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**AMELIORATION OF ORAL CONTRACEPTIVES INDUCED HEPATOTOXICITY BY
METHANOLIC EXTRACT OF TURMERIC (*CURCUMA LONGA LINN.*)**

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ABSTRACT

Oral contraceptive (OC) are widely used by postmenopausal women and women of child bearing age prolong use of which causes cholestasis and other complications. Turmeric is known for possessing antioxidant and anti-inflammatory properties. However its efficacy in contraceptive-induced liver damage and cholestasis is unknown. Female rabbit (n = 6; each group) were given, (a) Estradiol valerate, (b) Norethisterone (NETA), (c) Levonorgestrel / ethinyl estradiol daily for three weeks. Treatment groups were administered 220mg /kg turmeric extract. Highly significant (p<0.005) rise in liver enzymes and total bilirubin in oral contraceptives treated groups was observed. In case of lipid profile, significant changes were documented in the level of cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol. Body weight was declined by oral contraceptives. TC Significantly (P <0.005) reduced the levels of liver enzymes, increased body weight and resulted in improvement in lipid profile. TC effectively cures the oral contraceptives induced hepatotoxicity and hyperbilirubinemia. Women can use TC as food supplement with oral contraceptives providing beneficial effects to their Health.

**Keywords: Antioxidant, Hepatotoxicity, Oral Contraceptives (OCs), Turmeric Extract
(TC)**

INTRODUCTION

Oral contraceptives (OCs) were widely used by women of childbearing age to circumvent pregnancy and postmenopausal women to restore hormonal deficiency since 1960's. Continuous use of oral contraceptives leads to various adverse effects, for instance, myocardial infarction, thromboembolism, stroke, breast cancer, cholestasis, hepatocellular carcinoma, etc. Of numerous adverse effects the most serious one is hepatotoxicity caused by cholestasis[1]. Estrogen and progesterone induce cholestasis by disturbing the ability of liver to secrete bile[2]. Liver injury caused by drugs has a serious commencement. Their constant use results in histological and anatomical configurations characterized as hepatocellular necrosis and, cholestasis. Increased level of serum alanine aminotransferase indicates the liver injury. Cholestasis can be characterized increased levels of bilirubin, decrease level of albumin and clotting factors produced from liver. OCs induces hepatic cholestasis in experimental animals. Their use led to cholestasis, vascular lesions and hepatic neoplasm. The prevalence of cholestasis with OCs is 2.5 per 10,000 women [3]. The effects of estrogens have been attributed to the endogenous

estrogen metabolite 17β -estradiol glucuronide. [2]

Curcuma longa Linn.a perennial herb belonging to ginger family Zingiberaceae contains Curcumin that is a polyphenolic compound present naturally in its root. It is the most widely used in India as a spice and as an anti-inflammatory compound in old medication.[4] Turmeric oleoresin is the organic extract of turmeric-which is obtained by solvent extraction of the ground turmeric by solvents acetone, ethylene dichloride and ethanol for 4-5 hrs. It is orange red in color. It is composed of 37-55% curcuminoids and approximately 25% essential oils[5]. Curcumin is a bis- α - β -unsaturated β -diketone. At acidic and neutral pH, the bis-keto form of the compound predominates and at basic pH the enolate form is usually found. Hence at pH 3-7, it acts as the most potent H-atom donor and above pH 8; Mainly it acts as an electron donor-which is more suitable for antioxidant properties of curcumin[6].

The anti-oxidant properties are due to strong oxygen radical scavenging ability. Animal studies have shown that a dose of 100-200 mg/kg of body weight induced anti-inflammatory effects. Oral median lethal dose (LD50) in mice is higher than 2.0 g/kg body weight[7]. Curcuminoids, curcumin,

demethoxycurcumin, bisdemethoxycurcumin, 5-methoxycurcumin and dihydrocurcumin are famous antioxidants in turmeric. Regarded as curcuminoids are potent inhibitors of lipid peroxidation in rat brain homogenates, and rat liver micro omes than α -tocopherol [8]. Antioxidant enzymes in the blood are elevated after administration of turmeric oil (100-500mg / kg body weight) for a month. There is significant elevation in glutathione, catalase and glutathione reductase. Turmeric oil also has a significant effect on antioxidant enzymes in liver tissue of mice after 30 days treatment. Catalase is not raised but glutathione peroxidase and superoxide dismutases are significantly raised between doses of 100-500mg / kg bodyweight. The core agent of this activity is Arturmerone[5, 9].

