

## Attenuation of Female Reproductive Hormones in Piroxicam Administered Wistar Rats

### ABSTRACT

One of the most commonly used drugs in today's world is the Nonsteroidal Anti-inflammatory Drugs (NSAIDs). These drugs are renowned for their role in anti-inflammations, as well as in reducing muscular and joint pains. Though several studies have acknowledged the role of Piroxicam (an NSAID) in the treatment and/or management of rheumatoid arthritis, osteoarthritis, acute gout, ankylosing spondylitis, acute musculoskeletal disorders and dysmenorrhoea, very little or ruff information details its implications in the endocrine and reproductive systems. To this point, this study was geared towards investigating in wistar rats, the attenuated effect(s) of Piroxicam administration on female reproductive hormonal levels. Twenty (24) adult female wistar rats (between 160-220g) were divided into four (4) groups of six rats each (n=6). While group 1 served as control (fed with normal diet), group 2 animals were administered with Piroxicam (0.1mg/kg), with group 3 given 0.2mg/kg of Piroxicam. Group 4 rats were co-administered with 0.2mg/kg of Piroxicam and Vitamin E. following period of administration of test substance, rats were sacrificed, blood samples collected and centrifuged to obtain serum for biochemical analysis. Hormone levels were analysed using Enzyme Linked Immunosorbent assay (ELISA). Analysis of Variance (ANOVA) shows a significant decrease ( $p < .05$ ) in hormone levels of groups 2 and 3 rats upon comparison with those in control group. Antioxidant Vitamin E helped in antagonizing the effects of Piroxicam, showing less decrease in hormone levels. By decreasing oestrogen, progesterone and gonadotropins levels, NSAIDs can therefore be said to pose negative effects on female fertility.

**Keywords:** NSAIDs, Piroxicam, Hormones

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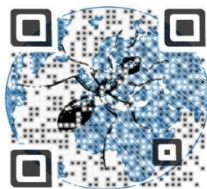
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## INTRODUCTION

Infertility is a disease of the reproductive system defined by the failure to achieve pregnancy after 12 months or more of regular unprotected sexual intercourse.<sup>1</sup> It is the inability of a person, animal or plant to reproduce by natural means. In humans, infertility may describe a woman who is unable to conceive as well as being unable to carry a pregnancy to full term.<sup>2</sup> Several causes of infertility have been documented. With most common cause of female infertility being ovulatory problems, primary infertility has mostly been linked with neuro-endocrine and/or hormonal problems.<sup>3</sup>

Available statistics posit that, about 20-30% of infertility cases are due to male infertility, 20-35% is due to female infertility, and 25-40% is due to combined problems in both parts. Though 10-20% of reported cases is idiopathic (unknown cause), the highest prevalence of infertility in Africa occurs south of the Sahara, but 5-8% of couples are estimated to experience infertility at some point in their reproductive lives (50-80

million people worldwide).<sup>4</sup> The average infertility in Africa is 10.1% of couples, with about 32% in some countries. While primary infertility is higher in other regions of the world, secondary infertility is more common in Africa.<sup>4</sup>

Diagnosis of infertility begins with a medical history and physical exam, hormone testing to measure levels of female hormones at certain times during a menstrual cycle, measure of FSH and oestrogen levels to assess ovarian reserve, measurements of thyroid function<sup>5</sup> through the thyroid stimulating hormone (TSH) level, measurement of progesterone level in the second half of the cycle to help confirm ovulation and Anti-Müllerian hormone to estimate ovarian reserve etc.<sup>5</sup>

Nonsteroidal anti-inflammatory drugs (NSAIDs) are a drug class that provide analgesic (pain-killing) and antipyretic (fever-reducing) effects, and, in higher doses, anti-inflammatory effects.<sup>6</sup>

