

## Effects of the Carbon Tetra Chloride on the Body Weight and Ovaries, Uterus, Testes of the Rabbit

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### ABSTRACT

**Aims:** To investigate the effects of carbon tetra chloride on the body weight and ovaries, uterus, testes of the rabbit for 30 days.

**Methodology:** The rabbit were randomly divided into two equal groups (control and one treated groups), and the animals were treated as follow: 1- Control group C. (n = 15) received Distilled water 1ml /kg b.w four times a week intrapertunium for 30 days. 2- Treatment group. (n=15) were treated with carbon tetrachloride (1 mg / kg b.w mixing with equal volume of olive oil (1ml/ kg b.w.) four times a week/ intrapertunium for 30 days. The body weight of the animals detected at the day 1<sup>th</sup>, 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>th</sup> and 30<sup>th</sup> of the experimental period. At30 days animals sacrificed and ovaries, uterus, testis were removed and weighted, Samples of ovaries, uterus & testis were taken for histopathological study.

**Results:** Treatment with carbon tetra chloride causes a significant decrease in body weight. Also a significant decreased of ovarian, testicular weight and non-significant increase of uterus weight's.

**Conclusion:** Carbon tetra chloride causes decrease body

weight, degeneration testes, ovaries followed by effect on fertility.

**Keywords:** Rabbits, Carbontetrachloride, Ovaries, Uterus, Testes.


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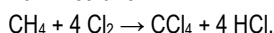
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### INTRODUCTION

Carbon tetra chloride was originally synthesized by the French chemist Henri Victor Regnault in 1839 but now produced from methane.<sup>1,2</sup>



It is ahaloalkane used extinguish fires cleaning solvent, a refrigerant, and in lavalamps.<sup>3,4</sup> CCl<sub>4</sub> leads to generation of free radicals that causes cell injury and necrosis.<sup>5,6</sup> It is also known to be hepatotoxic and nephrotoxic to experimental animals.<sup>7</sup> Acute exposure to carbon tetrachloride through any route cause gastrointestinal and neurological effects in the first 24 hours, liver damage occur after 24 hours and kidney damage can occur within 1-6 days in association with liver failure.<sup>8</sup> Chronic CCl<sub>4</sub> treatment induces hepatic fibrosis<sup>9</sup>, renal<sup>10</sup>, pulmonary<sup>11</sup> and testicular injuries<sup>12</sup> and cardiac tissue damage<sup>13</sup> in experimental animals. Tissue damage by CCl<sub>4</sub> depends on the amount of dosage and duration of exposure of the experimental animals to this toxicant.<sup>14</sup> Carbon tetrachloride also cause degeneration growth, decrease body weight and irritation of eyes, skin.<sup>15</sup> CCl<sub>4</sub> toxicity can affect the pituitary gland causing a reduction of FSH and LH leading to degeneration follicle in female. Also causes inhibition of testosterone with degeneration and decrease sperm count and testicular atrophy in male in experimental animals.<sup>16,17</sup> CCl<sub>4</sub> causes advance cirrhotic state that's lead to hypogonadism and gonadal dysfunction in male and female.<sup>18,19</sup>

The aim of the present study was to investigate the effects of Carbon tetrachloride on the body weight and weight of ovaries,

uterus, testes with histological changes of ovaries, uterus, testes in rabbits.

### MATERIALS AND METHODS

Thirty adult rabbit were obtained from market of Kut. Animals were placed at the animal house, College of science, Wasit University and fed with pellet during experimental periods; temperature was 25C°. Female was left in a separate cage with one male for the each cage ratio 2:1. Male and female couples were kept together in mating cage for four weeks.

The rabbit were randomly divided into two equal groups (control and one treated groups), and the animals were treated as follow:

1: Control group C. (n = 15) received Distilled water 1 ml /kg b.w four times week intrapertunium for for 30 days

2: Treatment group. (n=15) were treated with carbon tetrachloride (1 mg / kg b.w mixing with equal volume of olive oil (1ml/ kg b.w.) four times week intrapertunium for 30 days.<sup>20</sup>

The body weight of the animals detected at the day1<sup>th</sup>, 7<sup>th</sup>,14<sup>th</sup>, 21<sup>th</sup> and 30<sup>th</sup> days of the experimental period by electrical balance. After eight day until end of experiment period every day the females were examined for signs of pregnancy by palpating the abdomen. At end of experiment period rabbit weighted and sampling ovaries, uterus, testes weighted by sensitive balance then preserved in 10% formalin buffer solution until preparation of histopathological section. Tissue was cut at 7-8µm and embedded in paraffin and takes sections of ovaries, -uterus, testes were

stained with hematoxylin –Eiosin stain (H&E) for histopathological study.<sup>21</sup>

**Statistical Analysis**

Data were expressed as mean ± standard error of mean and were compared by one way ANOVA followed by LSD. P value more than 0.05 was considered as statistically significant.<sup>22</sup>