Curcumin promotes the protection of hemoglobin from oxidation at very low concentration. Superoxide dismutase, glutathione peroxide and catalase are the enzymes that confer antioxidant properties to curcumin. It has at times greater action than vitamin E. The antioxidant property of curcumin is due to the phenolic and methoxy group on the phenyl ring and the 1,3-diketone system [10]. Curcumin has anti-cancer, antioxidant and anti-inflammatory

properties. It makes purifying phase II enzymes alarmed with the decontamination or carcinogens [11].

2. MATERIAL AND METHODS

EXTRACT PREPARATION

Turmeric (*Curcuma longa* Linn.) family; Zingiberaceae, rhizomes were collected from Kahna, District Lahore, and Punjab, Pakistanduring November 2012 and was identified by an expert in the department of Pharmacognosy, University College of Pharmacy, University of the Punjab. The finely powdered crude turmeric (500g) was extracted with 1000 ml methanol by maceration at 37 ° C for one day. The Macerata was shaken at room temperature with mechanical stirrer for 60 minutes. The sample was filtered and Filtrate was collected in wide open mouthed Petri dish and was placed in oven at 37°C for evaporation. The thermostat of the oven was set at 37°C. Orange-red colored sticky gummy residue was obtained. In order to obtain a proper dosage the residue was again dissolved in 50 ml of methanol and 40 g starch was added to obtain a smooth paste. This paste was kept at room temperature for one day-which constantly methanol evaporated and fine powder was obtained.

ANIMALS

Doe rabbits having weight 1-2 kg were used in the course of whole work. The animals were placed in the animal house of the University College of Pharmacy. The use of animals and experimental design were approved by Animal Ethics Committee, University College of Pharmacy, PU, Lahore (No.D / AEC / UCP1010 / 4813). They were kept for acclimatization for five days before starting research work. The temperature was maintained at $37 \pm 0C$ and relative humidity between 30-70%. Fresh green fodder was provided to rabbits regularly and water ad libitum. Animals were randomized into control and treated groups and were retained separately.

DRUGS AND CHEMICALS

Estradiol valerate (EV) Norethisterone (NETA) (Bayer Schering Pharma AG, Germany) and Levonogestrel/ Ethinyl estradiol (LNG / EE) from Zafa Pharmaceuticals Laboratories Pvt Ltd, Karachi, Pakistan was obtained. All other chemicals used in this study were of analytical grade.

EXPERIMENTAL DESIGN

The female rabbits were randomly divided into seven groups (n=6). EV and EV + TC, NETA and NETA + TC, LNG / EE and LNG / EE + TC were given to rabbits orally for

three weeks and doses were calculated based on the weight of 70 kg adult human being.

Group 1 served as negative control (NC) treated with starch powder as placebo

Group 2 was treated with EV (40 micrograms / kg orally once daily).

Group 3 was treated with both EV and TC (40 micrograms / kg + 220 mg / kg orally once daily).

Group 4 was treated with NETA (140 micrograms / kg orally once daily).

Group 5 was treated with both NETA + TC Treated Group (140 micrograms / kg + 220 mg / kg orally once daily).

Group 6 was given LNG and EE (4 micrograms / kg orally once daily).

Group 7 rabbits were treated with both LNG and EE, and TC (4 micrograms / kg + 220 mg / kg orally once daily).

At the end of experimental all the animals were euthanized under anesthesia.

