

INVIVO EVIDENCES OF *CURCUMA LONGA* ON OXIDATIVE STRESS IN STZ-INDUCED DIABETES ON SPERM PARAMETERS IN MALE WISTAR RATS.

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ABSTRACT

Several conditions such as chemotherapy and toxins can interfere with spermatogenesis and reduce sperm quality and production. In the case where natural antioxidant response cannot manage oxidative stress and free radical overload, oxidative damage occurs and this begins the genesis of many diseases of which diabetes is one of them. This has awakened the interest of researchers to the use of an alternative source of medicine and herbal medicine. Medicinal use of *Curcuma longa* dates back to ancient China and India; its constituents are stated to have anti-hepatotoxic, anti-inflammatory, stimulant, and antioxidant and used since ancient time as medicinal and nutritive origins knowing to possess androgenic activities and have well effect in diseases treatment in more countries world-wide. As an antioxidant *Curcuma longa* possible has a useful effect on spermatogenesis and sperm parameters. Wistar male rats (n=24) were allocated into six groups, positive control (n=4), diabetic control (n=4) and experimental groups (n=20), that subdivided into groups of 4 that received treatment of *Curcuma longa* rhizome powder with or without STZ-induced diabetes in the dosages (25 and 100mg/kg/day) for 21 consecutive days. In twenty-second day, the testes were removed and semen was collected from epididymis and prepared for analysis. The percentage of sperm viability and motility in the treatment groups increased mildly with a significance of: (p<0.05) in comparison to control group and with the diabetic group being critically lower than those in control group. This suggested that *Curcuma longa* may be promising in enhancing sperm health parameters.

Keywords: Turmeric, oxidative stress, semen, STZ-induced diabetes, antioxidant

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both (Thévenod, 2008; Bal *et al.*, 2011; Wankeu-Nya *et al.*, 2014). Sustained higher levels of blood glucose cause damage to nerves and blood vessels, leading to complications such as erectile dysfunction (ED) (Thorve, 2011; Cao *et al.*, 2012). DM is one of the predominant risk factors of ED and also one of the most difficult to treat

(Chitale *et al.*, 2009). DM may cause ED through a number of pathophysiologic changes, including neuropathy, endothelial dysfunction and hormonal changes (Konstantinos and Dimitrios, 2009).

It is shown that DM has detrimental effects on sperm parameters in human and experimental animals. DM may affect male reproductive functions at multiple levels including its detrimental effects on endocrine control of

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spermatogenesis and/or by impairing erection and ejaculation (Petroianu *et al.*, 2009). Ricci *et al.* found that insulin-dependent diabetes is accompanied by reduced semen volume and decreased vitality and motility of the spermatozoa, but no change in seminal viscosity (Ricci *et al.*, 2009).

Infertility is one of the major health problems in life, and approximately 30 % of infertilities are due to a male factor (Carlsen *et al.*, 1992; Isidori *et al.*, 2006). Several conditions can interfere with spermatogenesis and reduce sperm quality and production. More factors such as drug treatment, chemotherapy, toxins, air pollutions and insufficient vitamins intake have harmful effects on spermatogenesis and sperm normal production (Mosher and Pratt, 1991). Several studies have reported that antioxidants and vitamin A, B, C, and E in diet can protect sperm DNA from free radicals and increase blood-testes barrier stability (Arash *et al.*, 2011).

Nowadays *Curcuma longa* rhizome is used worldwide as a spice. Both anti-oxidative and androgenic activity were reported in animal models. Besides, other researches showed that *Curcuma longa* has dominative protective effect on DNA damage induced by H₂O₂ and might act as a scavenger of oxygen radical and might be used as an antioxidant (Menon and Sudheer, 2007). Antioxidants protect DNA and other important molecules from oxidation and damage, and can improve sperm quality and consequently increase fertility rate in men (Rajeev *et al.*, 2006; Salvioli *et al.*, 2007). Therefore, the role of nutritional and biochemical factors in reproduction and sub-fertility treatment is very important. The present study was planned to assess the ability of *Curcuma longa* to promote sperm parameters and modulate follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone concentration, spermatogenesis and oxidative stress.