**RESULTS AND DISCUSSION**

Table 1 showed a significant decrease of body weight in carbon tetra chloride compared with the control along time of the experimental periods. Except one day non-significant between carbon tetra chloride compared with the control. Carbon tetrachloride is readily absorbed from the gastrointestinal tract causes vomiting and gastrointestinal pain apparent following acute exposure,<sup>23,24</sup> but in chronic exposure causes irritation to the gastrointestinal tract with cell injury and necrosis followed by

degeneration growth, decrease body weight gradually.<sup>25,26</sup>

Data in table 2 demonstrated that there was significant decrease in testicular weight with carbon tetrachloride when compared with control group. CCl4 toxicity cues inhibition of testosterone may cause a decrease in the number and function of somatic and germinal cells of testis followed by a testicular weight reduction.<sup>27,28</sup>

The result of Table 3 showed a significant decrease in the weight of the ovaries in CCL4 treated group when compared with that of normal control. CCl4toxicity affected the pituitary gland causing a reduction in level of FSH and LH leading to inhibition of follicles and hypogonadism.<sup>29</sup>

Table 4 indicated that, there was no significant increase in the weight of the uterus of CCL4 treated group when compared with of normal group. Hypertrophy and hyperplasia of endometrium and myometrium followed by enlargement of uterus.<sup>30</sup>

**Table 1: Effect of Carbon tetrachloride on the body weight of the adult rabbit in the different periods**

Time	Control Weight rabbit Kg	Carbon tetrachloride Weight rabbit Kg
1day	1.98 ± 0.017 a	1.96 ± 0.006 a
7 day	2.2 ± 0.029 a	1.60± 0.052 b
14 day	2.5 ± 0.097 a	1.44± 0.033 b
21 day	2.11 ± 0.077 a	1.20 ± 0.034 b
30 day	2.15± 0.08 a	1.15± 0.68 b

-The value represent Mean(gram) ±Standard Error-The different small letters show significant effect while the same small letters show insignificant effect between different groups.

**Table 2: Effect of CCl4 on testicular weight in adult male rabbit**

Groups Time	Control	Carbon tetrachloride
After 30 day of treatment	1.86 ± 0.054 a	0.34± 0.043 b

-The value represent Mean (gram) ±Standard Error

-The different small letters show significant effect

**Table 3: Effect of CCl4 on ovarian weight in adult female rabbit**

Groups Time	Control	Carbon tetrachloride
After 30 day of treatment	0.80 ± 0.057 a	0.36± 0.015 b

The value represents Mean (gram) ±Standard Error-

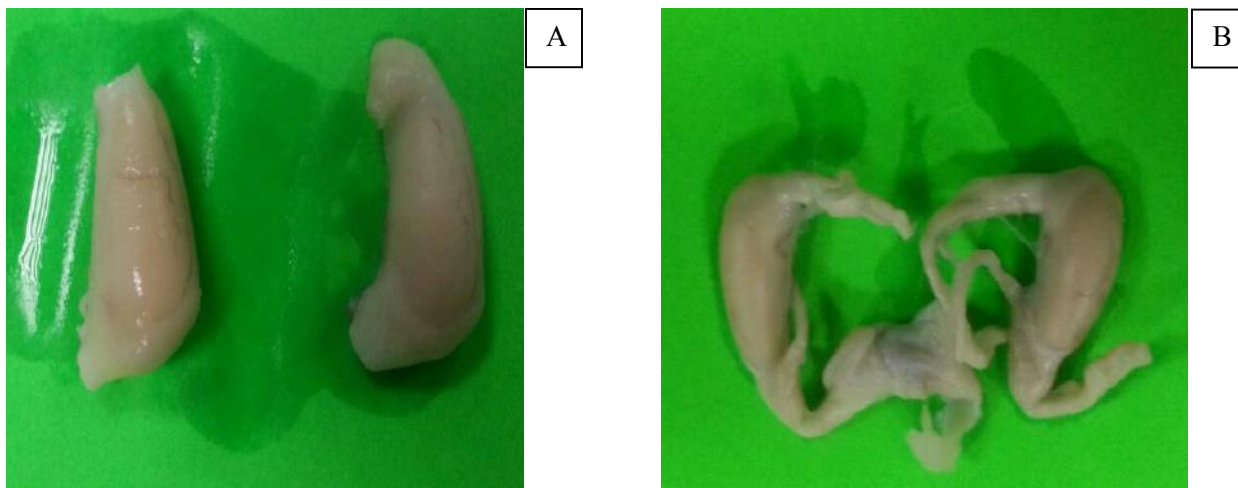
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**Table 4: Effect of CCl4 on uterus weight in adult female rabbit**