SERUM BIOCHEMICAL ASSAY

At the end of investigational schedule blood sample of each rabbit were collected under anesthesia. Blood samples were centrifuged (4000 rpm for 15 minutes). Serum was collected and used instantaneously for the estimation of ALT, AST, ALP, Total Bilirubin and cholesterol using commercial kits (Randox, country name), LDH (AMP kits, country name), HDL-C, LDL-c

(Analyticon, country name) and triglyceride (Crescent Diagnostics, country name).

HISTOLOGY

Liver tissue approximately 1 cm³ was harvested from the rabbits after cervical dislocation under anesthesia. Tissues were preserved in Formalin solution (4%). 5μ Paraffin sections of liver were cut and processed for Hematoxylin staining as per standard laboratory procedure. Stained slides were observed and representative picture of the tissue were obtained using microscope.

STATISTICS

The data was expressed as \pm SEM for six rabbits in each treatment group. Student T-test was used for find statistically significant difference among groups and Prism was used to draw histograms. P-values < 0:05 were considered significant.

RESULTS

Oral contraceptive induced liver damage is suppressed by turmeric extract

Liver damage and subsequent protective effect of Turmeric extract was analyzed by quantifying levels of transaminases, LDH and Alkaline Phosphatase in contraceptive-induced liver damage in female rabbits. The oral contraceptive treatment with EV and NETA alone significantly induced rise serum transaminases (SGOT and SGPT), ALP, Bilirubin and LDH-which was not observed

in groups where turmeric extract was co-administered Figure 1. However the levels of liver damage markers in LNG/EE treated group was not elevated to the similar extent whereas ALP levels were not increased at all. The co-treatment of TC with LNG/EE also reduced the elevated levels of liver damage markers as well as Bilirubin but did not affect the ALP levels (Figure 1).

EV and NETA have inconsistent effects on cholesterol metabolism (Fig 2a). EV administration reduced the levels of cholesterol and LDL while raised the levels of HDL and triglycerides (Figure 2). NETA and combination of LNG/EE had similar effects; they raised the levels of Cholesterol, LDL and triglycerides in the serum but no significant effect was on HDL. TC co-administration in generally lowered the levels of cholesterol and LDL and triglycerides while TC in NEAT treated rabbits restored the levels of HDL.

Animal's weights were also monitored during the course of experiment. There was no significant change in the weight of animal in the control group, however the treatment with EV, NEAT significantly decreased the weight of animals in the 3rd week by 9.77% and 11.04% respectively. Surprisingly LNG/EE treatment did not caused negative effect on the weight of animals. Co-treatment

of OC treated animals with TC had a positive effect on general health of the animals and a significant increase in weight was observed during the course of treatment. In EV+TC the weight was increased gradually from 2.46% (1st week) to 13.18% in the third week. In NETA+TC treated group the weight increment was 3.26% in 1st week that became 9.63% in 2nd week and then 8.01% in the third week. In the LNG/EE treated group the weight of animals increased by 6.06% in the first week and no further effect on weight was observed in the following weeks.

HISTOLOGY

Oral contraceptives induced hepatotoxicity was established by histological examination. EV induced coagulative necrosis, portal and periportal inflammation. Necrotic hepatocytes were also perceived as indicated by dead nuclei. EV + TC revealed significant hepatoprotective effect in which there was little or no inflammation. NETA induced inflammation was indicated by presence of macrophages in the cytoplasm of cells and ductal epithelium. NETA + TC group have no inflammation and necrosis due to hepatoprotective effect of turmeric. LNG/EE demonstrated portal to periportal inflammation as well as portal to portal inflammation. LNG / EE + TC had no

inflammation but venous congestion was observed (Fig 3).

DISCUSSION

The present investigation was developed to assess the effect of estrogen (EV), progesterone (NETA) and combination of estrogen and progesterone on biochemical changes in liver function and lipid profile. The hepatotoxicity produced by oral contraceptives was cured by the administration of turmeric extract.

