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Ameliorating Effect of *Tinospora Cordifolia* Leaf Extract Loaded Phytoniosome on Mifepristone Induced PCOS Rats – A Biochemical Study

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Abstract: The most common endocrinological reproductive disorder in women is Polycystic Ovarian Syndrome (PCOS).The herb*Tinospora cordifolia*, (Menispermaceae) is a famous medicinal plant which hashypoglycaemic effect.Niosomes are considered as novel drug carriers in drug delivery systems with bilayer assembly made up of self-assembled structures of cholesterol and non-ionic surfactants using aqueous phase. Thisstudy is performed to assess the ameliorative effect of ethanolic leaf extract of*Tinospora cordifolia*(TCELE) and ethanolic leaf extract of*Tinospora cordifolia* loaded phytoniosome (pnTCELE) on PCOS in mifepristone induced female rats. For a period of 8 days PCOS was induced by administrating of mifepristone daily to the female rats which was confirmedby persistent estrous cycle. 200mg /kg b. w of TCELE and pnTCELE extracts were given to the rats which attained PCOS condition for 28 days.Weight of the reproductive organs and the body weight were analysed after treatment along with estimation of biochemical parameters. Metformin was used as positive control.The body weight and weight of the reproductive organs were increased significantly. Additionally significant dyslipidaemiaand hyperglycaemiawere seen in PCOS rats which were normalized in pnTCELE drug treated animals and TCELE drug treated animals.Thus, pnTCELE can be effectively used for the supervision of PCOS which requires a control of endocrine and metabolic complication.

Keywords: Hyperglycemia, Phytoniosomes, Dyslipidemia, PCOS, *Tinospora cordifolia*

1. Introduction:

The most common endocrinological disorder Polycystic Ovarian Syndrome (PCOS) is the leading reproductive problems among women. In spent of being a “lifestyle” disease, PCOS has establishedlittle attention among women and gynaecologist because it has no known cure thus becoming a principalsource of female infertility worldwide(1). The metabolic issues associated with PCOS include obesity, dyslipidemia, type II diabetes mellitus, insulin resistance and hyperinsulinemia.Other problems associated with PCOS are cardiovascular problems, psychological effects like depression and anxiety as well as neurological and cancers in the regions of breast and endometrium. Therefore, PCOS is regarded a systemic disease of chronic nature, having increasedoxidative stress leading severe inflammation(2). The treatment and management of PCOS varies in different individuals which are mainly based on the symptoms in the patient. Usually, a multiple approach treatment such as weight reduction, ovulation induction and treatment of menstrual dysfunction is followed through lifestyle modification. But for the chronic management of PCOS the above-mentioned treatments are less helpful and hence chemical drugs in the form of synthetic hormones such as progestin as well as insulin sensitizing agents in the form of metformin are suggested. Ovulation induction therapy involves using drugs like letrozole, clomiphene or gonadotropin therapy are widely used for the treatment of infertility(3).

A powerful anti- inflammatory herb*Tinospora cordifolia*, (Menispermaceae) is a famous medicinal plant which has hypoglycaemic effect. Insulin imbalance and ovarian cysts occurs due to chronic inflammation in tissues. It aids in boosting a metabolism, revitalizing all the body tissues and lowering insulin resistance naturally(4). Traditionally it has been used as an anti-inflammatory, anti-

stress, anti-spasmodicand also used to treat urinary tract infection, seminal weakness, fever, jaundice, skin diseases and diabetes. Glycosides, diterpenoids, alkaloids, sesquiterpenoid, lactones, phenolics, steroids, polysaccharides and aliphatic compounds are the active ingredients present(5).