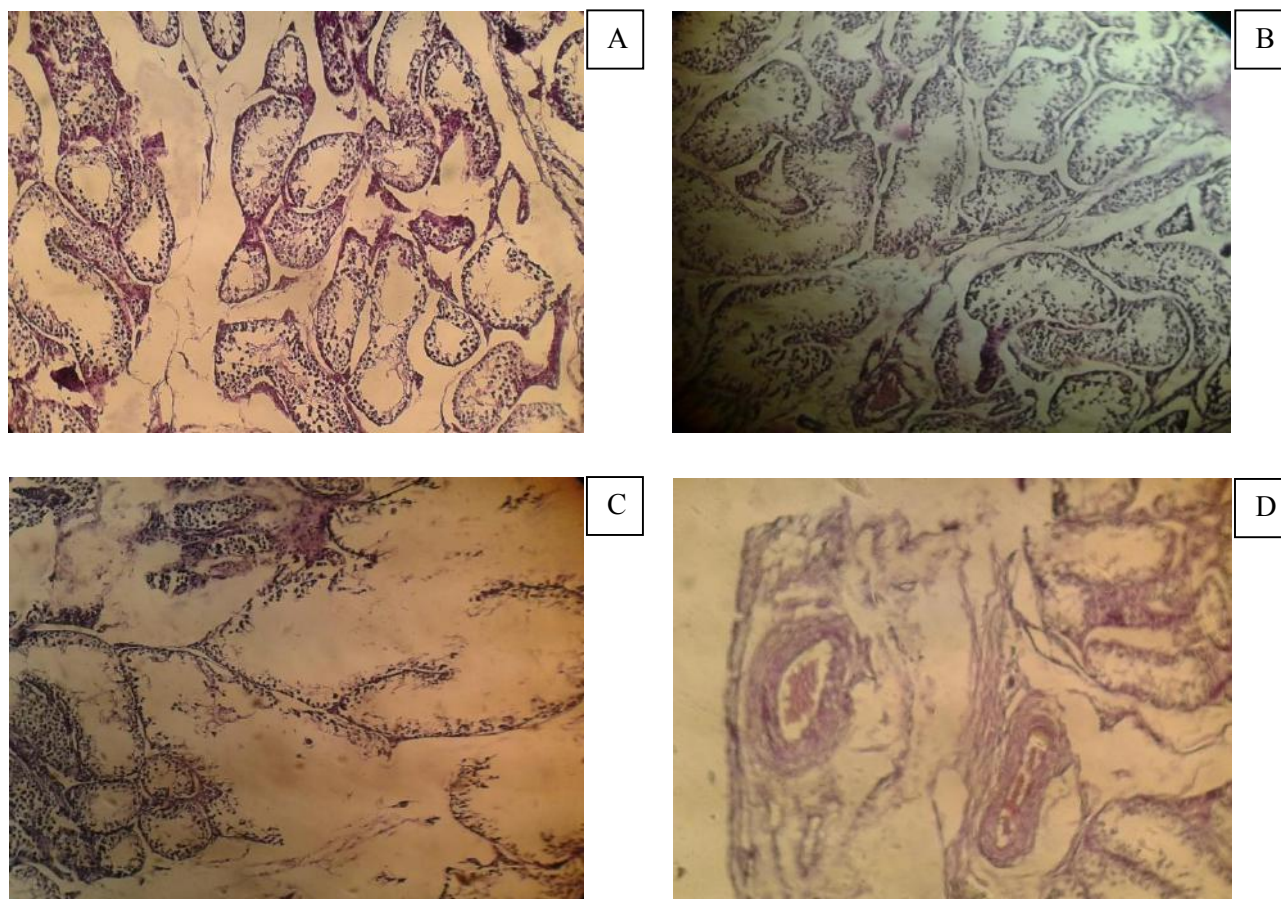
Groups Time	Control	Carbon tetrachloride
After 30 day of treatment	0.45 ± 0.068 a	0.55± 0.029 a

The values represent Mean (gram) ±Standard Error

-The same small letters show in significant effect



**Fig 1: Gross examination of testes rabbits (A) control group. (B) group treated with 1mg/kg CCL4 mixed with 1 ml of olive oil.(I.P)**