Oral contraceptive induced increased level of SGOT (serum glutamic oxaloacetic transaminase), SGPT (serum glutamic pyruvic transaminase), ALP (alkaline phosphatase), LDH (lactate dehydrogenase) and total Bilirubin signifying liver damage as compared to negative control. In case of lipid profile, there was a significant change in the level of cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol. Body weight was declined by oral contraceptives. Turmeric extract containing curcuminoids significantly ($P < 0.005$) reduced the level of liver enzymes, increased body weight and resulted in improvement in lipid profile. OCs comparison with OCs + TC treated groups showed highly significant difference in liver parameters. Histological examination confirmed the OCs induced hepatotoxicity.

Liver is a vital organ That metabolizes different drugs and other substances. Liver cells (hepatocytes) control the function of liver. Any injury to liver cells lead to different cell permeability and seeping out of tissue content into bloodstream. This results in increased serum aminotransferase levels [12].

In turmeric treated group liver enzymes were not elevated like OCs treated groups as shown in table 1. This was possibly due to antioxidant effect of curcuminoids That did not allow the OCs to induce liver injury That Appeared in OCs treated groups. The effect of curcumin on liver function was ook Investigated by Egyptian scholars And They Concluded That curcumin due to its several at functional groups providence antioxidant effect by enhancing the glutathione stores of the body. Curcumin effectively reduced the SGOT, SGPT, ALP and bilirubin due to its hepatoprotective effect [13]

In case of lipid profile methanolic extract of turmeric was highly significantly decreased total cholesterol and LDL-C due to strong hypolipdemic effect of turmeric. These results indicated the cardio protective effect of turmeric. Curcumin Reduces low density lipoprotein and very low level in liver along with an incensementor alpha-tocopherol level in rabbit plasma, signifying in vivo interface

between curcumin and alpha-tocopherol That May raise the bioavailability of vitamin E and declining cholesterol levels. Another investigation indicated the protective effect of curcumin on lipoproteins. Curcumin Reported to Increase the level of HDL-c and reduce LDL-C and serum total cholesterol [14, 15].

Weight of female rabbits wastakenat baseline and after every one week to assess the change in weight caused by oral contraceptives and combination of oral contraceptives with turmeric extract. EV Caused reduction in weight while combination or EV with turmeric increased weight. NETA reduced weight while combination or NETA with turmeric resulted in weight gain. LNG / EE and combination of LNG / EE with turmeric Caused elevation of weight throughout the experiment.

Drug metabolites can generate free radicals or electrophilic elements that can cause many chemical reactions, for illustration they can diminish reduced glutathione; fix covalently to proteins, phospholipids, or nucleic acids; or generation of lipid peroxidation. All of these have special effects on cellular organelles such as mitochondria, the endoplasmic reticulum, the cytoskeleton, microtubules, or on the core. Cellular structures are affected by them through the

instigation of signaling kinases, transcription factors, and gene-expression. The subsequent intracellular strain can encourage cell death caused by either cell contraction and nuclear disassembly (apoptosis) or inflammation and lysis (necrosis)[16]. Liver injury is considered as liver cell death, even though bile duct epithelium or sinusoidal endothelial cells may be tangled. Rabbits are more prone to hepatic toxicity as compared to rats. Within 2 weeks of treatment with EE alone at dose of 0015 mg / kg-day, rabbits became anorexic than the control, γ -glutamyl transferase (γ -GT), AST, and ALT were significantly elevated. EE and norethindrone alone or in combination cause low acute toxicity but at doses several at times higher than human therapeutic dose[17]. The phenolic structure of estrogens is responsible for their antioxidant activity but at elevated doses metabolism or phenolic structure results in harmful effects. The increment in H₂O₂ as prooxidant effect after E2 and E2V was more effective in 60 days old rats with high biotransformation activity. In vivo administration of estrogens cause dose and age dependent biotransformation that cause pro-oxidative effect in addition to antioxidant effect [13, 17-19]. These antioxidant activities are opposed by estrogens or progestins through the stimulation of the

NADPH oxidase and the inhibition of the expression and activity of MnSOD and ecSOD[16]. Oxidative stress in liver (Hepatocytes) predisposes the inactive hepatic steallet cells to become activated and resistant to oxidative stress causing liver fibrosis. [20] In experimental models in rat and Syrian hamster, estrogen reflects its pro-oxidant effect. Indeed, estrogens can be metabolically activated into catechol estrogens by the enzymes or cytochrome P450. These are easily auto-oxidized to ortho-quinone byproducts-which are powerful oxide-reducing agents capable of generating ROS. De Groote et al., 2009, in his study Concluded That estrogens significantly affect OS by increasing lipid peroxidation and Cu⁺² in serum [16].

