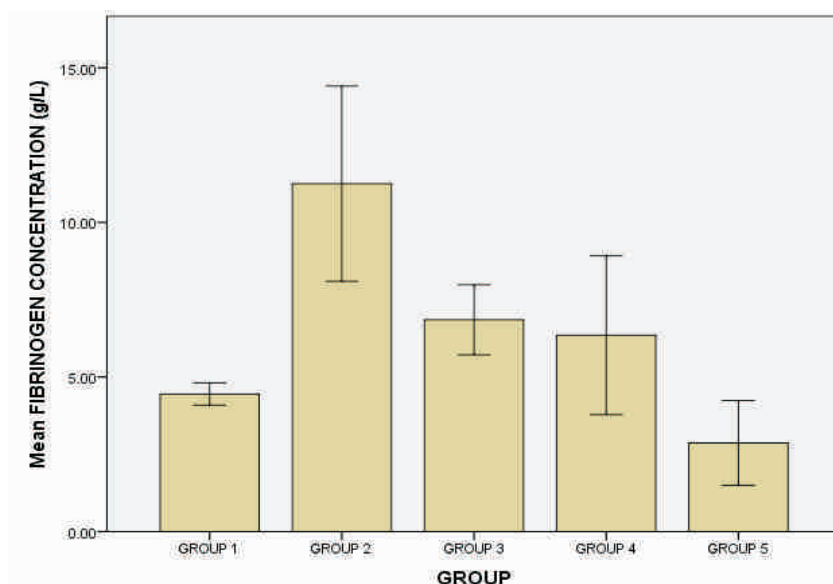
**Figure 1: Estimation of PTTK of control group and Tramadol treated groups (Error bars represent Mean  $\pm$  SEM).**

Figure 1 shows that treatment with Tramadol caused a difference in PTTK and this difference was statistically significant ( $p > 0.05$ ) and when we compared within groups, the difference was found to be between groups two and four, as there was a significant increase in group two and a significant decrease in group four when both groups were compared with the control group. There was however a difference in other groups when they were compared with the control, but this difference was not statistically significant ( $p > 0.05$ ).



**Figure 2: Estimation of PT of control groups and Tramadol treated groups (Error bars represent Mean ± SEM).**

Figure 2 shows that Tramadol also caused a statistically significant difference in Prothrombin Time test ( $p < 0.05$ ), and when compared within groups, it was shown that the increase was between groups three, four and five. There was however an increase in other groups when compared with the control, but this increase was not statistically significant ( $p > 0.05$ ).