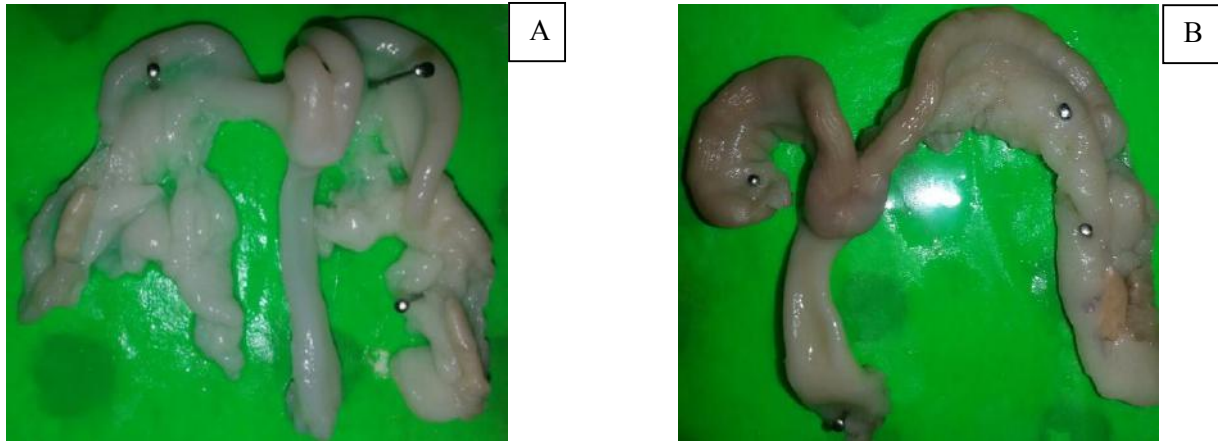


**Fig 2: Histopathological examination of testes rabbits (under fox 10\*0.25)**

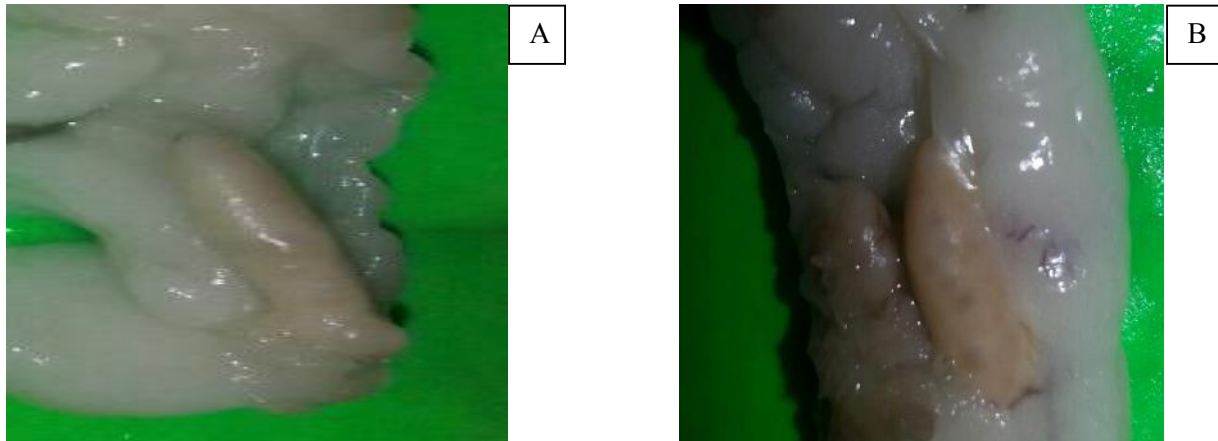
Cross examination of testis rabbits (Fig 1- A control group) normal size with no congestion while in (Fig 1- B treated with carbon tetra chloride) degeneration and atrophy testes with congestion. CCl<sub>4</sub> causes advance cirrhotic and necrosis by free radicals which lead to testicular atrophy and dysfunction.<sup>31</sup> Enlargement of the capillary by CCl<sub>4</sub> followed by congestion.<sup>32</sup> Histopathological examination of testes rabbits in (Fig 2-A) control group no degeneration in seminiferous tubule and in leydig cell. (Fig. 2-B) control group show normal size blood vessels (Fig. 2-C) treated group with 1mg/kg of CCL<sub>4</sub> mixed with 1 ml of olive oil.(I.P) showed degeneration in seminiferous tubule and leydig cell (Fig. 2-D) treated group hyperplasia blood vessels.

CCl<sub>4</sub> induce oxidative stress and free radical in testis may cause degeneration with atrophied of leydig cells with tubules except blood vessels cause enlargement.<sup>33,34</sup> Cross examination of ovaries rabbits in (Fig. 4-A control group) showed normal size with no hemorrhage while in (Fig. 4-B treated with carbon tetra chloride) showed degeneration and atrophy ovary and hemorrhage spot on ovarian surface. It is evidenced that metabolic activation of CCl<sub>4</sub> by cytochrome P450 resulted in the production of trichloromethyl radical ( $\cdot\text{CCl}_3$ ) and peroxy trichloromethylradical ( $\cdot\text{OCCl}_3$ ) that, in turn, initiate subsequent lipid peroxidation, responsible for ovarian injuries and degeneration.<sup>35</sup> Free radical by CCl<sub>4</sub> which lead hyper atrophy capillary followed by hemorrhage.<sup>36</sup>

