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EXPERIMENTAL ACETIC ACID-INDUCED COLITIS IMPAIRS FERTILITY INDICES THROUGH DISRUPTED HORMONAL FEEDBACK IN MALE RATS

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ABSTRACT

Although, men with ulcerative colitis demonstrate more infertility tendency than their healthy counterparts, the mechanisms through which colitis impairs fertility are not well understood. Therefore, the study investigated whether colitis associated impairments in fertility indices are characterized by intact or disrupted hormonal feedback. 12 male rats aged 53 days were randomly divided into control and colitic groups. Colitis was induced through intrarectal instillation of 1ml/200g of 7% acetic acid 5 times at an interval of 7 days. 72 hours after the fifth induction, colitic rats were characterized by increases in colonic thickness, colonic weight and rectal temperature. When compared with control group, colitic group exhibited a significant decrease ($P<0.05$) in sperm count, sperm motility, sperm morphology, testicular weight and percentage body weight change. Colitis significantly disrupted pituitary Follicle Stimulating Hormone (FSH) secretion but had no effect on luteinizing hormone. There was also a reduction in steroidogenic capacity of testes evidenced by significant decrease ($P<0.05$) in serum testosterone. The findings of this study indicated that colitis decreased fertility indices in male rats through disrupted hormonal feedback.

KEYWORDS: Infertility, Follicle Stimulating Hormone, Luteinizing Hormone, Colitis, Sperm count.

INTRODUCTION

Ulcerative colitis is a chronic inflammatory disease of the large intestine that affects 5-500 per 100,000 individuals each year (Danesse and Fiocchi, 2011; Ford *et al.*, 2013). The disease is more prevalent between ages 15 and 30 years (Danesse and Fiocchi, 2011). Diarrhea, abdominal pain, fever, anemia, anorexia and reduction in weight (Wanderas *et al.*, 2016) are among the primary symptoms of the disease.

In humans and animals, anorexia and reduction in weight are associated with decreased activity of gonadotropin releasing hormone secreting neurons of the hypothalamus and reductive secretion of gonadotropin from anterior pituitary (Ballinger *et al.*, 2003; Takeshi and Hivoshi 2016). This suggests that ulcerative colitis could lead to delayed sexual maturation and infertility, a medical condition that affect 7% of all men (Lotti and Maggi 2014).

Many studies have documented that colitis impairs reproductive function and delays onset of puberty in male children. For example, while the onset of puberty was found to be 13.2 years in boys with inflammatory bowel Disease (IBD), it was 12.4 years in healthy controls (Brain and Salvage 1994). In an experimental model of colitis using male rats, there was a significant reduction in seminal vesicle and prostate weight compared with pair-fed groups. Concentrations of testosterone in colitis rats were lower than in healthy free feeding group but similar to pair-fed groups (Ballinger *et al.*,2003; Takeshi and Hivoshi 2016).

In another report, plasma concentration of gonadotropins in colitic rats was found to be similar to that seen in healthy free-feeding controls (Azooz *et al.*, 2001; Tigas and Tsatsoulis, 2012). There is increasing evidence that colitis induced infertility is mediated by mechanisms other than under nutrition and underweight. For instance, Mark and Yongli (2011) noticed that colitic rats showed delayed puberty but exhibited the same weight and leptin levels with food restricted male rats.

Studies have highlighted the roles of inflammatory cytokines (Mizokami *et al.*, 2001) and very many works have claimed that testosterone levels become reduced in colitis induced infertility (Ballinger *et al.*,2003; Takeshi and Hivoshi 2016). It is unclear whether hormonal

feedback in colitis induced infertility is intact or disrupted. Therefore, the study is designed to determine the characteristics of hormonal feedback in ulcerative colitis induced infertility.

MATERIALS AND METHODS

Site of the Experiment

The experiment was done in the Department of Physiology, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.