Niosome–are the novel carriers of drug made up of bilayer structure, containing surfactants of non - ionic nature and cholesterol formed by self- association in aqueous phase.It is multilamellar vesicles or unilamellar formed from synthetic, non-ionic surfactants of the alkyl which offers a substitute to liposomes as drug carriers(6).Niosomes are nonimmunogenic,has long shelf life biocompatible,biodegradable and exhibits high stability which allows the target site drug delivery with required sustainability. The phytosome technology effectively encapsulates herbal extracts and phytochemicals which shows notable results both in-vitro and in-vivo pharmacokinetic studies(7). An attempt has been made to evaluate the ameliorative effect of (*Tinospora cordifolia* ethanolic leaf extract) TCELEand phytoniosomeloaded(*Tinospora cordifolia*ethanolic leaf extract)pnTCELE on PCOS in female rats induced by a antiprogesterone drug.

2. Methodology:

Chemicals:

Mifepristone procured from Merck Milliporewas used for assessment of biochemical parameters.Ranbaxy kits were used to carry out the biochemical tests. Analytical grade compounds were utilized in the current investigation.

Tinospora Cordifolia leaf extraction using ethanol (TCELE):

Tinospora cordifolia leaves were acquired fromCuddalore, Tamil Nadu.Dr. Mythreyi, Professor, K.K. College of Pharmacy's Pharmacognosy Department in Chennai, India identified and verified the leaves and the same was kept for reference as specimen voucher.The extraction of leaves was done using 90% ethanol, using cold maceration and stored in refrigerator until they were used. Rota flash evaporator was used to evaporate the solvent. The yield of samplewas1.25 %w/w.

Ethanolic extractionof Loaded Phytoniosomeof *Tinospora Cordifolia*leaves (nTCELE):

Thephytoniosome loaded ethanolic leaf extract of*Tinospora cordifolia*(pnTCELE) was produced using film hydration method(8).Tween 60 and cholesterol were mixed with chloroform in the ratio 1:1M, followed by addition of 1.0 mg/ml of *Tinospora cordifolia* ethanolic leaf extract. To get a thin film of pnTCELE, removal of extra chloroform was done by using rotary evaporator at 55ºC. This was hydrated using Phosphate Saline buffer followed by leaving it for 2hrs in water bath (55ºC). To prepare finer vesicles, the final solution was kept in bath sonicator for 20 min. Dialysis was done overnight to separate the phytoniosomes from untrapped elements.

Animals:

190-200 gm Adult Wistar strain female albino rats which are aged 12 weeks were used for the pharmacological studies. They were kept in rooms with good ventilation in a proper day-night cycle. A wholesome diet for rodents were provided in form of pellets and tap water was accessiblethroughout the duration of the experiment. Before the trial, the rats were kept in the lab for a week to get acquainted to their surrounding. The rats were housed in the lab for a week to acclimatize them prior to the trial. During a meeting at Saveetha Dental College and Hospitals in Chennai, the Institutional Animal Ethics Committee gave the project their clearance number BRULAC/SDCH/SIMATS/IAEC/3-2021/065.

In vivo Studies onToxicity:

The toxicity studies for *Tinospora cordifolia*ethanolic leaf extract were conducted previously (9) as per guidelines issued by the (Organization for Economic Co-operation and Development) OECD, and it was reported that there was no mortality upto the test dose of 2000mg/kg. 200 mg/kg of the extract, which is one-tenth of LD_{50} dose, was selected for the current investigation to ascertain the effectiveness of PCOS treatment.

PCOS induction in female ratsby Mifepristone:

Wistar female rats were administered with Mifepristone (4mg/ 0.2 ml oil) of RU486 grade, commencing from their estrous cycle for eight days straight (10). Following the injection of Mifepristone, a vaginal smear test was performed every day to track and detect any irregularities or changes in the estrous cycles. PVC (Persistent Vaginal Cornification), a sign for the growth of follicular cysts when attained, the animals were grouped for the therapy.

Treatment Methods and Animal Grouping:

Twenty female adult rats, of five groups each with four rats were randomly selected. The course of treatment was done for 28 days.