MATERIALS AND METHODS

Adult Wistar male rats (n=24) were included in the present study. The rats were 6-8 weeks old and weighing 220±10g each. They were obtained from animal facility of Babcock University, Nigeria. Male rats were housed in temperature controlled rooms (25°C) with constant humidity (40-70%) and 12h/12h light/dark cycle prior to experimental protocols. All animals were treated in accordance to the Principles of Laboratory Animal Care. All rats were fed with a standard diet and water ad libitum. Thereafter, the rats were randomly divided into 6 groups with 4 in each. The control group (1), received distilled water daily. However, the experimental groups split into 5 groups 2-6, Group 2: rats received high dose turmeric (100mg/kg) daily; Group 3: rats received low dose turmeric (25mg/kg) daily; Group 4: rats were induced with 30mg/kg of STZ intraperitoneally for 3 days; Group 5: rats were induced with STZ (30mg/kg) intraperitoneally for 3 days with high dose turmeric (100mg/kg) daily; Group 6: rats were induced with STZ

(30mg/kg) intraperitoneally for 3 days with low dose turmeric (25mg/kg) daily.

Sperms from the cauda epididymis were released by cutting into 2 ml of medium (Hams F10) containing 0.5% bovine serum albumin (11). After 5 min incubation at 37°C (with 5% CO₂), the cauda epididymis sperm reserves were determined using the standard hemocytometric method and sperm motility was analyzed with microscope (Olympus IX70) at 10 field and reported as mean of motile sperm according to WHO method (World Health Organization, 1999).

Statistical comparisons were made using the ANOVA test for comparison of data of the control group with the experimental groups. The results were expressed as mean ± S.E.M (standard error of mean). P<0.05 was considered statistically significant.

RESULTS

Sperm Count

The mean sperm count after three weeks of treatment in the control group was $(43.17 \pm 2.12, p < 0.05)$. The mean sperm count in high dose turmeric group was significantly higher $(49.83 \pm 0.75, p < 0.05)$ when compared to the control group. The mean sperm count in low dose turmeric group was significantly higher $(48.17 \pm 0.54, p < 0.05)$ when compared to the control group. The mean sperm count in STZ induced diabetes group was significantly lower $(30.50 \pm 3.66, p < 0.05)$ when compared to the control group. The mean sperm count in STZ induced diabetes+ high dose turmeric group was slightly higher $(44.33 \pm 1.36, p < 0.05)$ when compared to the control group. The mean sperm count in STZ induced diabetes+ low dose turmeric group was slightly lower $(42.00 \pm 1.08, p < 0.05)$ when compared to the control group.

Sperm Motility

The mean sperm motility after three weeks of treatment in the control group was $(95.67 \pm 0.67, p < 0.05)$. The mean sperm motility in high dose turmeric group was slightly higher $(96.67 \pm 0.33, p < 0.05)$ when compared to the control group. The mean sperm motility in low dose turmeric group was slightly lower $(94.67 \pm 0.33, p < 0.05)$ when compared to the control group. The mean sperm motility in STZ induced diabetes group was significantly lower $(49.00 \pm 22.00, p < 0.05)$ when compared to the control group. The mean sperm motility in STZ induced diabetes+ high dose of turmeric group was significantly lower $(91.33 \pm 0.67, p < 0.05)$ when compared to the control group. The mean sperm motility in STZ induced diabetes+ low dose of turmeric group was significantly lower $(88.50 \pm 1.50, p < 0.05)$ when compared to the control group.

