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KEY WORDS: Letrozole, Polycystic ovary syndrome, hyperandrogenism, insulin resistance, fish oil (Sardine oil), shell fish oil (Krill oil) and metformin.

COMPARATIVE STUDY OF HORMONE ANALYSIS IN PCOS INDUCED RAT AND TREATED WITH FISH OIL, SHELL FISH OIL AND METFORMIN

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ABSTRACT

Polycystic ovary syndrome (PCOS) is a very common endocrine disorder among women in their reproductive age, characterized by polycystic ovary, hyperandrogenism, insulin resistance and menstrual irregularity. The present study was undertaken to determine the effect of sardine oil and krill oil in the treatment of letrozole induced PCOS in Wistar albino female rats on Follicle Stimulating Hormone, Luteinizing Hormone, Thyroid Stimulating Hormone. Estradiol, Progesterone and Testosterone. The rats were induced PCOS with letrozole for 21 days and treated with fish oil, shell fish oil and Metformin for 30 days and 60 days. The results showed that the hormone levels altered after the treatment. Hence, it may be concluded that the hormonal fluctuation caused in PCOS was brought to nearly normal levels by Omega-3 fatty acid present in the sardine oil and krill oil thus showing its potential effect and an alternative to metformin in treating PCOS.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a very common endocrine pathology affecting 4-10%young women in their reproductive age (Carmina E et.al 1999, Teede H et.al, 2010). Polycystic ovary syndrome is associated with reproductive and metabolic disorders which includes infertility, hyperandrogenism, luteinizing hormone (LH) hypersecretion, low levels of Estradiol and Progesterone, polycystic ovaries, insulin resistance and dyslipidaemia, with an increased risk of cardiovascular disease and diabetes mellitus. (Strowitzki T et.al, 2010; Rajasekaran S. et.al2005). Women with PCOS may have underactive thyroid gland, causing hypothyr oid ism (Archana Shirsath, et.al, 2015). Often the treatment of PCOS includes the use of Metformin drug, but its prolong use causes many side effects. Therefore, alternative therapy to treat and manage PCOS is essential. Earlier workers have reported that fish oil improves several disorders associated with PCOS (M. Luisa Vargas, et.al, 2001).

Fish oil contains high amount of Omega-3 polyunsaturated fatty acids (n-3 PUFA) which contains long-chain n-3 polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which are the two most important omega-3 fatty acids for human health and are considered as the essential fatty acids necessary to maintain good health but cannot be synthesized by the human body (Yanli Yang 2012).

The present investigation was undertaken to study the effect of fish oil (Sardine oil) and shell fish oil (Krill oil) in the treatment of PCOS and to compare the much-preferred Metformin drug.

MATERIALS AND METHOD

Sixty Wistar albino female rats weighing 150g-200g were procured from Haffkine Biopharmaceuticals limited, Parel, Mumbai. All the animals were weighed and their health was verified. They were divided into 5 groups, each comprising of 6 rats. Animals were acclimatized for 7 days under laboratory conditions prior to initiation of study. They were housed in well-ventilated cages under standard environmental conditions (25 ± 2°C, 45 - 55% relative humidity, and 12 h dark/light cycle and were fed with Ragi pellets. Animals were assigned to cages and groups, two or three per cage and the individual animal was fur marked. The females were nulliparous and non-pregnant.

EXPERIMENTAL PROTOCOL

Oral dose of Letrozole was given for inducing PCOS in experimental animals, the dose was made at the concentration of 1.0 mg/kg body weight dissolved in 1% Carboxylmethyl Cellulose (CMC), 2.0 ml/kg of dose was administered once daily for a period of 21 days (Kafali, et al., 2004) and during the experimental period the vaginal smear was collected daily to study the estrous cycle. After inducing PCOS in rats for 21 days the animals were treated with Sardine oil and Krill oil, 240 mg /kg/orally/daily (Ouladsahebmadarek E. et al.,2014) for 30 days and 60 days respectively and one group was treated with Metformin drug for comparative studies.

Group I: Normal- administered with 2.0 ml/kg of1% Carboxylmethyl Cellulose (CMC)
Group II: Control-Letrozole Induced PCO without treatment (control)
Group III: Letrozole Induced PCO and treated with Sardine Oil at the dose levels of 240 mg /kg/orally/daily for 30 days and 60 days.
Group IV: Letrozole Induced PCO and treated with Krill Oil at the dose level 240 mg /kg/orally/daily for 30 days and 60 days.
Group V: Letrozole Induced PCO and treated with Metformin 2 mg/100g body weight for 30 days and 60 days.

At the end of 30 days and 60 days of treatment respectively, blood samples of the rats were collected by cardiac puncture from all the groups in EDTA containing vials which were centrifuged at 3000 rpm for 15 minutes and the plasma was separated for hormonal assay. Plasma estradiol, progesterone, testosterone, Thyroid Stimulating Hormone (TSH), Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) were analyzed using the commercially available kits.

Statistical Analysis:
One-way analysis of variance (ANOVA) and statistical assessment of result was carried out using SSPS software 16 version.

RESULT

In letrozole induced PCO rats, levels of FSH, LH and Testosterone showed an increase but after the treatment with fish oil and shell fish oil, their levels decreased. Whereas Estrogen Progesterone and TSH levels were observed to be low, which was elevated after the treatment with fish oil. Similar results were observed in rats treated with Metformin. The animals treated for prolonged period of 60 days showed better results as compared to 30 days. Figure 1, 2, 3, 4, 5 and 6 shows the hormone levels after the treatment for 30 days and 60 days.