ANIMAL CARE AND MANAGEMENT

12 fifty three days old male Sprague Dawley rats were obtained from Animal house of Ladoke Akintola University of Technology, Ogbomoso. They were housed in standard cages at room temperature and 12hr light/12hr dark cycle. The animals were acclimatized for 1 week. All rats were fed pelletized grower mash (standard chow) and distilled water *ad libitum.*

ETHICAL CERTIFICATION

The study was conducted in line with the guidelines of National Institute of Health (NIH) for the use of laboratory rats.

STUDY DESIGN

Experimental animals were divided into two groups of six rats each as follows:

Group A (control group): received standard chow and distill water orally (P.O.).

Group B (Colitis group): received intrarectal instillation of 1ml/100g 0f 7% acetic acid, standard chow and distilled water (P.O.).

DAILY DETERMINATION OF WEIGHT

Each rat was individually weighed daily using weighing balance (Labtech, Bihar India).

INDUCTION AND CONFIRMATION OF EXPERIMENTAL COLITIS

The rats were fasted for 24 hours and weighed prior to induction of colitis. Rectal flushing was done to remove fecal remnants. Colitis was induced through intrarectal instillation of 1ml/100g of 7% acetic acid using a flexible catheter in the trendelenburg position. To prevent the acetic acid from coming out, the rats were pressed rectally and held upside down for about 30s. The induction of colitis was done 5 times at an interval of 7 days.

Colitis was confirmed by determining rectal temperature, tissue thickness and colonic weight.

DETERMINATION OF COLONIC WEIGHT

Following the fifth induction, the rats were weighed and then sacrificed 72 hours after. The colon was excised and distal 6cm of the colon was removed. The colons were washed in normal saline and then weighed.

DETERMINATION OF PERCENTAGE WEIGHT GAIN OF THE ANIMAL

The % weight gain of the animal is obtained by the following formula:

% weight gain= (final weight – initial weight)/initial weight ×100%

DETERMINATION OF TESTICULAR WEIGHT

The testes were excised, washed in normal saline and then weighed.

DETERMINATION OF SEMEN ANALYSIS PARAMETERS

The right caudal epididymes were placed in a petri dish containing 1ml of normal saline. An incision of the caudal epididymes was done and semen was collected for the determination of sperm motility, sperm count and sperm morphology.

DETERMINATION OF TESTOSTERONE LEVEL

Serum testosterone was determined using Radioimmunoassay (RIA) using a testosterone 125I RIA Kit (ICN, Biochemical, Immunotech, Marseille, France).

DETERMINATION OF LUTEINIZING HORMONE (LH) AND FOLLICLE STIMULATING HORMONE (FSH)

Serum levels of luteinizing hormone (LH), and follicle-stimulating hormone (FSH) were estimated using the enzyme-linked immunosorbent assay (ELISA) kits from Fortress Diagnostics, Northern Ireland.

STATISTICAL ANALYSIS

Analysis of data was carried out using SPSS 20.0. Pairwise comparison was done using one sample independent T test.

RESULT

TABLE 1: CONFIRMATORY TESTS FOR COLITIS.

Group	Colonic Thickness (cm)	Colonic Weight (g)	Rectal Temperature(^{0}c)
Control	0.1±0	0.5150±0.1893	36.3±0.5578
Colitic	0.2667±0.2108*	0.6533±0.1229*	37.4±0.2933*

There was a significant increase in colonic thickness, colonic weight and rectal temperature in colitic group when compared with control at $P<0.05$.

PERCENTAGE WEIGHT GAIN

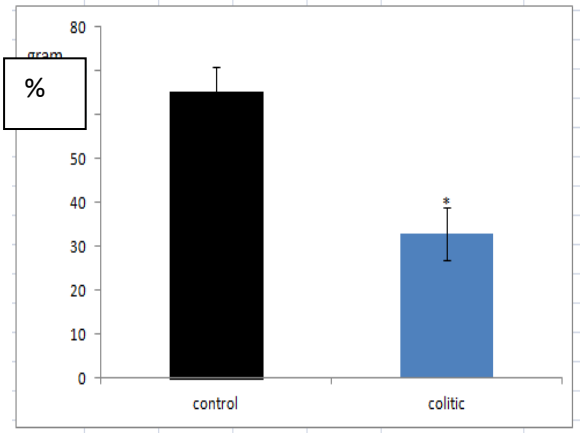


Figure 1: Effect of Experimental Acetic Acid Induced Colitis On Percentage Weight Gain.

