EFFECT OF AQUEOUS AND N-HEXANE EXTRACT OF NIGELLA SATIVA ON PREGNANCY HORMONES IN WISTAR RATS

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ABSTRACT

Nigella sativa (N.sativa) is generally called black seed. Regarding many recommendations of N. sativa for prevention and remedy of many diseases including anti-fertility, the present study was aimed at investigating the effect of N. sativa extracts on some pregnancy hormones in Wistar rats. The effect of aqueous and n-hexane extract of N. sativa on levels of progesterone and prolactin hormones in pregnant and non-pregnant rats was determined. Thirty-six female Wistar rats were used, eighteen pregnant and eighteen non-pregnant. They were divided into six groups (n=6): Non-pregnant Control; Non-pregnant Aqueous; Non-pregnant N-hexane; Pregnant Control; Pregnant Aqueous and Pregnant N-hexane. Aqueous extract of N. sativa at dose of 200mg/kg b.w., and N-hexane Extract of N. sativa at 50mg/kg were administered orally to respective groups of non-pregnant and pregnant Wistar rats for three weeks. Elisa kits were used to estimate the serum levels of progesterone and prolactin hormones. The serum levels of progesterone and prolactin were increased in the pregnant control groups compared with non-pregnant control group (p<0.05). Aqueous and n-hexane extract of N. sativa decreased progesterone and prolactin levels in the pregnant and non-pregnant groups (p<0.05) but the decrease levels of both hormones were more evident with aqueous than N-hexane extract (p<0.05). In conclusion aqueous and n-hexane extract N. sativa decreases progesterone and prolactin levels in pregnant and non-pregnant Wistar rats.

Keywords: Nigella sativa, Progesterone, Prolactin, Pregnancy

INTRODUCTION

Nigella sativa L. seed is commonly called black seed, studies have ascertain it’s numerous traditional claims on many diseases (Tembhurne1 et al., 2014). The major active phytochemical property of N. sativa has been identified as thymoquinone (TQ), which is present in the volatile oil fraction of the plant (Toma et al., 2015).

Scientific research studies have reported the pharmacological action of N. sativa as anti-cancer (Rooney and Ryan, 2005); anti-diabetic (Mohammed et al., 2009) and Hepatoprotective (Michel et al., 2010). Also, the fertility and anti-fertility effect of N. sativa has been reported (Parandin et al., 2012; Jasima et al., 2016).

Progesterone is a multifunctional female sex
hormone that promotes the development of mammary glands, ovulation, implantation of embryos, and maintenance of pregnancy (Dorniak and Spencer, 2013). The production of progesterone to maintain pregnancy, require first the release of prolactin from the anterior pituitary gland, which increases leutinizing hormone (LH) receptors on luteal cells to form corpus luteum and the enzymatic inhibition of progesterone conversion (Hamid and Zakaria, 2013). Other pregnancy hormones such as Human Chorionic Gonadotropin Hormone (hCG) stimulate the production of oestrogen and progesterone within the ovary but its production diminishes once the placenta take over production of oestrogen and progesterone (Spencer and Bazer, 2002). There is paucity of literature on the effect of N. sativa on pregnancy and pregnancy hormones. This study was therefore designed to investigate the effect of aqueous and n-hexane extract of N. sativa on progesterone and prolactin hormones in pregnant and non-pregnant Wistar rats.

MATERIAL AND METHODS

Chemicals and Reagents
Progesterone ELISA Kit (Cat# 2077-18), Prolactin ELISA Kit (Cat# 4226-16) Diagnostic Automation/cotez Diagnostic,inc California USA.

Plant Materials
Nigella sativa seeds (imported from Saudi Arabia) were purchased from herbal store at no. 25 Old Jobs Road, Tudun Wada Zaria, Kaduna Nigeria. The seed with voucher specimen no. 28092 was identified and authenticated by Namadi Sanusi in the herbarium unit of the Department of Biological science in faculty of science Ahmadu Bello University Zaria, Kaduna, Nigeria.