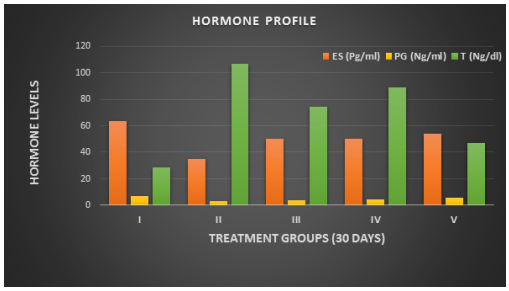

Figure1: Effect of fish and shell fish oil on Estradiol (ES), Progesterone (Pg) and Testosterone (T) along with group –V treated with Metformin treated for 30 days.

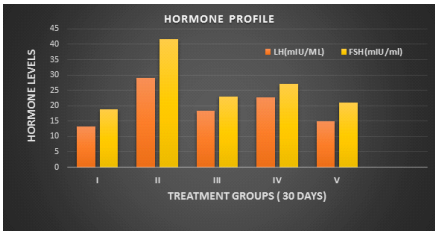

Figure 2: Effect of fish and shell fish oil on Follicle stimulating Hormone (FSH) and Luteinizing Hormone (LH) along with group –V treated with Metformin treated for 30 days.

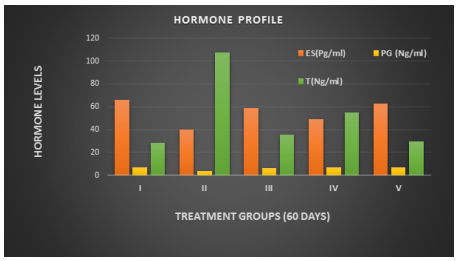

Figure 3: Effect of fish and shell fish oil on Estradiol (ES), Progesterone (Pg) and Testosterone (T) along with group –V treated with Metformin treated for 60 days.

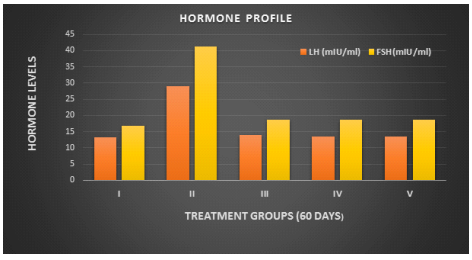

Figure 4: Effect of fish and shell fish oil on Follicle stimulating Hormone (FSH) and Luteinizing Hormone (LH) along with group –V treated with Metformin treated for 60 days

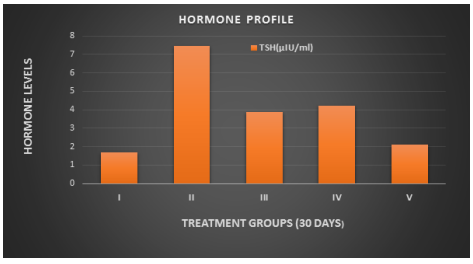

Figure 5: Indicates the effect of fish and shell fish oil on Thyroid Stimulating Hormone (TSH) along with group –V treated with Metformin treated for 60 days.

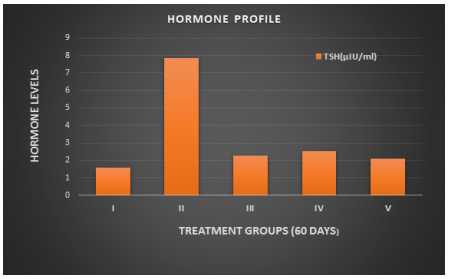

Figure 6: Indicates the effect of fish and shell fish oil on Thyroid Stimulating Hormone (TSH) along with group –V treated with Metformin treated for 60 days

DISCUSSION

PCOS has many clinical manifestations though its etiology still remains uncertain. (Riitta Koivunen,2001) In recent times ,allopathic medicines are used to treat PCOS of which Metformin is more recommended and its prolonged use causes enormous side effect. The need for better alternative is needed and therefore, in the present study fish (Sardine oil) and shell fish oil (Krill oil) was used for the treatment of PCOS. Earlier studies (Desai *et al.*, 2012; Jadhav *et al.*, 2013) reported that in nearly 80% of PCOS patients, the testosterone levels increase. Similar results were observed in the present study where both Testosterone and LH levels increased causing Hyperandrogenism, key feature of PCOS. Estrogen levels were found to be reduced in PCO induced group which results in increasing levels of LH by weakening the negative feedback mechanism on the LH production in the pituitary gland, which in turn further stimulates theca cells to secrete testosterone (Kafali *et al.*, 2004). The elevated levels of testosterone and LH decreased when treated with metformin. and it lowered the levels of androgens. Treatment with fish oil and shell fish oil showed similar result and marked improvement was observed in the animals treated for 60 days. Levels of testosterone and LH reduced to nearly normal levels. Higher levels of FSH was observed which is caused due to impairment of ovarian folliculogenesis, after the treatment with fish oil and shell fish oil the hormone levels decreased. Group-III which was treated with Sardine oil exhibited better results as compared to Krill oil. It could be said that the Omega-3 brought about the anti- androgenic effect and helped in normalizing the alteration of hormones in induced rats. The present study may provide a baseline data for further investigation on therapeutic benefits of fish oil and shell fish oil in management of PCOS.

CONCLUSION

Owing to the ever-increasing incidences of PCOS in the population during their reproductive age is one of the most common endocrine disorder. There is a need to find out an alternative therapeutic agent instead of the drug which are prescribed for the treatment of PCOS which after prolonged use results in various side effects. It may be concluded that fish oil and shell fish oil may be used as an alternative to drugs for treating PCOS.

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