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Changes in Some Reproductive Hormones Levels of Stress Induced Albino Rats Treated with Aqueous Extracts of *Syzygium Aromaticum*

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Abstract: ***The correlation between a stress exposed or a stress induced lifestyle and the etiology of reproductive challenges has come into focus, knowing that exposure to stress can lead to overproduction of reactive oxygen species which is the leading factor in the development of oxidative stress. Syzygiumaromaticum extracts have been discovered to increases sperm count but excessive intake caused damages to the testicular tissues and can reduce the oxidative stress in the body. This study examined changes in some reproductive hormones of stress induced rats exposed toSyzygiumaromaticum. Seven-two (72) albino rats comprising of 36 male and 36 female rats were used for the study. The Seven-two (72)were divided into six groups, consisting of three treatment groups of eight male and female albino rats. And a control group made up of four males and females. The control group was made up of the baseline group, positive control and a negative control group. The positive control received hormones analogs and feeds while the negative control did not receive any treatment apart from feeds. The treatment groups were given different dosages of 100mg/kg, 250mg/kg and 500mg/kg of Syzygiumaromaticum aqueous extract orally for 28days. All the rats were exposed to stress using the Analgesymeter which induced pain except the baseline control group. After the experimental duration, all the rats were sacrificed and blood samples collected and tissues harvested. This was followed by assaying the levels of some reproductive hormones. The result revealed that the reproductive hormones of all the various extract groups of male rats exposed to Syzygium aromaticum extract were significantly higher than the baseline and negative control except for the maximum dosage at p< 0.05). Similar trend was observed for the female rat. This study brought to bear that Syzygium aromaticum extract has the tendency of increasing reproductive hormone at minimal doses, but may retard reproductive hormone at higher doses.***

Keywords: oxidative, stress, reproductive, hormone, Syzygium aromaticum

1. Introduction

Recent advances in medical science involving the use of herbs or plants in therapy because of their vast medicinal value have gained recognition and increased in recent times (Bekalo*et al.*, 2009).

This study has expanded globally thereby creating concerns for the safety, efficacy as well as quality control of herbal medicines and traditional procedure-based therapies for both health authorities and the public, knowing that herbal medicine has several limitations such as poor quality control and standardization.

Current survey from the World Health Organization (WHO) indicated that about 70–80% of the world population rely on non-conventional medicine mainly of herbal sources in their primary healthcare (Saalu, 2016). This concern was also expressed by Amare (2009), who reported that herbal medicine will continue to play a central role in the healthcare system of large proportions of the world's population. This increased demand for herbal medicine can be attributed to several reasons such as, renewed preference or interest of consumers for natural therapies, cost of synthetic drugs, and the belief that herbal medicine is devoid of side effect and it is used for the treatment of certain diseases where conventional medicine fails (Saalu, 2016). Medicinal plants are therefore useful not only in the traditional system of medical care at the local level but are also used in the production of modern medicines.

Plants commonly used in traditional medicine are assumed to be safe and their safety is based on their long usage in the treatment of diseases according to knowledge accumulated over centuries. However, recent scientific research has shown that many plants used as food or in traditional medicine are potentially toxic, mutagenic and carcinogenic. In consideration of whether the benefits exceed the harm, the amount or quantity taken (dose effect) comes into significance (Rhun*et al.*, 2017).

In our today's competitive modern world, a lot of people often go through stress in various aspects of life. Research indicates that more than 80% of people report experiencing stress (Salam & Reetu, 2011). This may include the day to day social, emotional, psychological, financial, physical or even environmental stress due to poor living conditions, poor sanitation or poor power supply.

As stress increases in our lives, it is often accompanied by a number of chronic and debilitating health issues. Thus, as an adaptive response to stress, there is often a change in the serum level of various hormones in the body. These changes may be required for either the fight or flight response of the individual to stress. Long-term exposure to stress may lead to many deleterious consequences leading to various endocrine disorders (Salam &Reetu, 2011).

Stress can be considered as a state of disharmony or threatened homeostasis. Recently, the correlation between a stress exposed or a stress induced lifestyle and the etiology or pathology of reproductive diseases is being investigated knowing that exposure to stress can lead to overproduction of reactive oxygen species which is the leading factor in the development of oxidative stress (Nantia*et al.*, 2016).

Extreme stressis harmful for the body due to the negative effects of free radicals. These free radicals and reactive oxygen species (ROS) play a major role in affecting reproduction process if not controlled. Therefore, evaluation of oxidative stress markers is vital in the treatment of reproductive problems (Adewoyin*et al.*, 2017).

Currently oxidative stress research in the laboratory animals has assumed an important role in the biological and psychological sciences over the past decade due to the view that stressful stimulus may influence the pathogensis and progression of a variety of life-related diseases that includes psychiatric disorders such as depression and anxiety, immune suppression, metabolic disorders including diabetes mellitus, hypertension, endocrine disorders and reproductive problems (Adewoyin*et al.*, 2017).