Group I – Controlrats

Group II – Negative control (Mifepristone 4mg/kg b.w treated)

Group III – Treatment of PCOD induced rats with 200 mg/kg b.w. of TCELE

Group IV – Treatment of PCOD induced rats with 200 mg/kg b.w. of pnTCELE

Group V – Positive control (Metformin20 mg/kg b.w. treated)

Collection of Organs and blood samples:

Following the final pnTCELE dosage, the animals were slaughtered under moderate anesthesia. The dissection of ovaries and ovaries were dissected with connective tissues, blood and fats being removed. The reproductive organs weight was calculated instantly and using the retro-orbital puncture method the blood samples were also collected. The blood samples were collected eppondorf tubes with and without anticoagulant. For biochemicalassay to collect the serumblood samples were subjected to centrifugation at 3000rpm for the period of 10 min.The storage of obtained serum samples were done at -20℃.

Estimation of Biochemical Parameters:

Commercial kits from Ranbaxy India Ltd were used for determining the biochemical parameters. It includes serum glucose, total protein, urea, creatinine, total cholesterol, HDL, LDL, VLDL and triglycerides. Cyanmethemoglobin method was used for doing haemoglobin estimation.

Statistical Analysis:

Mean±SE is used to characterize the values. Dunnet's "t" test was employed after the statistical analysis was completed using the ANOVA approach. The p value <0.05 is significant.

3. Results:

Vaginal Smear for Estrous cycle:

The changes in the estrous cycle were completely regular and constant for group 1(control) rats indicating a normal estrous cycle. But the rats induced with PCOS completely showed a disrupted estrous cycle and mostly all the rats remained in the diestorus stage for prolonged period. The rats from Group III and Group IVshowed improvement in the estrous cyclicity. The (Group IV) pnTCELE treated rats restored the estrous cycle activity completely which was induced by mifepristone. The metformin treated animals of Group Vexhibitthediestorus phase.

Effect ofTCELEand pnTCELE on Reproductive Organsand Body Weight:

After 28 days of study, the Group II rats restored more body weight, uterus weight and ovary weight in comparison to the Group I rats. The TCELE and pnTCELE extract decreased the body weight($p<0.01$) when compared to Group II rats. The uterus weight of Group III and Group IV rats ($p<0.01$) also decreased significantly in accordance with the Group II induced animals and similar changes were seen for the weight of the ovaries ($p<0.01$). In the present investigation the significant reduction in the reproductive organ and body weight was observed in the rats which received pnTCELE drug treatment.Table 1 shows that the Group III and Group IVrats showed remarkable decrease when related to the metformin (positive control).

Table1: Effect of TCELE and pnTCELE extracts on reproductive organs weight and body weight in rats

Treatment groups	Body weight (gms)	Ovary weight (mgs)	Uterus weight (mgs)
GroupI – Control	105.12 ± 3.01a**	0.134 ± 0.10a**	0.200 ±1.34a**
Group II – Mifepristone4mg/kg b.w treated	236.5 ± 1.91	0.163 ± 0.16	0.174 ± 1.12
GroupIII – TCELE200mg/kg b.w treated	173.82 ±2.34 b**	0.150 ± 0.23b**	0.198 ± 1.91b**
GroupIV – pnTCELE200mg/kg b.w treated	164.54± 3.40c **	0.131 ± 2.20c**	0.174 ± 2.40c**
GroupV – Metformin20 mg/kg b.w treated	129.1 ± 0.14d**	0.187 ± 0.94d**	0.189 ±1.13d**

For each set of four animals, the values are presented in mean ± SEM form. Dunnet's 't" test was employed after the statistical significance was determined using the ANOVA approach. Comparisons were made between Group I and Group II (a), Group II and Group III (b), Group II and Group IV (c), and Group II and Group V (d). **p<0.01, *p<0.05, and ***p<0.001 are significant p values. NS: Not Significant

Estimation of Biochemical Parameters:

Table 2 shows the levels of biochemical parameters like creatinine, haemoglobin, total protein, glucose and urea with TCELE and pnTCELE extracts on rats induced with PCOS. The Group II rats exhibitednoteworthy increase in the level of glucose when related to the Group I (control) rats.Reduction in the level of glucose in blood ($p<0.01$) was seen in the animals treated with TCELE and pnTCELE (Group III and Group IV), which proves the hypoglycaemic activity of the plant. No significant variation was seen for other biochemical parameters like creatinine, totalproteins, urea and haemoglobin.