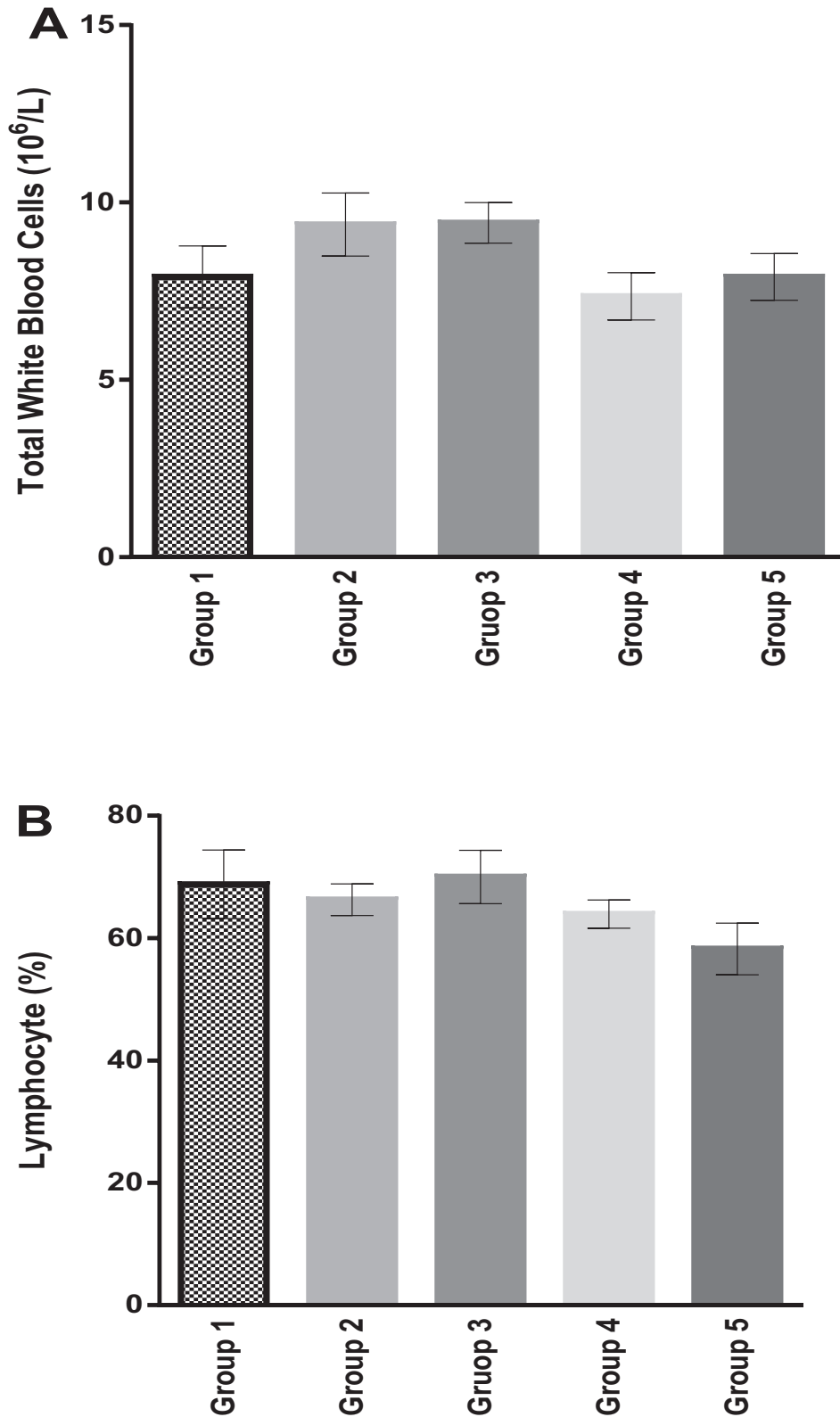
**Figure 3: Estimation of Fibrinogen Concentration of control group and Tramadol treated groups (Error bars represent Mean ± SEM).**

Figure 3 shows that Fibrinogen concentration increased in the groups that received low intramuscular, high intramuscular and low oral doses, this increase was however not significant. Fibrinogen concentration was reduced in the group that received high oral dose when this group was compared with the control, this decrease was not significant ( $p > 0.05$ ).

**Estimation of Haematological Indices**

A complete blood count was done for all subject both tramadol treated and control groups, though there was an increase and/decrease in some of the

parameters when they were compared with the control, none of the parameters showed a significant difference from the control groups ( $p > 0.05$ ).