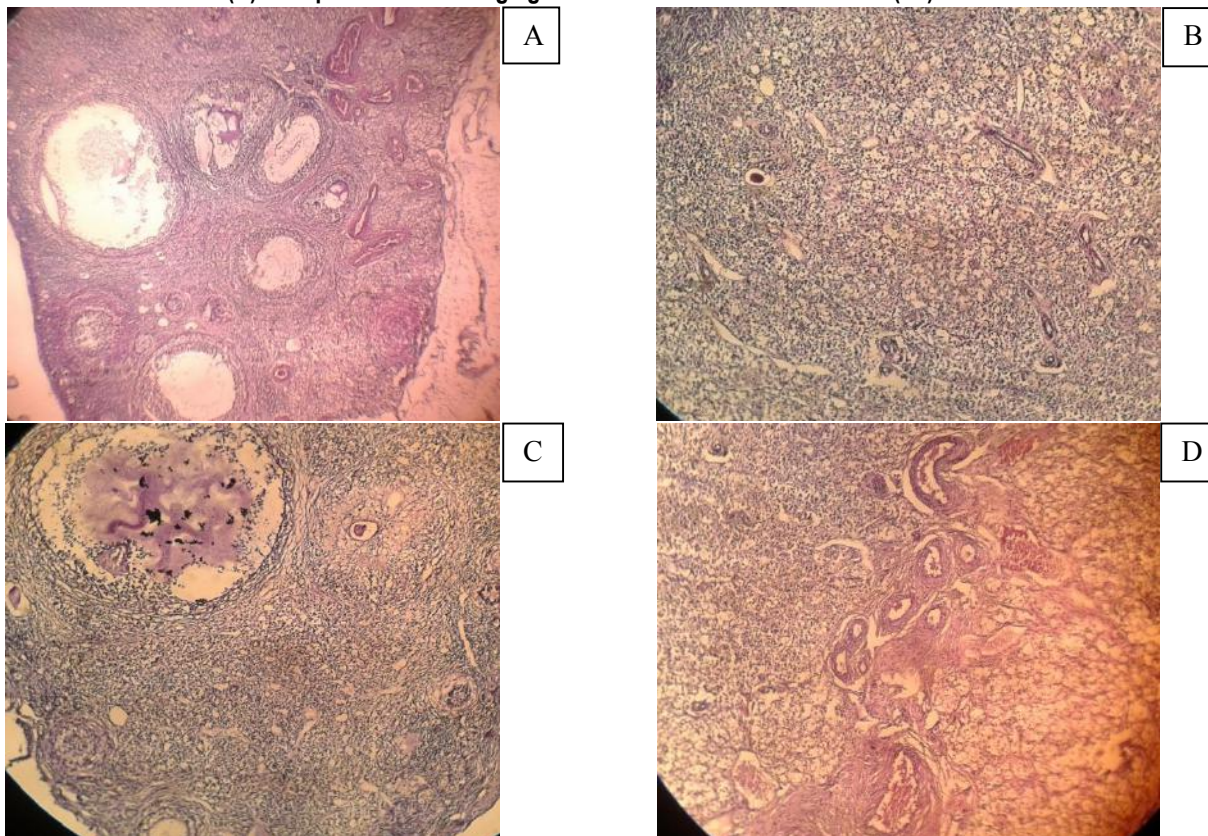




**Fig 3: Cross examination of ovaries and uterus rabbits (A) control group. (B) Group treated with 1mg/kg CCL4 mixed with 1 ml of olive oil. (I.P)**



**Fig 4: Cross examination of ovarian rabbits (A) control group. (B) Group treated with 1mg/kg CCL4 mixed with 1 ml of olive oil.(I.P)**



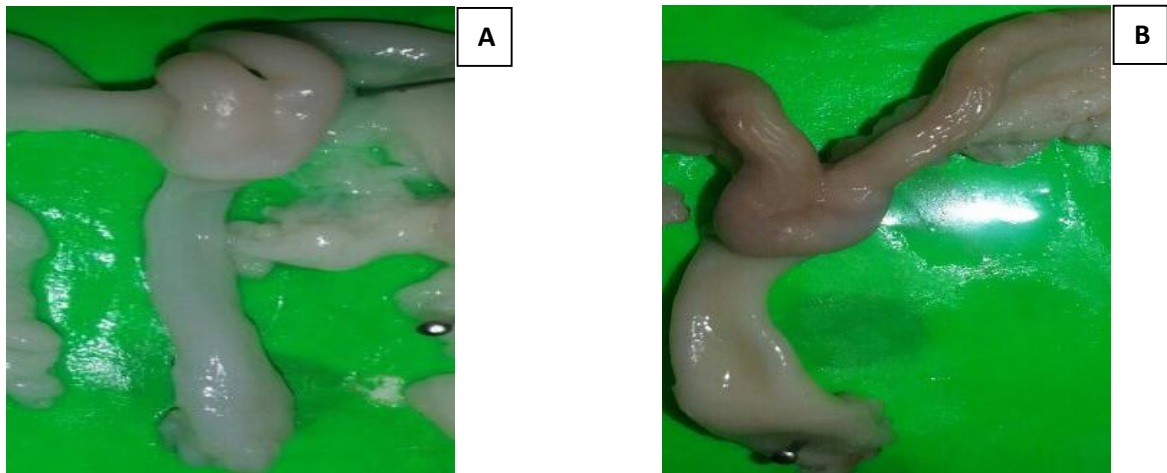
**Fig 5: Histopathological examination of ovarian rabbits (under fox 10\*0.25)**