There are reports of intrahepatic cholestasis when high dose of norethisterone were given to women with breast cancer. In rats, norethisterone can induce cholestasis associated with bile staining or hepatocytes. The mechanism of cholestasis induced by norethisterone is unknown. These reports confirmed that progestin pills are not free of risk of developing cholestasis [21].

Estrogens are well identified to effect intrahepatic cholestasis in vulnerable women during menopausal period management by oral contraceptives and postmenopausal

auxiliary therapy. Cholestasis due to estrogens may be caused by inhibition of bile secretion. These effects may be due to a direct effect on orphan nuclear receptors that modulate bile acid and bilirubin metabolism and transport [22, 23]. Estrogens induce hepatic cholestasis in experimental animals. The effect of estrogens has been attributed to the endogenous estrogen metabolite 17 β -estradiol glucuronide. This metabolite is one of a family of glucuronide conjugates of the estrogen D-ring that have been revealed to reduce bile flow and bile acid secretion in the rat in a dose-dependent manner. The mechanism involved in the diminishing of the bile flow is variable. Estrogen decreases sinusoidal uptake of bile acids, at least in part by inducing down-regulation of the expression of the sodium-taurocholate cotransporting polypeptide NTCP protein. Also, estrogen reduces hepatic capacity to expel bile salts and organic anions. Estrogen reduces bile salt synthesis, and also alleviates the endogenous bile salt pool and resulted in decreased bile salt secretion. Further More, estrogens and its 17 β -glucuronide administrations increase tight junction permeability in rat liver. This increased cellular absorbency permits for the paracellular arrival of bile constituents into the blood[2]. Curcumin, component of turmeric

positively regulates the expression of NTCP in IL1 β via JNK pathway.[24]. Turmeric-induced suppression in Bilirubin in OC treated rabbits may be a result of NTCP up-regulation, However, further experiments are required to find the exact molecular mechanism for the positive effects of turmeric on OC induced liver toxicity.

In conclusion, oral contraceptives induced elevation of liver parameters and Bilirubin and variation in lipid profile that was improved by Turmeric extract. TC seems to have some effect on the regulation of genes related to bile metabolism. Further research can be done to get deep insight. As a future perspective, turmeric can be used as a supplement in the diet of women using oral contraceptives providing beneficial effects to Their Health.

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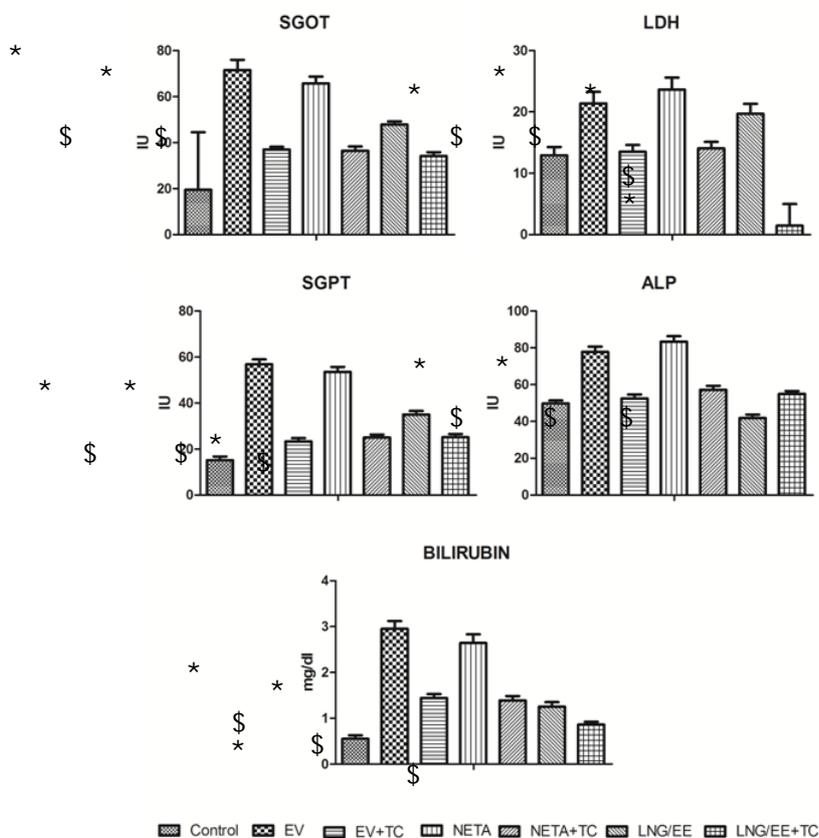