Unrelated to steroids, NSAIDs reduce inflammations by taming the production of prostaglandins; chemicals that promote

inflammation, pain, and fever. As a member of the oxycam family of NSAIDs, Piroxicam is most popular, with well-established roles in the treatment of rheumatoid arthritis and osteoarthritis. This drug seems to be highly effective in the treatment of acute gout, and useful in the management of ankylosing spondylitis, acute musculoskeletal disorders, dysmenorrhoea and as an analgesic.<sup>7</sup> Though beneficial and/or detrimental, there is not enough information on the role of Piroxicam on the reproductive hormones of the body; this study was therefore pioneered to the confirmatory findings on the dangers of this drug. The study examined the effect(s) of NSAID(Piroxicam) administration on female reproductive hormones. It determined the effects of NSAID (Piroxicam) on serum estrogen levels, investigated its effects on serum gonadotrophins {Follicle stimulating hormone (FSH) and Luteinizing hormone (LH)} and progesterone levels.

## **MATERIALS AND METHODS**

### **Ethics**

Ethical approval was sought and granted by the ethical committee of College of Health Sciences, Delta State University, Abraka, Delta state, Nigeria.

### **Study Design**

This experimental study involved twenty four (24) rats were used in the process, all of which were randomly divided into four (4) groups of six (6) rats each and represented as follows: Group 1 (Control rats were fed with chow and clean drinking water), Group 2 (Rats treated with 0.1mg/Kg of Piroxicam), Group 3 (Rats treated with 0.2mg/Kg of Piroxicam), Group 4 (Rats administered with 0.2mg/Kg Piroxicam and 15mg/Kg of Vitamin E)

### **Scope of Study**

Animal models, specifically the albino wistar rats were used for the study. These rats are biologically similar to humans and are susceptible to many of the same health problems in humans. Study would have been best carried



out on humans, but due to its invasive nature, it would be wrong to deliberately expose humans to such health risks. Hence, the need for wistar rats

### **Sample Collection**

Following period of administration of test substances, rats were euthanised by cervical dislocation. Blood samples were collected via cardiac puncture and centrifuged for 10 minutes at 4000rpm. Plasma was then aspirated into a well labelled plain bottle and stored in the freezer at 4°C. This plasma was used for the hormone analysis.

### **Piroxicam Administration**

20 mg of Piroxicam purchased under the drug brand Dolonex DT® was dissolved in distilled water. The wanted concentration used for this study was 7.5mg/Kg and 15mg/Kg body weight. Piroxicam was administered once daily for four weeks as stated by the experimental design.

### **Antioxidant Administration**

Vitamin E ( $\alpha$ -tocopherol) tablets were dissolved in distilled water at a dose of 150mg/kg and

administered orally via an oro-gastric canular once daily for four weeks.

### **Measurement of Hormones**

Serum LH, FSH, Progesterone, and oestrogen levels were measured with the Enzyme Linked Immuno-sorbent Assay (ELISA). They were carried out with instructions from the manufacturer of the assay kit (Randox Laboratories Limited, UK); 50  $\mu$ l of serum sample was added to well-labelled microtiter plates and 100  $\mu$ l. Enzyme conjugated detection antibody (horseradish peroxidase) was also mixed with the serum samples in the plates. The same procedure was performed for the standard experimental serum samples. They were incubated for 45 minutes at room temperature. The plates were rinsed 3 times with wash solution, prepared by diluting wash concentrate (supplied by manufacturer) with deionized water to remove unbound antibodies. 100 $\mu$ l TMB reagent was added and well incubated for the colour to develop in the dark for 20 minutes. 100 $\mu$ l of Hydrochloric acid HCL was added to