Table2: Effect of TCELE and pnTCELE extracts on Biochemical parameters in rats

Treatment groups	Hemoglobin (g/dl)	Glucose (mg/dl)	Total protein (g/dl)	Urea (mg/dl)	Creatinine (mg/dl)
GroupI – Control	12.99 ± 1.30a**	76.00±**3**.29a **	6.45± 0.37a ns	32.47 ± 1.33a ns	0.75±0.22a ns
Group II – Mifepristone4mg/kg b.w treated	11.45 ± 2.03	172.16± 2.00	6.44 ± 0.67	30.44 ± 1.32	0.80 ± 0.54
GroupIII – TCELE 200mg/kg b.w treated	12.51± 1.23b ns	137.23 ± 0.43b**	6.88 ± 0.84b ns	31.88 ± 1.65b ns	0.82 ± 0.33b ns
GroupIV – pnTCELE200mg/kg b.w treated	11.47 ± 0.32c *	113.9±1.85c**	6.33 ± 0.73c ns	30.98 ± 1.44c ns	0.78 ± 0.38c ns
GroupV – Metformin20 mg/kg b.w treated	13.01 ± 2.23d**	107.45 ±1.77d**	6.66 ± 0.70d ns	33.57 ± 1.76d **	0.84 ± 0.29d ns

For each set of four animals, the values are presented in mean ± SEM form. Dunnet's 't" test was employed after the statistical significance was determined using the ANOVA approach. Comparisons were made between Group I and Group II (a), Group II and Group III (b), Group II and Group IV (c), and Group II and Group V (d). **p<0.01, *p<0.05, and ***p<0.001 are significant p values. NS: Not Significant

Study of Lipid Profile:

The effect of TCELE and pnTCELE extracts on lipid profile can be seen from figure 1, 2. In Group II rats (p<0.01) total cholesterol, VLDL, LDL and triglycerides increased when related to the Group I (control) rats. In Group III and Group IV rats treated with TCELE and pnTCELE extracts these levels returned back to normalcy (p<0.01) when compared to treatment with metformin. However, HDL-cholesterol levels (p<0.01) showed a significant decrease in Group II rats compared to the control, whichnon-significantly increased (p<0.01) in Group III and Group IV rats and were close to the normal values.In the present study pnTCELE treated Group IV animals exhibited a significant correction towards dyslipidaemia in comparison to the TCELE treated Group III animals.

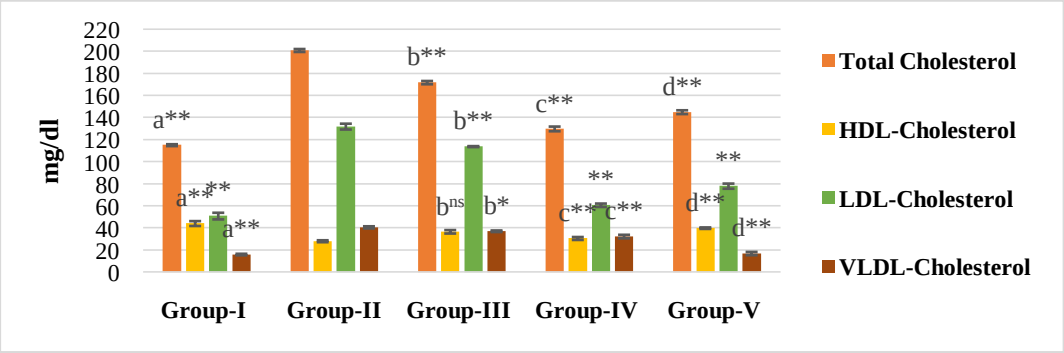

Figure 1: Effect of TCELE and pnTCELE extracts on cholesterol profile

For each set of four animals, the values are presented in mean ± SEM form. Dunnet's 't" test was employed after the statistical significance was determined using the ANOVA approach. Comparisons were made between Group I and Group II (a), Group II and Group III (b), Group II and Group IV (c), and Group II and Group V (d). **p<0.01, *p<0.05, and ***p<0.001 are significant p values. NS: Not Significant