There was a significant decrease in percentage weight gain in colitic group when compared with control at P<0.05.

TESTES WEIGHT

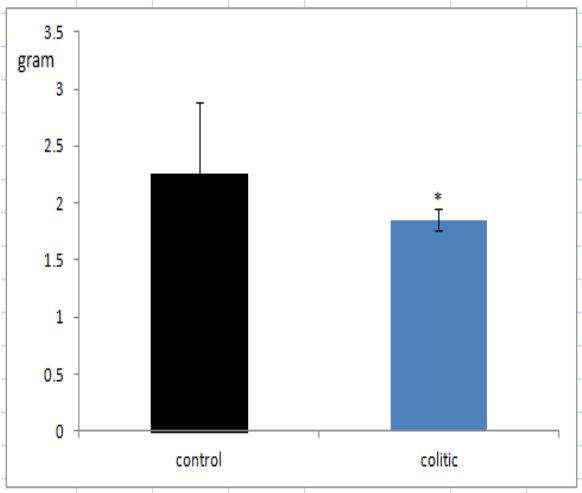


Figure 2: Effect of experimental acetic acid induced colitis on testes weight.

There was a significant decrease in testes weight in colitic group when compared with control at p<0.05.

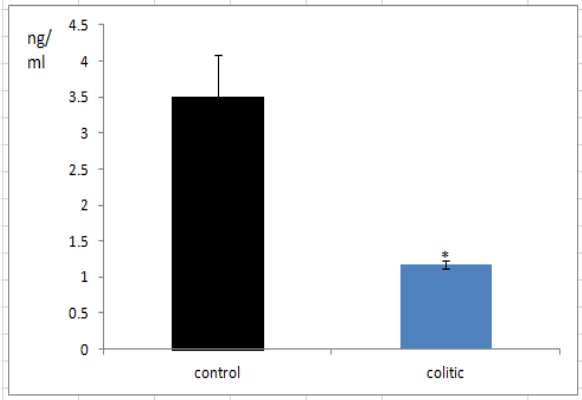
Table 2: Effect of Experimental Acetic Acid Induced Colitis On Semen Analysis.

Group	Sperm Count (cells/ml)	Sperm Motility (%)	Sperm Morphology (%)
Control	65.76±6.7176	77.552±1.7479	85.51±1.3353
Colitic	48.3±3.093*	71.32±1.07741*	74.93±1.1396*

***P<0.05 from control**

There was a significant decrease in sperm count, sperm motility and sperm morphology in colitic group when compared with control at P<0.05.

TESTOSTERONE

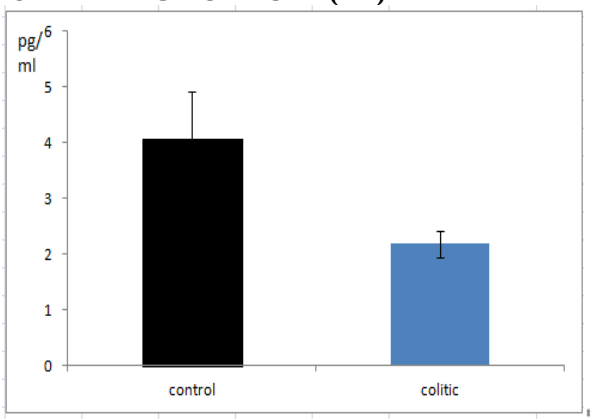


***P<0.05 from control**

Figure 3: Effect of Experimental Acetic Acid Induced Colitis On Testosterone.