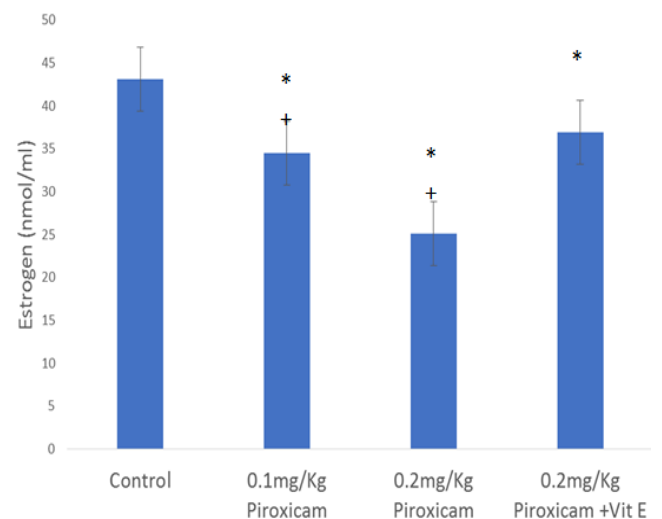
the various plates to stop further development of colour. Increased/decreased density was read at 450nm using ELISA machine and deionized water served as blank. The test and control samples concentrations were inferred from the standard curve. The standard curve was plotted from the optical density values and concentrations of series of LH (0, 5, 15, 50, 100 and 200 miu/ml), FSH (0, 5, 15, 50, 100 and 200 miu/ml), progesterone (0, 10, 30, 100 and 1000 mg/ml) and Oestrogen (0, 0.5, 3, 10, 25 and 50 mg/ml) standards provided by the manufacturer of the kit.

### Statistics

Results were expressed as mean  $\pm$  SD. Analysis of data for statistical significance between control and experimental groups was done using the one-way analysis of variance (ANOVA). Statistic software, SPSS 20 was used to analyze data. A p-level less than .05 was accepted as statistically significant.

## RESULTS

This study was undertaken to show alterations in ovarian hormone and gonadotropin levels of rats treated with graded doses of Piroxicam. The studied hormones were estrogen, progesterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH).

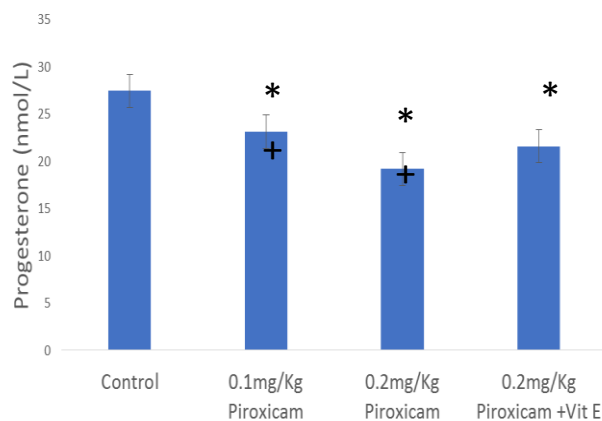


**FIGURE 1: Showing Oestrogen level for rats treated with Piroxicam**

\* $p < 0.05$  compared with control group; + $p < 0.05$  compared with 0.2mg/Kg Piroxicam + Vit E.

Here, changes on the mean serum level of estrogen in albino rats treated with graded dose of Piroxicam were examined. It was observed

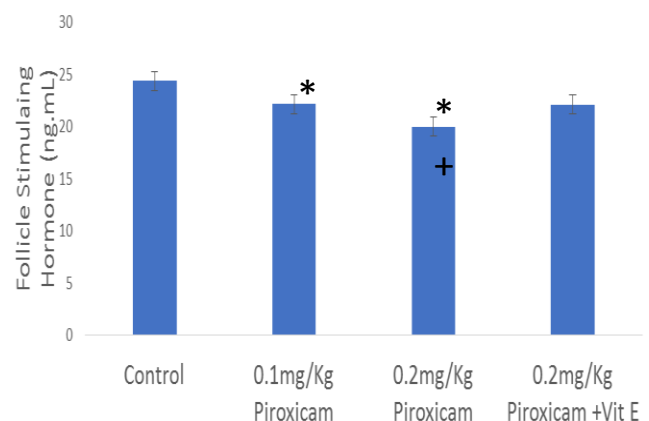
that Piroxicam significantly ( $p < .05$ ) decreased the serum oestrogen level in dose-dependent manner. This decrease was reversed with a recorded increase observed in the oestrogen level of Piroxicam treated rats administered with Vitamin E. The change in serum oestrogen level induced by Vitamin E was significant ( $p < .05$ ) compared to the oestrogen values of control rats and rats treated with Piroxicam.