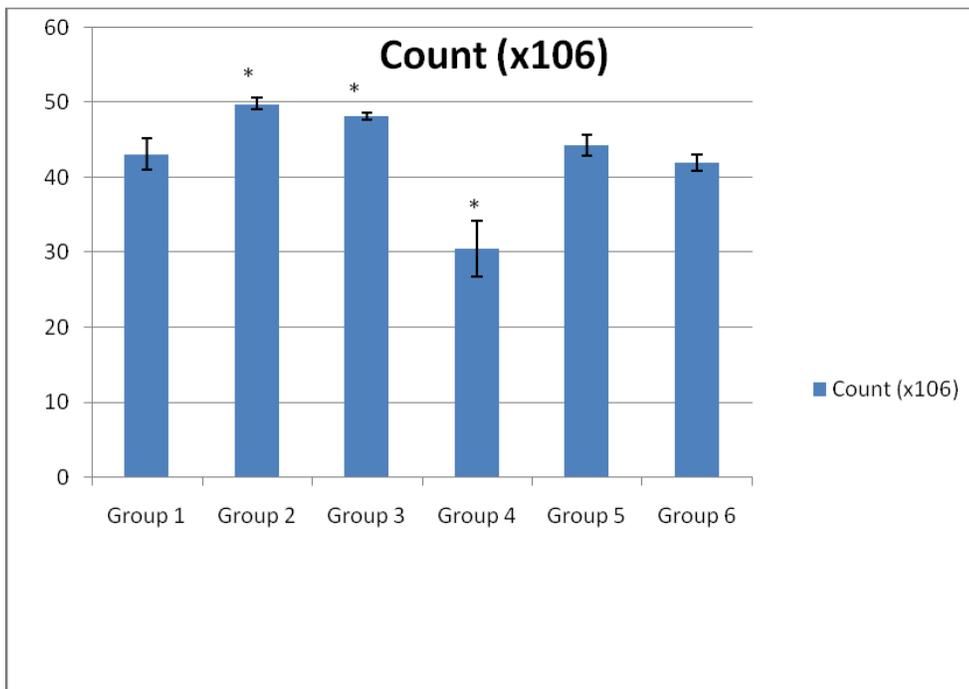


FIGURE 1: Chart shows levels of sperm count across the groups. **=P<0.05 when comparing with group 1 (this shows the significance in different levels in sperm count when compared to the control group).*