Figure 1. Effects of TC on liver damage markers. Rabbits were given oral contraceptive alone or in combination with TC for three weeks. Blood samples were collected and analyzed for (a) SGOT, (b) LDH, (c) SGPT (d) ALP and (d) Bilirubin. * Shows the significant different (P<0.05) Vs untreated control group.\$Shows significant difference (P<0.05) vs respective OC treated group

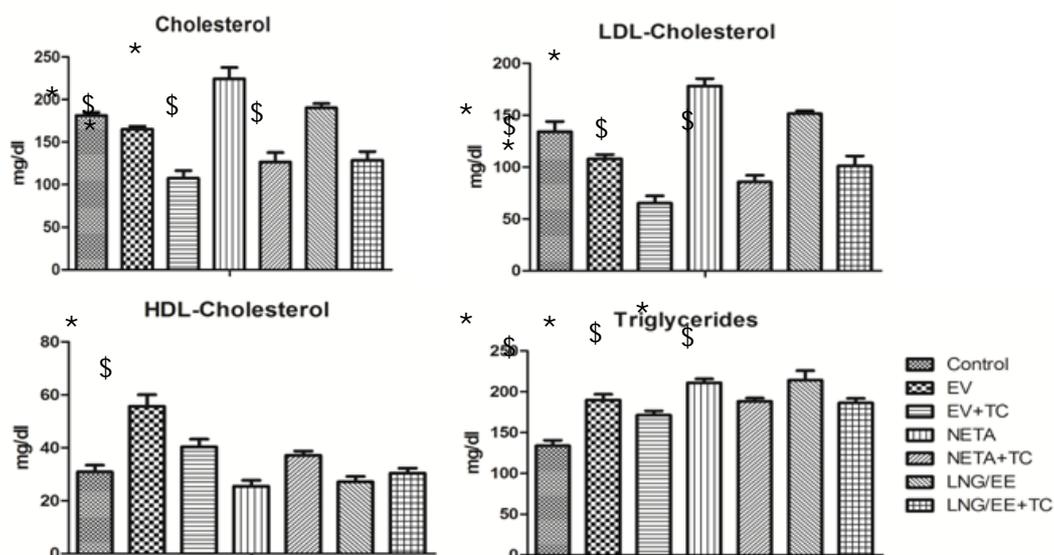


Figure 2.Effects of TC on lipid metabolites.Rabbits were given oral contraceptive alone or in combination with TC for three weeks. Blood samples were collected and analyzed for (a) Cholesterol, (b) LDL, (c) HDL and (d) Triglycerides. * Shows the significant different (P<0.05).\$Shows significant difference (P<0.05) vs respective OC treated group.