**Figure 4.4: Estimation of White Blood Cell and Lymphocyte Count (Error bars represent Mean ± SEM).**

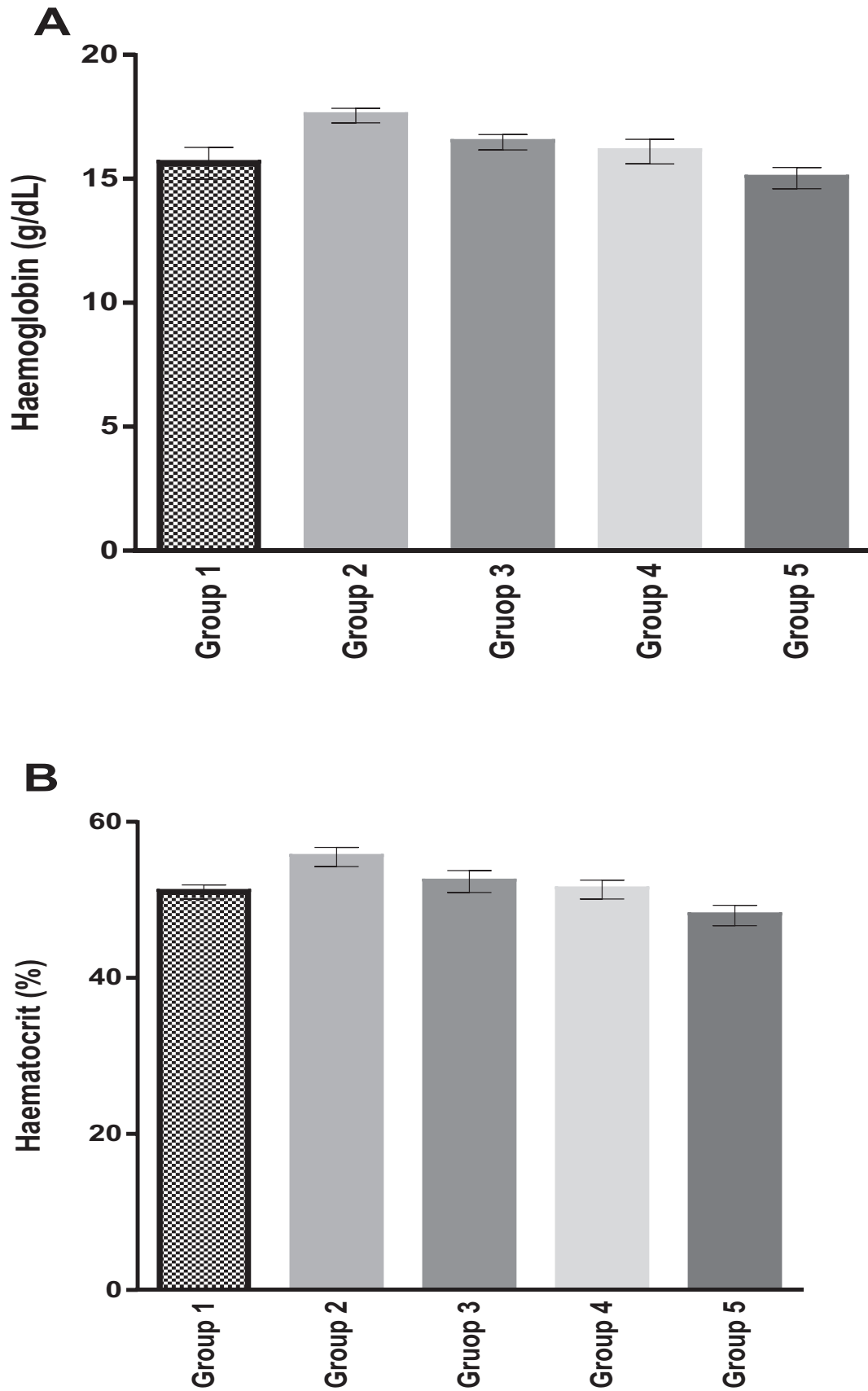
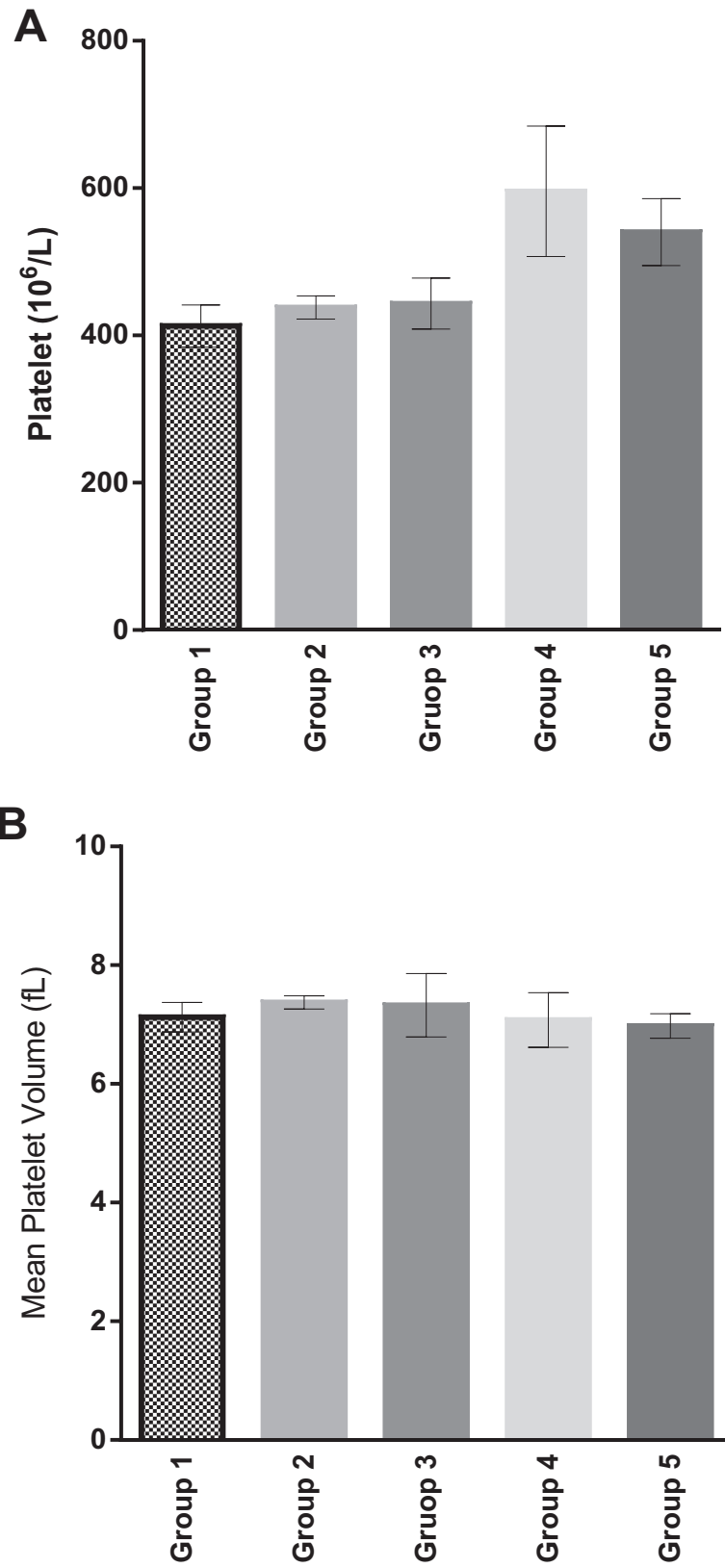