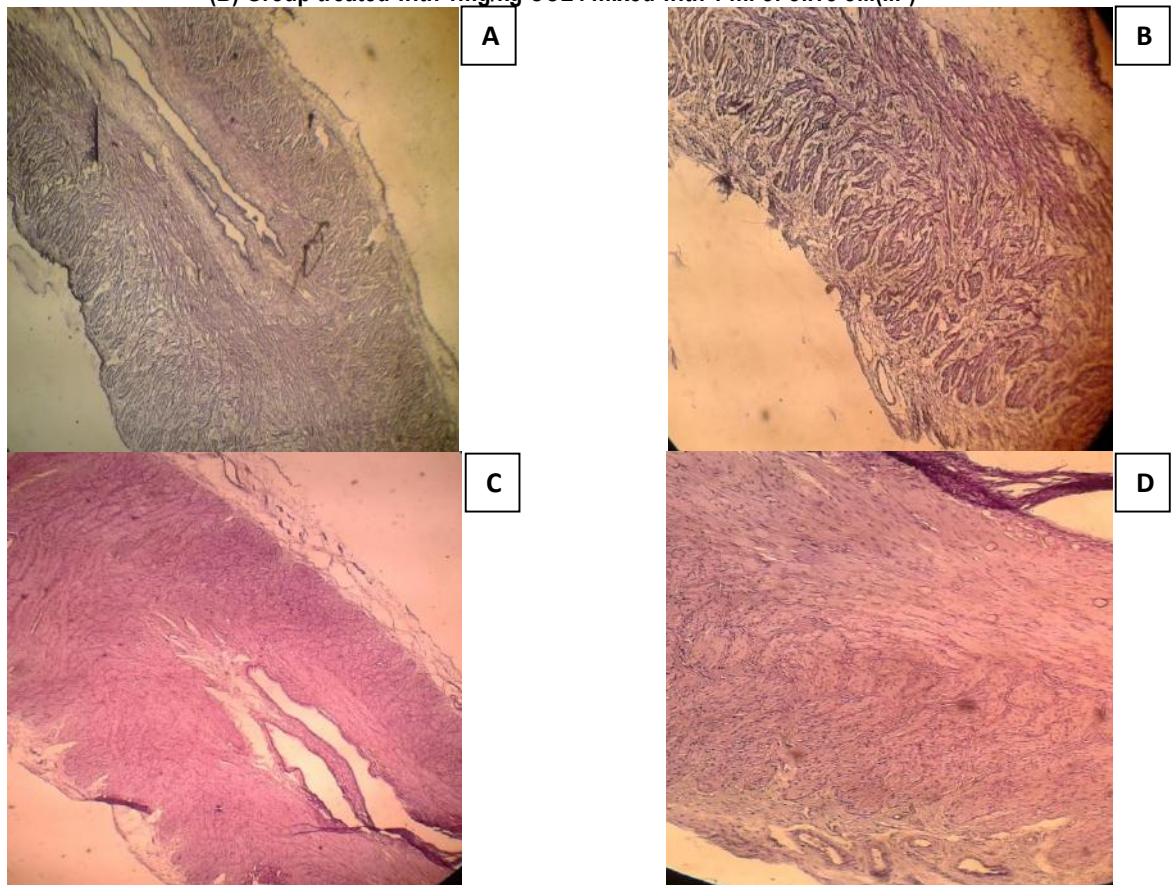
Histopathological examination of ovaries rabbits in (Fig. 5-A control group) normal size follicles and no degeneration. (Fig. 5-B control group) showed normal size blood vessels. (Fig. 5-C treated group with 1mg/kg CCL4 mixed with 1 ml of olive oil.(I.P) atrophy follicles and degeneration. (Fig. 5-D treated group) hyperplasia blood vessels. CCL4 lead to generation oxidative stress, which produces major interconnected changes of cellular metabolism, increases fragmentation, and destruction with necrosis and degeneration germinal cell with layers but blood vessels may hyperplasia.<sup>37,38</sup>