Syzygiumaromaticum (Clove) has elicited so much interest from agriculturists, pharmacologists, scientists and a whole lot of medical and research personnel as one of the most important herbs in medicine, having a wide spectrum of medicinal activity (Parle and Khanna 2011). It is one of the most valuable spices that has been used as food condiment and for its many therapeutic purposes around the world due to its antioxidant, antibacterial, antifungal, antiviral, anticarcinogenic, antipyretic, anti-inflammatory and analgesic activities. Whole and ground cloves are used to enhance the flavor of meat and rice dishes and used widely in curry powders and masalas as well as in the local Zobo drink. It is highly valued in medicine as a carminative and stimulant and is said to be a natural anti-helmintic. Recently, it has been recognized as an effective anesthetic for sedating fish for a number of invasive and noninvasive fisheries management and research procedures (Khatri*et al.*, 2014). *Syzygiumaromaticum* extracts have been discovered to exhibit potent antioxidant activity against various antioxidant systems in vitro, and can reduce the oxidative stress in the body. It can be used as easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical applications (Gulcin*et al.*, 2004).

Syzygiumaromaticum in particular has attracted much attention recently due to its potent antioxidant activity thereby standing it out among the other spices (Diego *et al.*, 2014). Furthermore, it has been categorized as the champion of all known antioxidants (Parle and Khanna 2011 &Perez-Jimenez *et al.*, 2010).

An overview of the pharmacological activities of *Syzygiumaromaticum* showed that moderate intake increases sperm count but excessive intake caused damages to the testicular tissues (Rhun*et al.*, 2017). Mishra and Singh (2013) proposed that higher doses had adverse effects on sperm dynamics and that the extract might also affect the secretory activities of epididymis and seminal vesicle. However, there seem to be paucity of data on the effect of *Syzygiumaromaticum* on fertility status of stress induced rats thus creates interest owing to review of its rich vegetal source of antioxidant and flavonoids.

This study aims to determine the effect of aqueous extracts of *Syzygium aromaticum* on some reproductive hormones levels in stress-induced male and female albino rats.

2. Materials And Method

2.1 Plant material

The dried flower buds of *Syzygiumaromaticum*spice was bought from market and was identified by a certified botanist.

2.2 Extraction and Processing

Extraction of the aqueous extract of the dried flower buds of *Syzygium aromaticum* spice was done by maceration of 400grams of the dried flower buds in a maceration jar and 1500mls of distilled water was added to it and macerated for 24hours under room temperature. During maceration, it was repeatedly shaken three times daily to enable proper extraction. This was followed by filtration using a white handkerchief first and then with a Whatman filters paper to have a clear filtrate. The filtrate was then allowed to stand for about five hours after which it was carefully poured into an evaporating dish and placed on a water bath for drying at a temperature of 65°C. This process makes it to dry eventually into a powder form. The extract was then keptin a desiccator at room temperature till it was used.

2.3 Experimental Animals

Seven-two (72) albino rats comprising of 36 male and 36 female rats were used for the study. The rats weighed averagely between 150 to 250grams. The rats were acclimatized for 10 days to the environment of the animal house and fed with standard commercial diet and water *ad libitum*. They were maintained at standard conditions of temperature of 25± 2°c until the commencement of the study.

2.4 Study Design

In this experimental study, 72 albino rats comprising of 36 male and 36 femalewere divided into six groups of consisting of three treatment groups of eight male and female albino rats.And a control group made up of four males and females. The control group was made up of the baseline group, positive control and a negative control group. The positive control received hormones analogs and feeds while the negative control did not receive any treatment apart from feeds. The treatment groups were given different dosages of 100mg/kg, 250mg/kg and 500mg/kg of *Syzygiumaromaticum* aqueous extract orally for 28days. Importantly, all the rats were exposed to stress using the Analgesymeter which induced pain except the baseline control group. After the experimental duration, all the rats were sacrificed and blood samples collected andtissues harvested. This was followed by assaying the levels of some reproductive hormones and total antioxidant capacity in both the treated and control animals. Semen analysis was also carried out on the male rats.

2.5 Determination of Hormone and oxidative profile levels

The hormonal assay was determined by enzyme-linked immune sorbent assay technique (ELISA), while the

oxidative variables were determined by standard spectrophotometric procedure. The hormones determined were Follicle stimulating hormone FSH, Luetinizing hormone LH, Testosterone and Progesterone.

3. Results

The Mean and Standard Error of Mean (SEM) of the controls and the treatment groups for reproductive hormone levels in stress induced male albino rats treated with *Syzygiumaromaticum* extract (SAE) are shown in table 4.1. The results show that the hormones of all the various groups are significant in reference to the controls even at minimal dosage administered to the rats at (p-value < 0.05).Turkey's test of multiple comparisons of serum hormone levels (post hocs analysis) in stress induced male albino rats treated with *Syzygium aromaticum* extract, depicted no significant difference in follicle stimulating hormone amongst the baseline, negative, positive control and the 100mg/kg SAE extract. There was significant increase in the 250mg/kg SAE extract, while at 500mg/kg SAE extract there was significant reduction. The various concentration of the extracts depicted significant increase in the luteinizing hormone when compared with the baseline and negative control. However, the concentration of this hormone for the various extract was significantly lower than the positive control (p = 0.001, F= 16.53). The testosterone levels for the various extracts were significantly higher than the baseline, negative and positive control (p=0.002, F= 22.24).