Figure 4.5: Estimation of Haemoglobin concentration and Haematocrit (Error bars represent Mean  $\pm$  SEM).



**4.4 Determination of Weight**

When the weight of the treated Groups was compared with that of the control Group, a difference was observed. There was a constant decrease in the weight of all treated Groups, but only the weight of Groups four and five showed a statistically significant difference

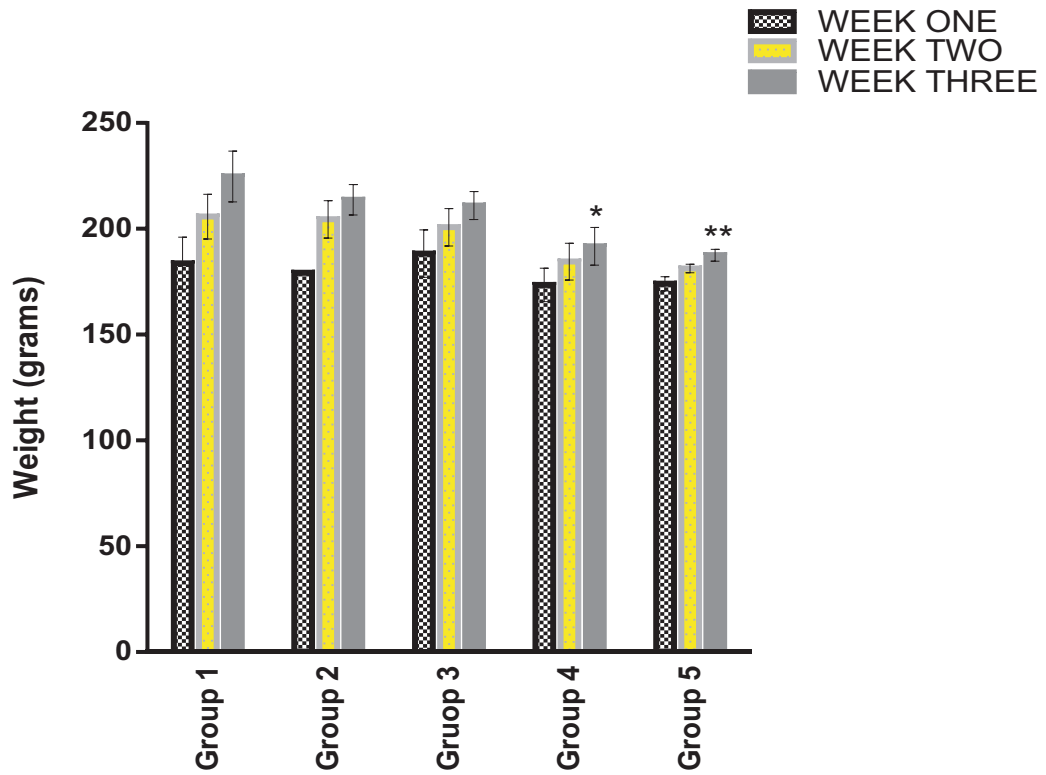


Figure 4.6: Effect of Tramadol on the body weight of study animals (Error bars represent Mean ± SEM).

