



# The Effective Role of Phitofert<sup>®</sup> Supplement in Male Reproductive Hormones and Epididymal Sperm Quality in Carbon Tetrachloride (CCL<sub>4</sub>) Treated Rats

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**Abstract** | The goal of this study was to see if Phitofert<sup>®</sup> might help improve sperm function in carbon tetrachloride (CCL<sub>4</sub>) -treated and healthy adult rats. Twenty-four adult rats were randomly divided into four groups and given the following treatments for 28 days: G1: as a control, the animals were oral administration with DW. G2: healthy rats were given 0.035 mg/kg BW of Phitofert<sup>®</sup> every day by gavage. G3: twice a week, they were given 500 mg/kg B.W. of CCL<sub>4</sub> mixed with an equivalent volume of olive oil (0.5 ml/kg B.W.). G4: were treated twice a week with 500 mg/kg BW of CCL<sub>4</sub> mixed with an equivalent amount of olive oil (0.5 ml/kg BW) and gavaged with (0.035 mg/kg BW) of Phitofert<sup>®</sup> daily. In the tenth and twenty-eighth of the experiment, blood samples were taken by heart puncture for serum collection. In comparison to all other groups, the current results reveal a significant ( $p \leq 0.05$ ) decrease in testosterone, somatic testicular index, and sperm quality in the G3 group, nevertheless, there was a significant ( $P < 0.05$ ) increase in LH, FSH, sperm abnormalities percent, and dead sperm percent in the G3 group. The capacity of Phitofert<sup>®</sup> to restore all passive effects of CCL<sub>4</sub> and increase epididymal sperm quality was demonstrated in G4 groups.

**Keywords** | CCL<sub>4</sub>, Phitofert<sup>®</sup>, Epididymal sperm, Testicular somatic index, Macca rote

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## INTRODUCTION

Infertility and its complications are important sources of distress in people's lives (Eskandari and Momeni, 2016). Male infertility is commonly caused by an inability to generate a sufficient quantity of active sperms (Amin and Hamza, 2006; Hamid et al., 2018). Many studies have found that oxidative stress can influence sperm generation and count by producing free radicals and oxidizing germ cells in the testis tissue (Agarwal et al., 2014). Tetrachloromethane, commonly known as carbon tetrachloride (CCL<sub>4</sub>), was thought to be colorless, nonflammable, and a heavy liquid with a pleasant, fragrant, nonirritating odor (Lide, 2006). It is employed as a fumigant agriculture and as a solvent in semiconductor manufacture; in fat production in oil, rubber and laboratory uses (Kauppinen et al., 2000). The central nervous system, hepatocyte and renal system can be

affected by high amounts of CCL<sub>4</sub> (Sönmez et al., 2014). Chronic [CCL<sub>4</sub>] exposed can induce kidney impairment and cancer in the liver (Masuda, 2006). In addition, sperm motility, sperm concentration and sperm destruction was produced by CCL<sub>4</sub> and relative or absolute alteration in reproductive organ weights (Türk et al., 2016). The nutritional supplement, such as Phitofert<sup>®</sup>, helped improve fertility and adaptogenic and sexual qualities in males due to its components, macca and selenium (Al-Shahery, 2019). Maca dry extract (Lepidium meyenii W., tuber), Colloidal, Maca (Lepidium meyenii W.) Silica, L-methionine (<https://www.promopharma.it/en/phitofert-men>) are the Phitofert<sup>®</sup> components. The ingredients are: The Maca plant is found in and belongs to Brassica in South America. It has also been used for the treatment of infertility in several animals, traditional medicine for many illnesses (Gonzales, 2012). For the second component, selenium,

this was a mineral that was able to alleviate oxidative stress and improve the quality of the sperm (Hamza and Al-Harbi, 2014). Current study was aimed for the first time to explore the underlying CCL4 mechanism to impact male fertility as well as to investigate the possibility of the Phitofert® product in males for medicating the adverse effects of CCL4 exposure.