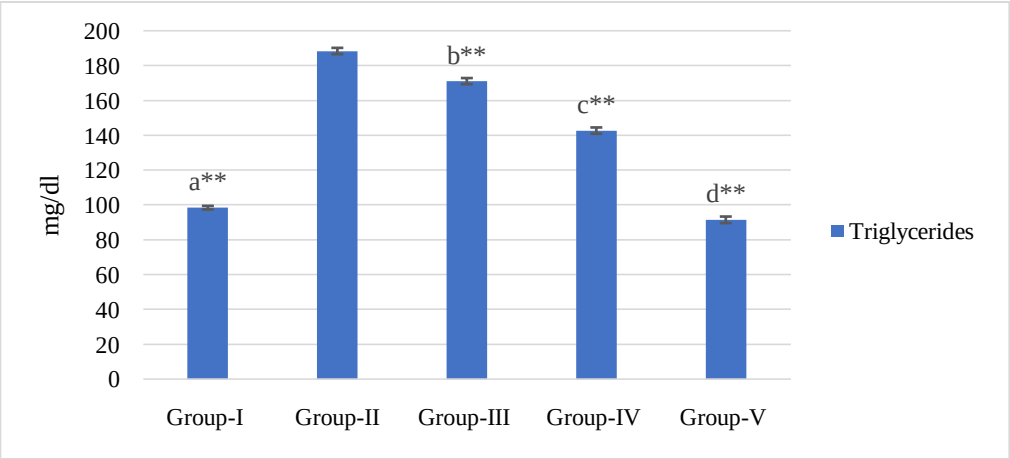

Figure 2: Effect of TCELE and pnTCELE extracts on Triglycerides

For each set of four animals, the values are presented in mean ± SEM form. Dunnet's 't" test was employed after the statistical significance was determined using the ANOVA approach. Comparisons were made between Group I and Group II (a), Group II and Group III (b), Group II and Group IV (c), and Group II and Group V (d). **p<0.01, *p<0.05, and ***p<0.001 are significant p values. NS: Not Significant

4. Discussion:

Polycystic ovarian syndrome is a condition seen in reproductive aged women, with multifaceted endocrinopathy.This is a complex endocrine and metabolic disorder consisting of hyperandrogenism, insulin resistance, anovulation and obesity(11). Mifepristone (RU486) is a contraceptive and an antagonistic progesterone receptor drug, which inhibits the growth and maturing of eggs resulting in delayed ovulation(12). Studies observed by Wessel *etal* (13)also states that RU486 impairs corpus luteum and decreases progesterone production.Mifepristone (RU486) the progesterone antagonistic drug,was used to induce PCOS and a comparative curative effect of ethanolic leaf extracts of *Tinospora cordifolia* (TCELE) and its loaded niosomes (pnTCELE) on PCOS induced rats were evaluated.

Adult female rats treated with mifepristone for 8 days developed features very much similar to women affected with PCOS. Normally the Ovarian functional physiology purely depends on vaginal smear histology. The mifepristone induced PCOS rats, in their vaginal smear showed anoccurrence of cornified cells of epithelial nature for a longer period indicating the extended estrous cycle in comparison with the drug treated rats which showed normal estrous cycle with the presence of epithelial cells for one day(14). Aprominent increase in ovary weight, body weight and also ballooning of uterus was seen in rats induced with PCOS, which correlates with the increased visceral fat distribution and abdominal obesity in women who are obese with PCOS. Active endocrine factor, thevisceral adipose tissue affects the normal and metabolic functions of reproduction. In the current study we detected that the dosing of pnTCELE reduced the body weight along with the weight of uterus and ovary.This demonstrates the shielding nature of the plant against PCOS by correcting the endocrinological disturbances. Devi*et al*(15)have reportedthe presence of phytochemicals such as berberine, rumphioside 1, syringin, tinocordiside, palmatine in the leaves by GCMs analysis. These

phyto constituents amendsthe impaired metabolic functions and accounts for the decrease in body weight and reproductive organ weight.