Table 4.1: Multiple comparisons of mean serum hormone levels in stress induced male albino rats treated with *syzygium aromaticum* extract

Groups	FSH(IU/L)	LH(IU/L)	Testosterone (ng/ml)
Baseline Control	0.20 ± 0.01	0.55 ± 0.02	0.35 ± 0.02
Negative control	0.21± 0.02	0.62 ± 0.02#	0.37 ± 0.01
Positive control	0.23 ± 0.01	0.93± 0.01*	0.92 ± 0.02#
100mg/kg SAE extract	0.23± 0.02	0.65± 0.02 #	2.07 ± 0.02* #
250mg/kg SAE extract	0.27 ± 0.02*	0.76± 0.01* #	2.18 ± 0.02* #
500mg/kg SAE extract	0.19± 0.01	0.73± 0.01* #	1.19 ± 0.02*
F-Value	3.22	16.53	22.24
p-value	0.03	0.00	0.00

Key: FSH =Follicle Stimulating Hormone, LH = Luteinizing Hormone, SAE= *Syzygium aromaticum* extract.

The Mean and Standard Error of Mean (SEM) of the controls and the treatment groups for reproductive hormone levels in stress induced female albino rats treated with *Syzygiumaromaticum* extract (SAE) are shown in table 4.2. Values are presented in mean ± standard error of mean (sem). * means values are statistically significant when compared to the Baseline and negative control group. # means values are statistically significant when compared with the Baseline and positive control group. The table shows that the negative control and positive control of FSH administered to the subjects orally are significantly higher than the baseline, negative and positive controls at p-value < 0.05. Similarly, the same presentation was observed for LH and Progesterone administered to the subjects as well as the different doses of *Syzygium aromaticum* extract at p-value < 0.05 were significant.

Table 4.2: Multiple comparisons of serum hormone levels in stress induced female albino rats treated with Syzygium aromaticum extract

Groups	FSH(IU/L)	LH(IU/L)	Progesterone (ng/ml)
Baseline Control	0.20 ± 0.02	0.50 ± 0.02	10.0 ± 0.02
Negative control	0.22± 0.01	0.66± 0.01	12.22± 0.02*
Positive control	0.15± 0.02	0.33 ± 0.02	18.95± 0.02*
100mg/kg SAE extract	0.28± 0.02 #	0.67± 0.01 #	13.41± 0.02* #
250mg/kg SAE extract	0.25± 0.01 #	0.84± 0.01 #	9.91± 0.02* #
500mg/kg SAE extract	0.37± 0.01* #	1.02± 0.02* #	5.42± 0.02* #
F-value	9.85	13.69	345.92
p-value	0.00	0.00	0.00

Key: FSH =Follicle Stimulating Hormone, LH = Luteinizing Hormone, SAE= *Syzygium aromaticum* extract.

4. Discussion

In this study, the reproductive hormones of all the various groups of male rats exposed to *Syzygium aromaticum* extract were significantly higher than the baseline and negative control except for the maximum dosage. Similar trend was observed for the female rat. This finding is in tandem with the report of Negendra *et al.,* (2014). They posited that *Syzygium aromaticum* extract as an aphrodisiac should have the potential to arouse sexual desire, increase spermatogenesis, increase fertility hormones level, treat infertility and enhance sexual performance. Conversely, *Syzygium aromaticum* Mishra and Singh (2016), and Saalu (2016) in separate research reports presented as a plant extract culpable of inducing infertility effect in mouse in chronic oral high dose exposure. However, the mechanism in which the extract exerts these effects is not known. Earlier, Dehghani *et al.,* (2012) in their evaluation of the toxic effects of water/alcoholic extract of *Syzygium aromaticum* on sperm quality, sex hormones and reproductive tissues in male mouse indicated that the high dose-treated animals showed significant decline in sperm count, motility and testosterone levels but a significant increase in estradiol concentration when compared with the control group. This report was similar to that of Mishra and Singh (2008) on the antifertility potential of *Syzygium aromaticum* at high dose. Gulcin (2011), thus said that although *Syzygium aromaticum* extract is rich in antioxidant that should help reduce lipid peroxidation and boost spermatogenesis, it is however inhibitory to spermatogenesis or spermicidal at high dosage.

Yakubu *et al.,* (2011) however presented a contrary report on similar study of the same doses of *Syzygium aromaticum* extract on Rats for a shorter period of time. They observed that groups with doses of 500 and 1000mg/kg showed increased serum testosterone concentration.

Sequel to the findings in this study, it seems that*Syzygium aromaticum*to at high dose lowers reproductive hormones level in both male and female rats.

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