## MATERIALS AND METHODS

### EXPERIMENTAL ANIMALS DESIGN

Twenty-four adult rats aged three and a half-four months, 350-400 gm weight were used in the experiment. They were placed six per cage and put in the acclimatization room for two weeks. Air in the room was kept at (21-25 KF), ventilation air and a light/ dark cycle of 12:12 h/day were used to continually alter the air of the room. Every 2 days, the cage litter was replaced. Pellet of a freshly made meal was used to fed animals. Randomly designed and handled the experimental animals as follows: Randomly, 24 adult rats were placed into four groups and treated as follows for 28 days, G1: animals were given DW and served as control. G2: healthy rats were gavage daily with 0.035 mg/kg BW of Phitofert® (Yousif et al., 2018). G3: were treated I.P with 500 mg / kg B.W. of CCL4 mixing with olive oil (equal volume of olive oil) (0.5 ml/kg B.W.) twice a week (Arrack and Wassan, 2017). G4: were treated I.P with 500 mg / kg B.W. of CCL4 mixing with (0.5 ml/kg B.W.) of olive oil twice a week and gavage daily with 0.035 mg/kg BW of Phitofert®.

### SPECIMEN PREPARATION

Intra muscular injection of (90mg/kg) of ketamine and (40 mg/kg) of xylacine were used to anesthetizing the animal on the animals. At (Zero, 10, 28) days of research, blood samples were collected using heart puncture methods. Serum was isolated by centrifuged from coagulated blood specimen at 2500 rpm for 15 min and maintained at -20 °C by freezing until needed (David, 2005). ELISA kit used to measurement of Testosterone, follicular stimulating hormone (FSH), luteinizing hormone (LH) concentration according to Kit manufacturer's instructions. At the end of research rats were anesthetized and weighted, testes weighted by sensitive balance. Somatic testicular index was study through the following calculation:

$$\text{Relative testicular weight} = (\text{testes weight (gm)} / \text{body weight (gm)}) \times 100$$

### SPERM PARAMETER

rats epididymal of four groups were removed and prepared. The epididymal caudae were individually crushed by microsurgical scissor in one ml of Ham's solution to release the spermatozoa. The sperms suspension was kept at (37°C) in a humid atmosphere of (5% CO<sub>2</sub>), one drop of sperms

suspension was examined under microscope to determine the sperm quantity such as morphology, concentration, viability and motility (Al-Shahery, 2019).

### STATISTICAL ANALYSIS

The program was used to Least significant difference –LSD test and Duncan Multiple Range (ANOVA) was used to significant compare between means in this study by using Statistical Analysis System- SAS (2012) program.

## RESULTS AND DISCUSSION

### SERUM HORMONES PARAMETERS

#### TESTOSTERONE CONCENTRATION (NG\ML)

Table 1 revealed a significant ( $p \leq 0.05$ ) decline in testosterone levels in G3 when compared to the control and all other administered groups during the period of this study; moreover, testosterone levels within groups showed a significant decrease in (10 and 28) days when compared to zero time. Whereas testosterone levels increased ( $P < 0.05$ ) significantly in the G2 group when compared to the control group, they also increased ( $P < 0.05$ ) significantly within the same group when compared to zero time and ten days of administration. The G4 group exhibited no significant changes when compared to the control group, but showed a significant rise ( $P < 0.05$ ) in testosterone levels when compared to the G3 groups. The G4 group exhibited no significant changes when compared to the control group, but showed a significant ( $P < 0.05$ ) rise in testosterone levels when compared to the G3 groups. In contrast, testosterone levels in G4 were significantly ( $P < 0.05$ ) higher in (28 day) as compared to (zero and 10) days of the study period. Finally, testosterone level in G3 group showed a significant decrement ( $P > 0.05$ ) when compared zero time in the same group and when compared with all treated groups.

**Table 1:** Effect of CCl<sub>4</sub>, Phitofert® and (Phitofert® + CCl<sub>4</sub>) on testosterone hormone level (ng/ml) in adult male rats M±S.E. (n = 6).

Groups	Zero time	10 days	28 days
G1	1.50 ± 0.04 A a	1.68 ± 0.23 B b	1.61 ± 0.51 B a
G2	1.47 ± 0.05 A c	2.73 ± 0.16 A b	3.83 ± 0.12 A a
G3	1.49 ± 0.21 A a	0.59 ± 1.10 C b	0.37 ± 0.31 C b
G4	1.51 ± 0.23 A b	1.45 ± 0.41 B b	2.47 ± 0.56 B a

LSD = 1.02; G1: healthy control group; G2: animals received (0.035) of Phitofert®; G3: animals received (500 mg / kg. B.W.) of ccl4; G4: animals received (500 mg of ccl4 + 0.035 mg of Phitofert®). Various capital letter denote significant differences between groups ( $P < 0.05$ ). Various small letter denote significant differences within groups ( $P < 0.05$ ).