It is commonly recognized that the co morbidity associated with PCOS is type 2 diabeteswhich is responsible for reproductive as well as metabolic disorders. As stated byJanssen(16), the insulin resistance is present in affected women in spite of hyper insulinemia and devoid of obesity.Thus, it is a very critical task to reduce the hyperglycemic condition in the PCOS individuals. In our present investigation also, we observed an increased blood glucose level in rats induced with mifepristone leading to metabolic complications. Both TCELE and pnTCELE extracts reduced the blood glucose level effectively in the present study. Study by Navin*et al*(Navin S et al., 202)further confirms that *Tinospora cordifolia* leaves maintains glucose levels very effectively when compared to metformin indicating the glucose sensitization effect of the*Tinospora cordifolia* leaves.In the current study, the phytoniosomes loaded ethanolic leaf extracts of *Tinospora cordifolia* (pnTCELE) showed amplifiedreduction in the level of glucose in blood. The glucose lowering effect of *Tinospora cordifolia* leaves loaded niosomes (pnTCELE) was reported by Hasan*et al*[18]and as per his studies the glucose lowering effect was high due to the high penetration property of the niosomes. This is due to the lipid bilayer encapsulated drug which resist the hydrophobic interaction and aids for the leisurely release of drug and mimics the biological membrane structure as stated byAkbarzadeh*etal*[19].

According to Manzoor*etal*[20]hyperglycemia and increased androgen levels were also responsible for, hypercholesterolemia, increased serum LDL, decreased serum HDL level and hyper-triglyceridemia. In the present study it is seen that there is dyslipidemic condition with decreased HDL cholesterol, increased VLDL and LDL cholesterol as well as triglycerides, in rats induced with PCOS. The phytoniosomes loaded ethanolic leaf extract of *Tinospora cordifolia* (pnTCELE) changed this dyslipidemic condition by showing a decrease in triglycerides, VLDL as well as LDL cholesterol with HDL-Cholesterol increase. This could be because of the existence of saponins, flavonoids,sterols and tannins present in the leaves[21]. According to Zeka*et al*[22]the flavonoids have the ability to enhance the lecithin cholesterol acyl transferase enzyme (LCAT) activity, which is important for the synthesis ofHDL-C.The same effect may be observed in our present study in increasing the HDL concentration. Kumar *et al*[23]has reported the enhanced activity of lipoprotein lipase activity by saponins which will aid in thefaster removal of free fatty acid from circulation and the tannins have the capacity to increase the activity of endothelium bound lipoprotein lipase activity. The dyslipidemic correcting action of the phytoniosomes loaded ethanolic leaf extract of *Tinospora cordifolia* (pnTCELE) may accounted by its flavanoid, saponin and tannin content. According toAl-Awaida *et al*[24]plant sterolsare also shown to decrease cholesterol absorptionand increases its excretion. This cumulative effect of increase in lipoprotein lipase enzyme which will consequently hydrolyse the cholesterol and triglycerides thereby increase their utilization as free fatty acids, as well as enhanced cholesterol excretion by plant sterols will reduce their level considerably in the blood to correct the dyslipidemia condition.

5. CONCLUSION:

In conclusion it is stated that leaves of *Tinospora cordifolia* ethanolic extract loaded phytoniosomes (pnTCELE) is an excellent drug carrier in reducing the blood glucose level in PCOS condition when compared to the normal ethanolic extract of *Tinospora cordifolia* (TCELE). It also has the potential to correct the dyslipidemic condition and can be used as a cardio protective agent. The pnTCELE treatment restored the glucose level, lipid profile and altered morphologyin mifepristone intoxicated

animals when compared with Metformin.This shows that pnTCELEcan be used as an effective drug either by itself or in conjunction with Metformin to effectively treat PCOSwhich requires a diversified therapy to control hormonal and metabolic complication and by inducing ovulation regains the fertility in women.

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CONFLICT OF INTEREST:

The authors individually declare that there are no conflicts of interest in publishing the paper.

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