Gross examination uterus rabbit of in (Fig. 6-A control group) normal shape and no congestion while in (Fig. 6-B treated with carbon tetra chloride) enlargement of uterine horns, body which were swollen and extensive edema of the organ with congestion. CCl4 causes extensive hemorrhages due to hyperplasia and

hypertrophy of the capillary which leads congestion. Hypertrophy of mucosa and muscular layer that cause enlargement of uterus.<sup>39</sup> Histopathological examination of uterus rabbits in (Fig. 7-A control group under fox 4\*0.10) consist endometrium, myometrium, with normal size. (Fig7-B control group under fox 10\*0.25) normal size glands with blood vessels were seen. (Fig. 7-C treated group under fox 4\*0.10 .consist of 1mg/kg CCL4 mixed with 1 ml of olive oil.(I.P) proliferation endometrium and myometrium. (Fig. 7-D treated group under fox 10\*0.25) hyper atrophy gland with blood vessels. CCl4 generates free radicals that causes hyperplastic or metaplastic changes of lining of epithelium of the endometrium, which was that of uterine mucous cuboidal type with proliferation endometrium, myometrium. As well as capillaries and glands there was hypertrophy.<sup>40,41</sup>



**Fig 6: Cross examination of uterus rabbits (A) control group. (B) Group treated with 1mg/kg CCL4 mixed with 1 ml of olive oil.(I.P)**



**Fig 7: Histopathological examination of uterus rabbits**

**CONCLUSION**

The present study has demonstrated the induction of Carbon tetrachloride in intraperitoneum in rabbit animal affects body weight, testes, ovaries, uterus.

The results also recorded decrease weight of (body, testes, ovaries) while increased in uterus weight induced by carbon tetrachloride.