## LUTEINIZING HORMONE CONCENTRATION (LH) CONCENTRATION (MLU\ML)

As opposed to controls and all administered groups, Table 2 shows a significant ( $P < 0.05$ ) rise in LH concentration in the G3 group. In addition, the concentration of LH was significantly ( $P < 0.05$ ) increased in the 28-day period compared with 0 and 10 days of the administration, whereas G2 showed a significant ( $P > 0.05$ ) decline compared to the control, and all animals were given and over zero and 10 days in the same group. The result from the G4 Group revealed that there were significant variations between the control group and G4 group in the concentration of LH and within 28 days when compared with zero time and 10 days after administered.

**Table 2:** Effect of CCl<sub>4</sub>, Phitofert<sup>®</sup> and (Phitofert<sup>®</sup> + CCl<sub>4</sub>) on LH hormone level (ng/ml) in adult male rats M±S.E. (n = 6).

Groups	Zero time	10 days	28 days
G1	1.81 ± 0.31 A a	1.85 ± 0.03 B a	1.78 ± 0.01 B a
G2	1.83 ± 0.02 A a	1.28 ± 0.16 C a	0.70 ± 0.13 C b
G3	1.79 ± 0.11 A c	2.59 ± 0.13 A b	3.67 ± 0.16 A a
G4	1.80 ± 0.21 A b	1.83 ± 0.31 B b	2.02 ± 0.27 B a

LSD = 0.55; G1: healthy control group; G2: animals received (0.035) of Phitofert<sup>®</sup>; G3: animals received (500 mg / kg. B.W.) of ccl<sub>4</sub>; G4: animals received (500 mg of ccl<sub>4</sub> + 0.035 mg of Phitofert<sup>®</sup>). Various capital letter denote significant differences between groups ( $P < 0.05$ ). Various small letter denote significant differences within groups ( $P < 0.05$ ).

## FOLLICULAR STIMULATING HORMONE (FSH) CONCENTRATION (MLU\ML)

Table 3 depicted FSH level in rats during the testing period, in comparison with control and other managed groups, the G3 group exhibited a significant ( $P < 0.05$ ) rise in FSH level. In 28 days of administration within the G3 group, FSH was also significantly ( $P < 0.05$ ) increased compared to zero and 10 days of the administration, while in comparison to control and all managed animals and in relation to zero and 10 days of administration within the same group, FSH level showed a significant ( $P > 0.05$ ) decrease in G2. FSH levels in the 10 and 28 days administration in comparison to zero-times are shown by the results from G4 group rats.

## SPERM PARAMETERS

Table 4 shows a significant ( $P < 0.05$ ) increase in sperm percentage and sperm concentration in G2 as opposed to control groups and other groups, and there was a significant ( $P > 0.05$ ) decrease in sperm abnormalities as a percentage and death sperm percentage in G2 groups. The results show that the percentage of dead sperms and abnormalities in G3 have significantly ( $P < 0.05$ ) increased.

In contrast, the outcome of the G4 sperm parameter showed non- significant differences in comparison to the control group, but the G4 sperm motility percentage and sperm concentration increased significantly ( $P < 0.05$ ) in comparison to the G3 sperm group, while the sperm abnormality percentage declined significantly ( $P > 0.05$ ) as compared with the G3 group.

**Table 3:** Effect of CCl<sub>4</sub>, Phitofert<sup>®</sup> and (Phitofert<sup>®</sup> + CCl<sub>4</sub>) on FSH hormone level (ng/ml) in adult male rats. M±S.E. (n= 6).

Groups	Zero time	10 days	28 days
G1	5.8 ± 0.31 A a	5.56 ± 0.23 B a	5.50 ± 0.21 B a
G2	5.72 ± 0.03 A a	4.36 ± 0.14 C b	3.16 ± 0.11 C c
G3	5.80 ± 0.13 A c	6.76 ± 0.12 A b	8.13 ± 0.01 A a
G4	5.74 ± 0.23 A a	5.24 ± 0.22 B a	4.32 ± 0.02 B b

LSD = 0.55; G1: healthy control group; G2: animals received (0.035) of Phitofert<sup>®</sup>; G3: animals received (500 mg / kg. B.W.) of CCl<sub>4</sub>; G4: animals received (500 mg of ccl<sub>4</sub> + 0.035 mg of Phitofert<sup>®</sup>). Various capital letter denotes significant differences between groups ( $P < 0.05$ ). Various small letter denotes significant differences within groups ( $P < 0.05$ ).