Aqueous and N-Hexane Extraction of N. Sativa
The N. sativa seeds were cleaned under running tap water for 10 min, they were rinsed twice with distilled water and air-dried in an oven at 40 °C overnight then grounded into powder form.

A manual screw oil-expelling machine was used for the aqueous extraction of N. sativa through cool pressing method. About 150ml of N. sativa oil was extracted from 900g of the grounded powder form of N. sativa seeds. Concentration of 200mg was made with Tween 80%, which was used in the experiment.

The N. sativa seed powder weighing 150g to 1liter of n-hexane was used repeatedly for 5 rounds for the n-hexane extraction of N. sativa using the soxhlet apparatus. After the extraction, the extract was placed in water bath at temperature of 45 °C to concentrate the oil and evaporate the solvent. Concentration of 50mg was made with Tween 80%.

Animals
Thirty-six female Wistar rats weighing between 200 and 250g aged 8weeks were supplied by animal house of Faculty of Medicine, Ahmadu Bello University Zaria, Kaduna, Nigeria. Animals were allowed to acclimatize for 1week prior to experiment.

Determination of Estrous Circle and Impregnating Animals
The estrous cycle was determined by collection of vaginal smear from female Wistar Rats as described by Marcondes et al. (2002). And mating was done on twenty female rats at ratio 1:2 male/female rats. Pregnancy was predicted by the presence of vaginal plug that indicates the occurrence of mating (Hamid and Zakaria, 2013). A total of 18 female rats were impregnated.
Experimental Design

Thirty-six female Wistar rats (18-pregnant and 18-non-pregnant) were divided into six groups (n= 6); designated AENS: Aqueous extract of *N. sativa*, N: non.

Group 1. N-pregnant Control was given Tween 80% (0.1ml/rat) orally for 3 weeks.

Group 2. N-pregnant aqueous was given Aqueous Extract of *N. sativa* (200mg/kg) orally for 3 weeks.

Group 3. N-pregnant n-hexane was given N-hexane Extract of *N. sativa* (50mg/kg) orally for 3 weeks.

Group 4. Pregnant Control was given Tween 80% (0.1ml/rat) orally for 3 weeks.

Group 5. Pregnant AENS was given Aqueous Extract of *N. sativa* (200mg/kg) orally for 3 weeks.

Group 6. Pregnant n-hexane was given N-hexane Extract of *N. sativa* (50mg/kg) orally for 3 weeks.

Sample Collection

Blood samples were collected via retro-orbital puncture (from optic vein) in sterile tubes without anticoagulant. The blood was left to clot at room temperature and then centrifuged; the serum was separated and used for biochemical assays.

Evaluation of Serum Progesterone And Prolactin.

Serum levels of progesterone and prolactin were measured using enzyme-linked immunoassay kits (Catalogue no. 2077-18, Catalogue no. 4226-16) respectively, according to the manufacturer’s description.

RESULTS

The serum level of progesterone significantly (p< 0.05) increased in the pregnant control group compared to non-pregnant control (Table 1). Treatment with aqueous and n-hexane extract of *N. sativa* in non-pregnant rats significantly (p< 0.05) decreased progesterone level compared to non-pregnant control, and the decrease was significantly lower with the aqueous extract than the n-hexane (p< 0.05). Also, treatment with aqueous and n-hexane extracts of *N. sativa* in pregnant rats significantly (p< 0.05) decreases progesterone level compared to pregnant control, but there was no significant difference between the two extract in treated pregnant rat (p>0.05) (figure 1).