**Pictures of Animals Showing Their Testes**



Slide 1: Shows the enlargement of testes of treated groups



**Slide 2: Shows the testes of the control group**

### Discussion

Haemostasis is the body's natural way of preventing excessive bleeding and maintaining the blood's fluid balance. Stopping excessive bleeding is a function of the coagulation system, which is composed of the vascular system, coagulation factors, and platelet count and function. Deficits or abnormalities in one or more of the aforementioned may lead to hemorrhagic diatheses (Bain *et al.*, 2012).

This study examined how body weight, fibrinogen concentration, full blood count (FBC), prothrombin time (PT), and partial thromboplastin time with kaolin (PTTK) were affected by oral and intramuscular injection of Tramadol.

The ability of opioids to reduce pain is well known. They do this by triggering opioid receptors in the brain, gastrointestinal tract, and spinal cord. Long-term opiate usage, however, has been thought to have a detrimental effect on haemostasis (Marquardt *et al.*, 2005). Tramadol was the opioid used in this experiment.

No discernible variations from the control were found when comparing the test groups' haematological markers, such as mean platelet

volume, white blood cell count, and platelet count. This suggests that tramadol has no effect on haematological markers. In a study on healthy dogs given large doses of tramadol, Akhtardanesh and Colleagues (2014) discovered similar outcomes, despite the fact that the effects of such a dosage on platelet counts were not stated. This is in line with what they discovered. Rita and Colleagues (2015) discovered significant haematological changes in laparotomized albino rats in research comparing single vs. recurrent Tramadol administration, which is in contrast to the findings of this inquiry. Similarly, Tramadol considerably changes haematological markers, according to a study by Elyazji and Colleagues (2013). Platelets are known to aid in the haemostatic process by exposing a sufficient phospholipidic surface, which initiates coagulation processes, and by using their cohesive and adhesive qualities to form a haemostatic plug. This surface catalyses the development of coagulation and the creation of the haemostatic plug (Hoffbrand *et al.*, 2012). Despite not being statistically significant, the platelet levels in this study were greater than those in the control group. However, tramadol induces thrombocytopenia, according to a previous report by Fardan and Colleagues (2016). Platelet values (in treated groups) suggest that Tramadol may improve

blood coagulation by encouraging platelet production. The research animals' PT changed when the treatment groups were compared to the control group. Comparing groups one and three revealed a statistically significant increase. This finding is consistent with a previous report (Harsherm, 2014).

The prothrombin time test may detect abnormalities in the coagulation components of the common and extrinsic blood coagulation pathways, as well as inhibitors of these processes (Bain *et al.*, 2012). In light of this, the increase in PT may suggest that Tramadol reduces the levels of one or more coagulation factors involved in the common and/or extrinsic clotting pathways. In comparison to the control, PTTK increased in groups two, three, and five; however, only the rise in group three was statistically significant. According to Bain *et al.* (2012), prolonged PTTK may be a sign of the existence of inhibitors of the intrinsic or common coagulation pathways or the absence of one or more of its coagulation components. Group 4's PTTK dropped in comparison to the control, and this difference was statistically significant.

According to Nna and Colleagues (2014), using tramadol causes genital growth to rise. The results of the investigation, which demonstrated that the testes of the study animals in the treated groups became bigger and had a reddish tint, corroborate this conclusion.

The animals in the treatment groups weighed much less than the control group, which is consistent with the results of Jablonski *et al.* (2002) and Lilies and Flecknell (1992), who observed that rats treated with Tramadol lost weight as a consequence of eating less. Rats administered Tramadol also had noticeably reduced mean weights, according to Rita and Colleagues (2015). Furthermore, it was shown that oral therapy had more noticeable results than intramuscular injections. This result is in line with a research by Lintzet and Associates (1986) that showed that oral tramadol is almost completely absorbed. No matter how much food is consumed at the time of administration, the oral absorption rate of tramadol is about 100%, and its typical bioavailability is 70%.

## Conclusion

Tramadol is becoming a widely used drug to treat acute and sub-acute pain. It is also being given to treat fertility issues in males as it increases sexual function. Since oral administration works faster, Tramadol should be administered orally for its proper function, but this should be at controlled dosage so that a negative side effect does not occur.

## Limitation to Study

The following are some of the difficulties encountered during the study:

1. Lack of a proper animal house which led to the use of a make-shift animal house.
2. Lack of needed laboratory equipments for proper assay.
3. Lack of funding for proper execution of research.

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