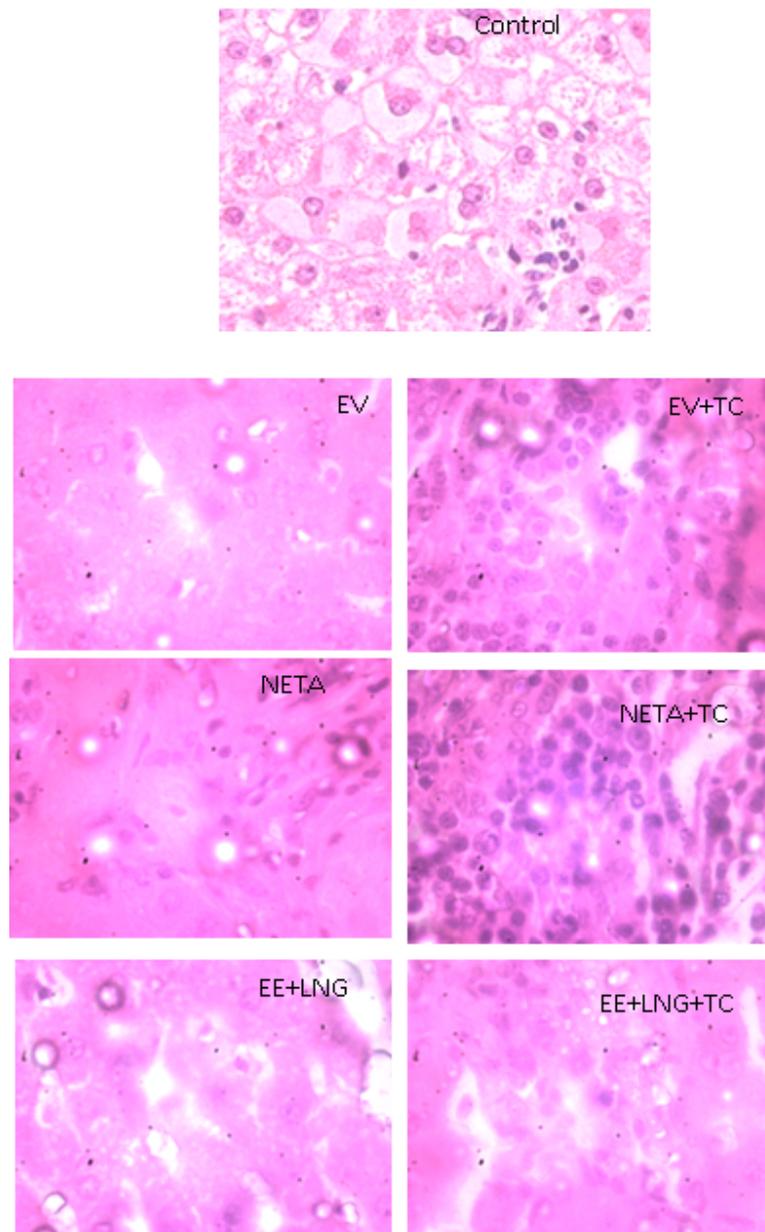


Figure 3: TC treatment reverses the morphological damage induced by OC.

Table 1: Weight of Female Rabbits in Different Treatment Groups

Drugs	Initial Weight	Weight 1 st week	Weight 2 nd week	Weight 3 rd week
Negative Control	1201	1203±1 ^{NS} (↑0.16%)	1206±2 ^{NS} (↑0.42%)	1210±3 ^{NS} (↑0.75%)
EV	1217	1247±39 ***(↑2.46%)	1210±58 ^{NS} (↓0.57%)	1098±38****(↓9.77%)
EV+TC	1206	1245±13****(↑3.23%)	1293±44****(↑7.21)	1365±39****(↑13.18%)
NETA	1205	1250±30****(↑3.73%)	1235±69 ^{NS} (↑2.49%)	1072±37****(↓11.04%)
NETA+TC	1298	1345±14****(↑3.62%)	1423±42****(↑9.63%)	1402±66****(↑8.01%)
LNG/EE	1285	1348±50****(↑4.90%)	1358±69*(↑5.68%)	1345±77*(↑3.62%)
LNG/EE+TC	1188	1260±33****(↑6.06%)	1278±43****(↑7.57%)	1253±36****(↑5.47%)

Changes in weight of rabbits All values are expressed as ±SEM. Highly Significant: *** 0.005, Very Significant: **0.01, Significant: *0.05, Non-Significant NS. Negative Control (NC), Estradiol valerate (EV), Norethisterone (NETA)