Table 1. The Effect of Aqueous or N-hexane Extract of *Nigella sativa* on Progesterone level in Pregnant and Non-pregnant Wistar Rats

<table>
<thead>
<tr>
<th>GROUP</th>
<th>PROGESTERONE (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N- Pregnant Control</td>
<td>3.42 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>N- Pregnant AENS</td>
<td>1.86 ± 1.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>N- Pregnant n-hexane</td>
<td>2.50 ± 0.15&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pregnant Control</td>
<td>3.65 ± 0.09&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pregnant AENS</td>
<td>3.30 ± 0.24&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pregnant n-Hexane</td>
<td>3.20 ± 0.17&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data presented as mean ± SEM, (n=6), values with different superscript are significant (p≤ 0.05); AENS: Aqueous extract of *N. sativa*, N: non.
The serum prolactin level significantly (p< 0.05) increased in pregnant control group compared to non-pregnant control (Table 2). Treatment with aqueous and n-hexane extract of *N. sativa* in non-pregnant group significantly (p< 0.05) decreased prolactin level compared to non-pregnant control, there was no significant difference within the treated groups. Treatment with aqueous and n-hexane extract in pregnant rats significantly (p< 0.05) decreased prolactin level compared to pregnant control. However, the decrease was more with the aqueous group than the n-hexane extracts treated group (p< 0.05) (Table 2).

**Table 2. The Effects of Aqueous or N-Hexane Extract of Nigella sativa on Prolactin level in Pregnant and Non-pregnant Wistar Rats**

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>PROLACTIN (mg/ml)</th>
</tr>
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<tbody>
<tr>
<td>N- Pregnant Control</td>
<td>1.10 ± 0.35a</td>
</tr>
<tr>
<td>N- Pregnant AENS</td>
<td>0.63 ± 0.37b</td>
</tr>
<tr>
<td>N- Pregnant n-Hexane</td>
<td>0.56 ± 0.50b</td>
</tr>
<tr>
<td>Pregnant Control</td>
<td>1.83 ± 0.21ab</td>
</tr>
<tr>
<td>Pregnant AENS</td>
<td>0.25 ± 0.45c</td>
</tr>
<tr>
<td>Pregnant N-Hexane</td>
<td>0.75 ± 0.47b</td>
</tr>
</tbody>
</table>

Data presented as mean ± SEM, (n=6), values with different superscript are significant (p≤ 0.05); AENS: Aqueous extract of *Nigella sativa*, N:non.

**DISCUSSION**

Pregnancy is the gestational period, which involves hormonal changes from conception to birth. Hormonal disorder during pregnancy can influence the outcome of pregnancy and fetal development. Aqueous and n-hexane extract of *Nigella sativa* in the present study indicated a significant decrease in the level of progesterone in pregnant and non-pregnant Wistar rats. The decreased progesterone level by *N. sativa* in the non-pregnant rats in this study was also reported by Ghalandari et al. (2012). Decrease in progesterone level can leads to infertility while, during pregnancy may leads to poor fetal development and its subsequent miscarriage (Karamardian et al., 2011). An active constituent of *N. sativa*, linoleic acid has been reported to decrease estrogen level, which can in turn, lowers the progesterone level (Khadii, et al., 2009). On the contrary, recent study by Parhizkar et al. (2016) showed that *N. sativa* at a low dose induced more estrogenic- like effects. Therefore it can be deduced that *N. sativa* has a variable effect on the serum level of progesterone hormone and the dosage as well as duration of *N. sativa* cannot be disregarded.

In this study there was a decrease level of prolactin hormone by aqueous or n-hexane extract *N. Sativa* in both the non-pregnant as well as the pregnant rats. This study is contrary to reports of Al-Snafi (2014) that indicated an increase in serum prolactin level with *N. sativa* containing diet in lactating mice, because assessment of prolactin level in their study and many others, are on lactating animals, it cannot absolutely be comparable to pregnant and non-pregnant animals as in the case of the present study. Prolactin is responsible for maintaining and regulates the secretory activity of corpus luteum (Bharj, et al., 2011). Decrease prolactin may result in inadequate endometrial maturation and subsequent low production of progesterone by the corpus luteum, as observed in the present study. Therefore it can be suggested that the decreased progesterone level by aqueous or n-hexane extract of *N. Sativa* in the present study might be associated with factors that affect endometrial development, which may set the stage for low progesterone level.
In conclusion aqueous or n-Hexane extract of *N. Sativa* causes decrease serum progesterone and prolactin hormones in pregnant and non-pregnant Wistar rats. So, should be used with caution during pregnancy.

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**REFERENCES.**