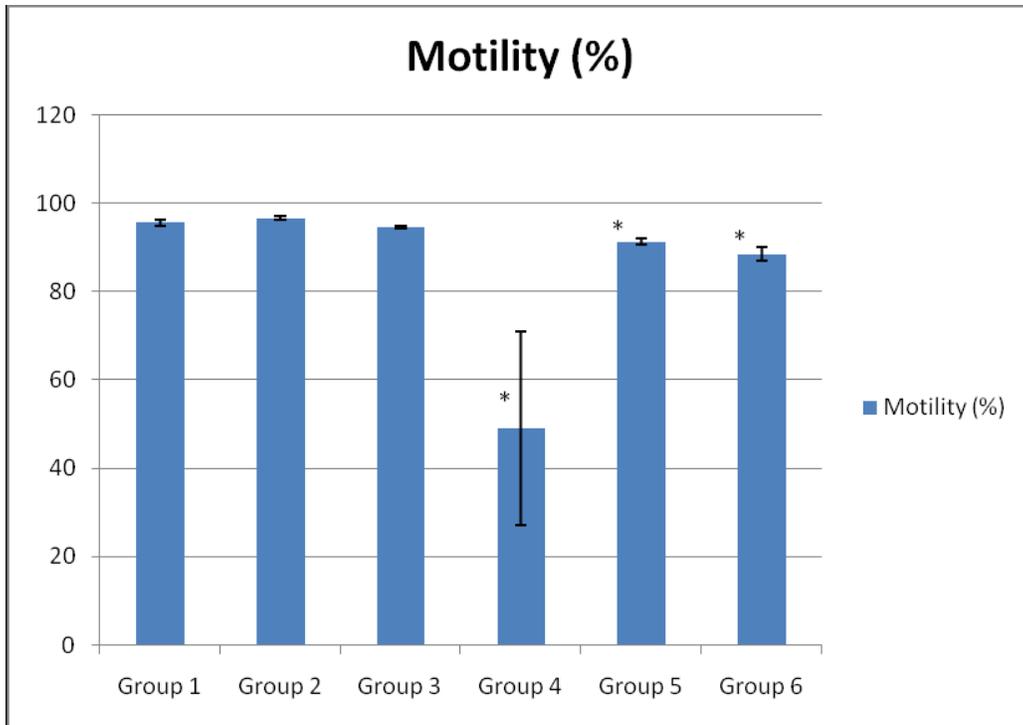


FIGURE 2: Chart shows levels of sperm motility across the groups. *=P<0.05 when comparing with group 1(this shows the significance in different levels in sperm motility when compared to the control group).

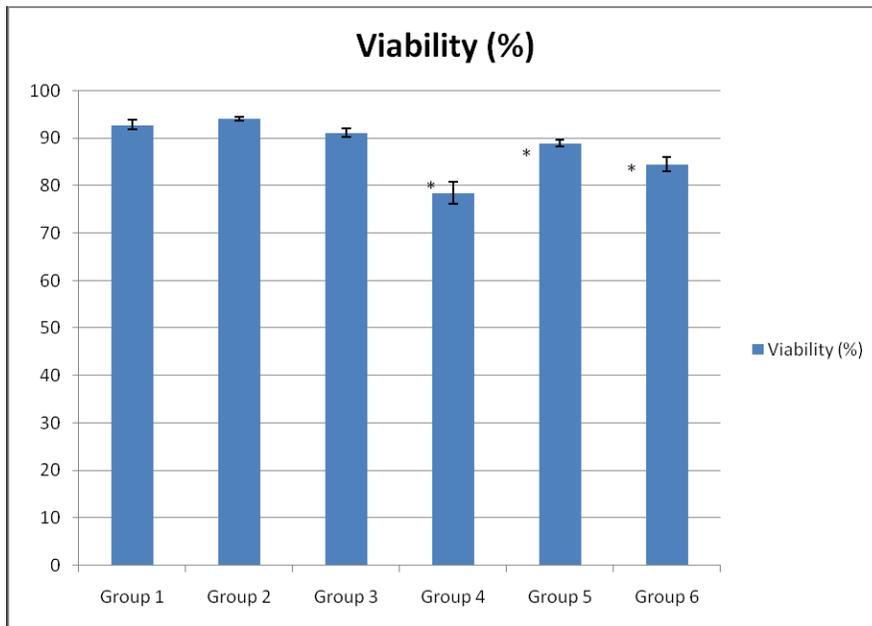


FIGURE 3: Chart shows levels of sperm viability across the groups. *=P<0.05 when comparing with group 1(this shows the significance in different levels in sperm viability when compared to the control group).

Sperm Viability

The mean sperm viability after three weeks of treatment in the control group was (92.83 ± 1.01 , $p < 0.05$). The mean sperm viability in high dose turmeric group was slightly higher (94.17 ± 0.40 , $p < 0.05$) when compared to the control group. The mean sperm viability in low dose turmeric group was slightly lower (91.17 ± 0.95 , $p < 0.05$) when compared to the control group. The mean sperm viability in STZ induced diabetes group

was significantly lower (78.50 ± 2.40 , $p < 0.05$) when compared to the control group. The mean sperm viability in STZ induced diabetes+ high dose of turmeric group was significantly lower (89.00 ± 0.68 , $p < 0.05$) when compared to the control group. The mean sperm viability in STZ induced diabetes+ low dose of turmeric group was significantly lower (84.50 ± 1.55 , $p < 0.05$) when compared to the control group.

DISCUSSION

This study was designed to evaluate the protective role of turmeric on the effect of type 1 diabetes on some reproductive parameters in the male reproduction using adult male Wistar rats.