**FIGURE 2: Showing Changes in progesterone levels for rats treated with graded doses of Piroxicam**

*\*p < 0.05 compared with control group;*  
*+p < 0.05 compared with*  
*0.2mg/Kg Piroxicam + Vit E*

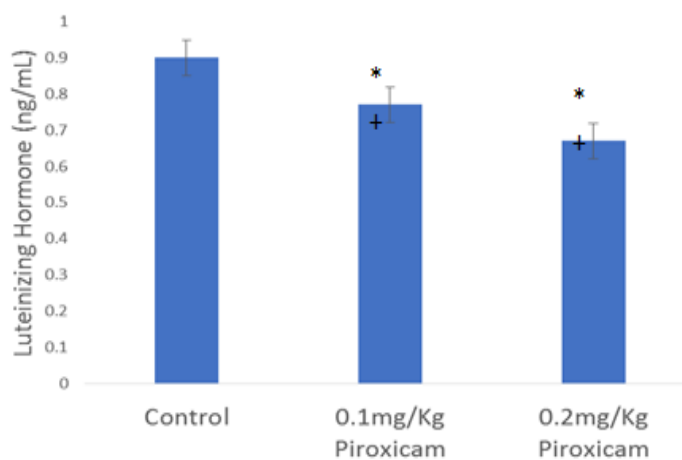
Chart 2 shows changes in serum progesterone level of rats treated with graded doses of Piroxicam. Results from this study show that Piroxicam caused a significant ( $p < .05$ ) dose dependent decrease in serum progesterone. Though co-administration of Vitamin E limited this effect with higher level of progesterone in rats recorded, significance ( $p < .05$ ) with control was established. The increased serum progesterone level induced by Vitamin E was also significant ( $p < .05$ ) when compared to progesterone of rats treated with 0.1mg/Kg and 0.2mg/Kg of Piroxicam.



**FIGURE 3: Showing Alterations in serum follicle stimulating hormone level due to the effect of Piroxicam**

*\*p < 0.05 compared with control group; +p < 0.05 compared with 0.2mg/Kg Piroxicam + Vit E*

From above chart, it was observed that Piroxicam decreased serum concentration of follicle stimulating hormone (FSH) with increase in dosage of drug administered. Similar to findings of charts 1 and 2, Vitamin E co-administration reversed the effect of Piroxicam with increased serum FSH observed, this increase was significant ( $p < .05$ ) when compared to serum FSH concentrations of rats treated with 0.2mg/Kg of Piroxicam.



**FIGURE 4: Showing Changes in serum Luteinizing hormone level of rats treated with Piroxicam**

*\*p < 0.05 compared with control group; +p < 0.05 compared with 0.2mg/Kg Piroxicam + Vit E*

Above chart shows changes in serum luteinizing hormone (LH) level of rats treated with Piroxicam. As observed, luteinizing hormone level significantly ( $p < .05$ ) decreased in dose dependent manner following administration of Piroxicam. Vitamin E co-administration reversed this effect with a recorded increase in serum LH concentration. This increase was significant ( $p < .05$ ) as compared to serum LH levels of control and rats treated with Piroxicam.