**Table 4:** Effect of CCl<sub>4</sub>, Phitofert<sup>®</sup> and (Phitofert<sup>®</sup> + CCl<sub>4</sub>) on sperms parameters in adult male rats after 28 days of experiment M±S.E. (n = 6).

Groups	Motility %	Dead %	Abnormality %	Concentration (x10 <sup>7</sup> )
G1	88.31±0.05 B	6.40±0.31 C	8.30±0.51 D	32.40±0.52 B
G2	93.05±0.30 A	10.04±0.51 B	5.03±0.13 C	40.30±0.41 A
G3	40.02±1.02 A	12.15±0.36 A	20.31±0.51 A	15.02±0.13 C
G4	73.21±1.01 C	7.02±0.61 C	15.00±0.53 B	30.21±0.83 B
LSD	37.4	1.93	2.20	5.32

G1: healthy control group; G2: animals received (0.035) of Phitofert<sup>®</sup>; G3: animals received (500 mg / kg. B.W.) of ccl<sub>4</sub>; G4: animals received (500 mg of ccl<sub>4</sub> + 0.035 mg of Phitofert<sup>®</sup>). Various capital letter denote significant differences between groups ( $P < 0.05$ ). Various small letter denote significant differences within groups ( $P < 0.05$ ).

## TESTICULAR SOMATIC INDEX

In Table 5 the testicular somatic index was shown, the associated weight of the tests showed a significant ( $P < 0.05$ ) increase in G2 rats compared to the administrative groups, while the G3 group showed a significant ( $P > 0.05$ ) decline in the weight of the tests compared to all administrated groups.



**Table 5:** Effect of CCl<sub>4</sub>, Phitofert® and (Phitofert® + CCl<sub>4</sub>) on testicular index (gm) in adult male rats after 28 days of experiment M±S.E. (n = 6).

Groups	G1	G2	G3	G4
Testicular weight to body weight ratio	0.364±0.01 B	0.606±0.01 A	0.300±0.02 C	0.432±0.01 B

LSD = 0.12; G1: healthy control group; G2: animals received (0.035) of Phitofert®; G3: animals received (500 mg / kg. B.W.) of ccl<sub>4</sub>; G4: animals received (500 mg of ccl<sub>4</sub> + 0.035 mg of Phitofert®). Various capital letter denote significant differences between groups (P < 0.05).

The significant (P < 0.05) decrease in the testicular somatic index also testosterone level following CCL<sub>4</sub> administration (Tables 1 and 5) may be due to CCL<sub>4</sub> toxicity. It may be producing ROS and influencing testicular weight, germ-line, and testosterone levels. Indeed, male infertility can be induced directly by free radicals or indirectly by altering male hormone homeostasis, resulting in reproductive failure (Hashem, 2021). Furthermore, a decrease in testosterone levels may be caused by the suppression of enzymatic pathways of hormones synthesis in the testes or adrenal gland, as well as interference with gonadotropin releasing hormone (GnRH) production, both of which induce a decrease in testosterone synthesis and release (Pineda and Dooley, 2003). The active ingredients maca plant extract and Selenium may contribute to the beneficial effect of Phitofert® on testicular tissue via a significant (P > 0.05) increase in testicular somatic index and testosterone production. According to the most recent research on the effects of maca on testicular gene expression for luteinizing hormone receptor, steroidogenic acute regulatory protein, and steroidogenic enzymes, maca increases testicular mRNA level for 3-hydroxysteroid dehydrogenase (3 β-HSD), an enzyme involved in androgen production. Enhanced 3β-HSD activity may enhance androstenedione, a precursor of testosterone, improving testosterone synthesis, this may also be linked to changes in the activity of reactive oxygen species (Sánchez et al., 2017). Yoshida et al. (2018) reported the same results of temporary testosterone rise by Leydig cells after feeding maca extracts for lengthy periods of time, indicating that maca may relieve the loss in steroidogenic capacity of Leydig cells with age. The treatment of Phitofert® with CCL<sub>4</sub> resulted in the restoration of normal testicular weight and testosterone levels. This finding indicated the involvement of Phitofert® as a scavenger of free radicals produced by CCL<sub>4</sub>. Phitofert® is a dietary supplement that contains numerous components such as adaptogenic and tonic plant extracts Maca, Ginseng and Withania. On the other hand, this medication contains certain vitamins, such as C and B12, that play a role in cell division (Al-Shahery 2019).