Type 1 diabetes was established in group four to six by the administration of small multiple doses of STZ. This confirms that STZ is a diabetogenic agent which selectively destroys the insulin producing beta cells of the pancreas which are responsible for the production of insulin. The absence of this would result to hyperglycaemia as seen in this study as there is no means of the blood glucose going into the cells. This findings supports previous report that STZ can be used to induce type 1 diabetes in rats (Arikawe *et al.*, 2012).

Results from this study showed that diabetes could actually be a predisposing factor to male infertility as recorded in the semen analysis. This could have been due to the fact that hyperglycaemia which is seen in diabetes was a consequence of glucose not entering the cells of the testes could have grossly affected the Sertoli and Leydig cells which are to produce testosterone and sperm cells through spermatogenesis, with this the possibilities of developing low testosterone level/hypogonadism becomes very high and hypogonadism would result to a reduction in the rate of spermatogenesis and finally the sperm count, motility and viability. This finding supports previous report of researchers who established a bidirectional relationship between diabetes and hypogonadism, stating that diabetes could

indeed result to infertility in male rats (Dockery *et al.*, 2003; Nishiyama *et al.*, 2005). Another possible mechanism though not explored in this study is the fact that diabetes is chiefly as a consequence of hyperglycemia which has been known to induce oxidative stress through the increase production of reactive oxygen species (ROS), this ROS tends to down regulate the activities of the antioxidant enzymes such as catalase, superoxide dismutase and glutathione, the reduction in this enzymes would lead to a decrease in the capacity and activity of the testis and sperm produced.

Diabetes-induced oxidative-stress has been reported to cause peroxidation of sperm membrane lipid which might interfere with membrane fluidity and transport processes (Sanocka and Kurpisz, 2004). In view of this, appearance of various abnormal sperm shapes could be due to abnormal membrane or cellular and nuclear changes induced by diabetes (Suresh *et al.*, 2013). More studies could be needed to elucidate mechanisms underlying abnormal sperm appearances in diabetes. Treatment with *Curcuma longa* extract prevents the increase in the amount of testis lipid peroxidation oxidation (LPO) in diabetic rats. In both diabetic rats (Nelli *et al.*, 2013) and humans (Karimi *et al.*, 2011), LPO was the major cause of sperm damage. Administration of Turmeric extract to diabetic rats alleviates oxidative stress via several mechanisms which include reduced amount of free radicals such as superoxide and preservation of total antioxidant capacity via main tainting near normal activity level of

endogenous enzymatic/non-enzymatic antioxidant. The later effects may be attributed to higher amount of total phenolic content in the Turmeric extract as revealed by phytochemical analysis. Meanwhile, ability of Turmeric extract to lower lipid profile levels in diabetic rats could also help to reduce the risk of acquiring abnormal sperm morphology and characteristics and sperm oxidative stress (Kanter *et al.*, 2012). Scarano *et al.* (2006) reported that sperm counts in diabetic rats diminished following short-term exposure to hyperglycemia, while Amaral *et al.* (2006) reported that prolonged hyperglycemia in rats adversely affect sperm concentration and motility due to oxidative stress.

Oxidative Stress as experimentally evident in the diabetic group is associated with detrimental effects on male reproductive function. It is empirically established that stress increases

reactive oxygen species (ROS) generation in the male reproductive tract. High ROS levels may be linked to low sperm quality and male infertility. However, it is still not clear if ROS are generated by oxidative stress in the testis.

In conclusion, the present work confirm that the treatment of rats with curcumin in a dose of 25mg/kg body weight led to a good protection against the oxidative effects of diabetogenic agent, STZ while at higher dose of curcumin (50mg/kg body weight) the protective effects was more pronounced.

Therefore, normalization of metabolic functions in diabetic rats treated with Turmeric extract may be partly due to its stimulatory effect on insulin secretion from pancreatic β -cells (confirmed by serum insulin levels).

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