## DISCUSSION

Globally, Analgesics are among the most commonly used medications with proper use considered safe and effective<sup>8</sup>. It has been hypothesized that certain Non-Steroidal Anti-inflammatory Drugs (NSAIDs) use could

improve fertility outcomes, possibly due to increased ovarian and/or endometrial vascular perfusion<sup>9</sup>. Other medications used for pain relief (i.e. analgesics), have been reported to inhibit ovulatory functions in human and animal studies. This study shows that Piroxicam caused a significant ( $p < .05$ ) dose dependent decrease in serum oestrogen, progesterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) concentrations. This data generated showed that Piroxicam alters hormone secreting functions of the ovary and hypothalamus.

Piroxicam administration in dose dependent manner caused a complete cessation of ovulation with significant decrease in both FSH and LH, this decrease was thought to be due to suppression of PGE2 by Piroxicam centrally in hypothalamus and locally on the ovarian level PGs.<sup>10</sup>

Studies have shown that prostaglandins have minimal direct effects on gonadotropin secreted from the pituitary, while NSAIDs seems to

suppress these hormones at the hypothalamic level by inhibition on GnRH release,<sup>11</sup> and this what happened when high doses of aspirin are used. PG levels are elevated in the mature ovarian follicles due to the ovulatory LH surge.<sup>12</sup> Several reports have shown that, ovulation can be inhibited by NSAIDs despite of undetectable changes in several key hormones of ovulation (FSH, LH, Estrogen and Progesterone), suggesting that local ovarian factors are the predominant driving force in ovulation. Many authors reported that, there is a marked increase in intrafollicular levels of PGs (E and F series) shortly before ovulation. Moreover it has also been reported that Piroxicam partially reduced follicular level of PGE2 and PGF2 $\alpha$ .<sup>13</sup> Women taking anti-PGs drugs, suffer from luteinisation of graafian follicle, to produce a syndrome referred to as luteinized un-ruptured follicle syndrome.<sup>1</sup>

Though animal studies consistently have shown that non-steroidal anti-inflammatory drugs (NSAIDs) are associated with inhibition of



ovulation, studies among women are less clear.<sup>15</sup>,  
<sup>16</sup> In a randomized crossover trial of ibuprofen use (800 mg, three times per day for 10 days beginning during the follicular phase), no associations with reproductive hormones were observed, though significant delays in the timing of ovulation were found, supporting the theory that ovulation may occur through inflammatory-related processes that lead to the targeted rupture of a follicle and that anti-inflammatory agents can interfere with that process.<sup>17</sup>

Reversal effect caused by administration of Vitamin E, implicates oxidative stress a possible mechanism for the hormone reducing effect of Piroxicam. Vitamin E significantly ( $p < 0.05$ ) increased the serum concentrations of oestrogen, progesterone, follicle stimulating hormone and luteinizing hormone.

It is a well-established fact that the oxidative stress developed due to the introduction of the xenobiotics, causing damage to the cells via oxidative stress-mediated lipid peroxidation (LPO) <sup>18&19</sup>. The use of various dietary

antioxidant treatments in terminating or reducing free radical attacks that are involved in various diseases are also an important part of the antioxidant mechanism. The antioxidant may act as free radical scavengers, reducing agents, and activators of anti-oxidative defense enzymes system to suppress the radical damage in biological system<sup>20</sup>.

### **Significance of Study**

This study could provide information on the issues of infertility particularly on its susceptibility to Non-steroidal anti-inflammatory drugs. Further, this study would explain the mechanism behind the effect on each hormone following Piroxicam administration. This would expectedly heighten the awareness of patients on NSAIDs, and the possible threats it poses.

### **Conclusion**

Present study showed that Piroxicam has negative effects on female fertility by decreasing oestrogen, progesterone and gonadotropin levels.



However, a combination of Vitamin E and piroxicam antagonized this effect, showing lesser decrease in hormonal levels.

### Recommendations

In the nearest future, clinical Piroxicam contraceptive or antifertility potential should be put into consideration whilst prescribing for other purposes such as analgesics.

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