The other component was selenium, which inhibits mitophagy in Sertoli cells, regulates immunological genes and proteins in the blood-testis barrier, enhances testosterone production from Leydig cells, and increases

the width of the seminiferous tubules (Khoshbakht et al., 2020). Our investigation found a significant (P > 0.05) increase in both LH and FSH levels in CCL<sub>4</sub>-treated rats and a significant (P < 0.05) decrease in both hormones in Phitofert®-treated rats, with no significant changes in the CCL<sub>4</sub> and Phitofert®-treated groups (Tables 2 and 3).

These hormonal alterations are caused by the feedback mechanism and hormonal control associated with testosterone levels in these populations. Gonadal malfunction and testicular atrophy caused by a drop in testosterone concentration were prompted pituitary gland to produce LH and FSH in rats in the G4 group, restoring the level to normal. Furthermore, CCL<sub>4</sub> toxicity alters the pituitary-gonadal system, causing an increase in LH and FSH production to promote spermatogenesis (Arrack and Wassan, 2017). The restoration of LH and FSH production in the G4 group suggested that Phitofert® has antioxidant action due to bioactive constituents in this product such as selenium, which controls oxidative stress and increases anti-apoptotic proteins (like as bcl2) (Khoshbakht et al., 2020).

Finally, (Table 4) shows the effect of Phitofert® on sperm parameters, as well as the effect of CCL<sub>4</sub> on testicular sperm production and sperm parameters. Following that, the unfavorable changes in the functional characteristics of the testes caused by oxidative stress produced by CCL<sub>4</sub> in (G2) were passively reflected in the testes' ability to produce sperms and sperm quality. As a result, it was shown to have a decrease in sperm concentration, viability, normal morphology, and a regression in motility when compared to G1. On the other hand, it was discovered that the Phitofert® supplement can reduce CCL<sub>4</sub> toxicity in the testicles and epididymis. The administration of Phitofert® (G4) to CCL<sub>4</sub> rats reduced oxidative stress in testicular and epididymal tissue via a significant (p < 0.05) increase in testicular sperm production. Our findings might be attributable to a balance of oxidant-antioxidant state in testicular and epididymal tissue via its components, which included maca plant extract and selenium supplement (Al-Shahery, 2019).

Furthermore, our findings were consistent with many earlier studies that demonstrated the efficacy of Phitofert® in modulating the failure of various reproductive aspects

through the significant function of its component, maca. According to Gonzales et al. (2003) treatment with alcoholic extract of maca at dosages (48mg/day or 96 mg/day) for three weeks produced a progression of spermatogenesis when the distances of stages of seminiferous epithelium in rats were raised. Additionally, Gonzales et al. (2006) discovered that treating rats using aqueous extract of black maca (2mg.Kg-1) over 12 days resulted in improved impacts on spermatogenesis; hence, in the spermatogenic cycles, daily spermatogenesis was continuous until day (12) when compared to the control. Finally, all of the negative effects of CCL4 treatment on testicular function were caused by an oxidative deficiency in this tissue, which reflected on sperm parameters. Meanwhile, Phitofer<sup>®</sup> was able to repair this damage through the systemization of its components such as maca and selenium, both of which have antioxidant effects. As a result, Phitofer<sup>®</sup> may be used as a supplement to minimize the effect of free radicals on testicular tissue and restore testicular function.

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## NOVELTY STATEMENT

This is the first report to conduct a study using rats to determine effect of Phitofer<sup>®</sup> on CCL4 treated rats and their effect on sperm quality.

## AUTHOR'S CONTRIBUTION

All authors design and performing experiment. Zainab Sattar Ali, performed data analysis, interpretation, and literature. All of the author preparing and writing the manuscript

## CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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