There was a significant decrease in testosterone in colitic group when compared with control at P<0.05.

LUTEINIZING HORMONE (LH)

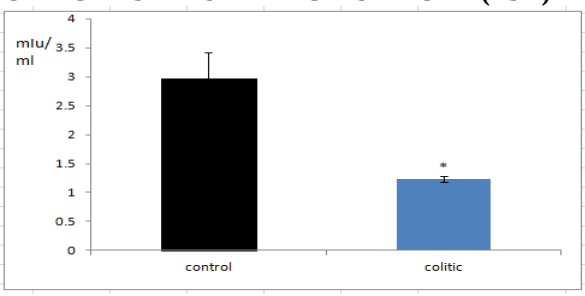


***P<0.05 from control**

Figure 4: Effect of Experimental Acetic Acid Induced Colitis On Luteinizing Hormone.

There was a insignificant decrease in LH in colitic group when compared with control at P<0.05.

FOLLICLE STIMULATING HORMONE (FSH)



***P<0.05 from control**

Figure 5: Effect of Experimental Acetic Acid Induced Colitis On Follicle Stimulating Hormone.

There was a significant decrease in FSH in colitic group when compared with control at P<0.05.

DISCUSSION AND CONCLUSION

In endocrine physiology and reproduction, the importance of communication between endocrine glands and target tissues cannot be undersized. Feedback mechanisms most especially negative feedback helps in preventing excess or underactivity of endocrine glands (Ballinger *et al.*,2003: Barrett *et al.*,2010). The present study examined the characteristic of hormonal feedback in colitis induced infertility using male Sprague dawley rats.

Colitic rats in this study exhibited high rectal temperature, increased colonic thickness, and elevated colonic weight. These results concur with human and animals reports of experimental colitis (Mascolo *et al.*, 1996; Tan-No *et al.*,2006; Adeniyi *et al.*,2016). While the high rectal temperature depicts an increased metabolic rate in colonic tissue, the increases in colonic thickness and weight may be connected with escape of macromolecules into microcirculation.

Alteration in semen quality in terms of volume, concentration, motility, progression, total motile count and normal oval forms depict impairment in fertility (WHO, 1991; Mahadevan 2006). We observed that male colitic rats showed marked alterations in semen analysis. In a similar report, Valer *et al.*,(2017) showed that men with inflammatory bowel diseases exhibited low quality of semen.

As far as this study is concerned, male colitic rats exhibit reduction in sperm motility, morphology and sperm count. The low sperm count may be due to low percentage body weight gain. Many studies have demonstrated the relationship between weight loss and infertility in male (Ballinger *et al.*,2003; El-Tawil 2003; Takeshi and Hivoshi 2016). In addition to weight loss, there is growing evidence that inflammation on reproductive axis and disruption of testicular function by cytokines can hinder spermatogenesis in colitis (Hales *et al.*,1995; Terranora *et al.*, 1997; Warren *et al.*,1999; Azooz *et al.*, 2001; Tigas and Tsatsoulis 2012).

The stimulatory effect of FSH on sertoli cells is widely known to contribute to testicular volume (Sairam and Krishnamurthy 2001; Mark and Yongli 2011). Therefore, the decreased testicular weight in this study may be connected to low FSH secretion. Interestingly, the result of gonadotropin test indicated that plasma FSH level was low.

Evidence abounds to suggest that the steroidogenic function of testes can be disrupted despite intact FSH secretion (Barrett *et al.*, 2010). The low plasma testosterone level observed in this study clearly confirms that colitis induced male infertility is characterized by both attenuated steroidogenic activity of testes and depressed FSH secretion. Although there was an insignificant decrease in plasma LH level, our study shows that in experimental acetic acid-induced colitis; there is an absence of physiological communication between the adenohypophysis and testes.

In conclusion, the findings of this study indicate that acetic acid-induced colitis impairs male fertility indices by disrupting hormonal feedback in sprague dawley rats.

CONFLICT OF INTEREST: Nil.

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