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**REFERENCES**

- Fasanmade O. (2008). Carbon tetrachloride Archived, at the Wayback Machine.23 (11): 2437- 2448.
- Seifert W, Bosma A and Brouwer A. (2004). Vitamin A deficiency potentiates carbon tetrachloride-induced liver fibrosis in rabbits. *Hepatology* 19 (1): 193–201.
- Rowe V, Collister D and Irish D. (2002) Carbon tetrachloride determined by experiments on laboratory animals. *Arch. Indust. Hyg. Occup. Med.* 6(1):55-68.
- Manibusan M, Jinot J, Kopylev L and Schlosser P. (2010). Toxicological Review of Carbon Tetrachloride. U.S. Environmental Protection Agency.56:23-25.
- Naidoo V and Eloff J. (2014). The antibacterial activity, antioxidant activity and selectivity index of leaf extracts of thirteen South African tree species used in ethnoveterinary medicin to treat helminth infections. *BMC Veterinary research.* 10: 52-63.
- Abdou H, Salah F, Hoda B and Abdel Rahim E. (2012). Antioxidant effect of celery against carbon tetra chloride induced hepatic damage. *African Journal of Microbiology Research.* 6(27): 57-67.
- Yoshimura A, Ideura T, Shirai M, Tiara T and Koshikawa S. (2003). The distribution of H-labeled endotoxin in the kidney of liver cirrhotic rabbit. *Scand. J. Urol. Nephrol.* 8(28):295-299.
- Adams E, Spencer H, Rowe V, McCollister D and Irish D. (1992). Vapor toxicity of carbon tetrachloride determined by experiments on laboratory animals. *Arch. Indust. Hyg. Occup. Med.* 6(1):50-66.
- Boll M, Weber LWD and Becker E. (2001a) Pathogenesis of carbon tetrachloride-induced hepatocyte injury: Bioactivation of CCl<sub>4</sub> by cytochrome P450 and effects on lipid homeostasis. *Z Naturforsch [C]* 56:111–121.
- Boll M, Weber LWD, Becker E. (2001b) Hepatocyte damage induced by carbon tetrachloride: inhibited lipoprotein secretion and changed lipoprotein composition. *Z Naturforsch [C]* 56:283–290
- Kim J and Muralidhara S. (1990). Influence of route and pattern exposure on the pharmacokinetics and hepatotoxicity of carbon tetrachloride. Principle of route-to-route extrapolation for risk assessment. New York, NY: Elsevier Science Publishing Co., Inc., pp. 271–284
- Bruckner J, Ramanathan R and Lee M. (2002). Mechanisms of circadian rhythmicity of carbon tetrachloride hepatotoxicity. *J Pharmacol Exp Ther* 30:273–281.
- Castro J and Fenos O. (2002) Effect of cystamine on the mixed function oxygenase system from rat liver microsomes and its preventive effect on the destruction of cytochrome P450 by carbon tetrachloride. *Pharmacol Res Commun* 4):185–190.)
- Condie L, Laurie R and Mills T. (1996) Effect of gavage vehicle on hepatotoxicity of carbon tetrachloride in CD-1 mice: corn oil versus Tween-60 aqueous emulsion. *Fundam Appl Toxicol* 7:199–206.
- Varela-Moreiras G, Alonso-Aperte E and Rubio M. (1995) Carbon tetrachloride-induced hepatic injury is associated with global DNA hypomethylation and homocysteinemia: effect of S-adenosylmethionine treatment. *Hepatology* 22:1310–1315.
- Wang MY and Liehr J. (1995) Lipid hydroperoxide-induced endogenous DNA adducts in hamsters:possible mechanism of lipid hydroperoxide-mediated carcinogenesis. *Arch Biochem Biophys*16:38–46.
- Mills T and Klein B. (2004). Textbook of Veterinary Physiology. 3rd edition. Elsevier Saunders Company, USA, Pp:420-421
- Recknagel R and Glende E. (2004). Mechanisms of carbon tetrachloride toxicity. *Pharmacol Therapeut*; 43:139-154.
- Weber L, Boll M and Stampfl A. (2003) .Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. *Crit Rev Toxicol.* 33: 135-136.
- Nabeshima Y and Kanno K. (2006). Antifibrogenic function of angiotensin II type 2 receptor in CCl<sub>4</sub>-induced liver fibrosis. *Biochem Biophys Res Commun.* 346: 658-664.
- Shyu M, Kao R and Yen G. (2008). Prevents against rat liver fibrosis induced by CCl<sub>4</sub> via inhibition of hepatic stellate cells activation. *Food Chem Toxicol.*46: 3707-3713.
- Torries JH and Tazuma S. (1990). Principles and Procedures of Statistics. Biometrical approach, 2nd edition. McGraw-Hill Book Co. New York, USA.33:5-8
- Chance B and Maehly A. (1995). Assay of catalase and peroxidases. *Methods Enzymol.* 11: 764-775.
- Kakkar P, Das B, Viswanathan P. (2004). Modified spectrophotometric assay of superoxide dismutase. *Indian J Biochem Biophys.* 21: 130-132.
- Habig W, Pabst M and Jakoby W. (1997). Glutathione-S-transferases:the first enzymatic step in mercapturic acid formation. *J BiolChem.* 249: 7130-7139.
- Carlberg I and Mannervik, E. (1998). Carbon tetra chloride level in experimental animal. *J BiolChem.* 250: 4475-4480.
- Mohandas J, Marshal J, Duggin G, Horvath J and Tiller D. (2004) Differential distribution of glutathione and glutathione-related enzymes in rabbit kidney. Possible implications in analgesic nephropathy. *Biochem Pharmacol.* 33: 1801-1807
- Benson A, Hunkeler M and Talalay P. (1990). Increase of NADPH, quinone reductase activity by dietary antioxidant: possible role in protection against carcinogenesis and toxicity. *Proc Natl Acad Sci U S A.* 77: 5216-5220.
- Jollow D, Mitchell J, Zampaglione N and Gillete J. (1998) Bromobenzene induced liver necrosis. Protective role of glutathione and evidence for 3, 4 bromobenzene oxide as a hepatotoxic metabolite. *Pharmacology* 1: 151-169.
- Iqbal M, Sharma S, Zadeh H, Hasan N and Abdulla M. (2006) Glutathione metabolizing enzymes and oxidative stress in ferric nitrile triacetate (Fe-NTA) mediated hepatic injury. *RedoxReport.* 2: 385-391.
- Pick A and Keisari Y. (1991). Superoxide anion and hydrogen peroxide production by chemically elicited peritoneal macrophages induction by multiple nonphagocytic stimuli. *Cell Immunol* 59: 3011-3018
- Jorsaraei A, Firoozjaee A, Pasha Y, Marzony T and Sarabi E. (2010). Histopathological Effects of single dose treatment of diazinon on testes structure in rat. *Yakhteh Med. J.,* 12 (1):39-42.
- Cunningham J and Klein B. (2007). Textbook of Veterinary Physiology. 4rd edition. Elsevier Saunders Company, USA, :421-422
- Ismail H and Al-nahari H. (2009). Therapeutic and protective role of Panax ginseng on pituitary and testicular axis in *Pharmacology*1: 177-179.

35. Maritim A, Sanders R and Watkins J. (2003). Effects of  $\alpha$ -lipoic acid on biomarkers of oxidative stress in streptozotocin-induced diabetic rats. *J Nutr Biochem.* 14:288–294.
36. Marnett J. (2000). Oxyradicals and DNA damage. *Carcinogenesis.* 21:61–70.
37. Murugesan G, Sathishkumar M, Jayabalan R, Binupriya A and Swaminathan K. (2009). Hepatoprotective and curative properties of Kombucha tea against carbon tetra chloride-induced toxicity. *J Microbiol Biotechnol.* 19:397–402
38. Sipes IG, Krishna G and Gillette J. (1977). Bioactivation of carbon tetrachloride, chloroform and bromotrichloromethane: role of cytochrome P-450. *Life Sci* 20:1541–1548.
39. Sivikova K, Piesova E and Dianovsky J. (2001) The protection of vitamin E and selenium against carbon tetrachloride-induced genotoxicity in ovine peripheral blood lymphocytes. *Mutat Res* 494:135–142.
40. Slater T. (1981) Free radicals as reactive intermediates in tissue injury. *Adv Exp Med Biol* 136:575–589.
41. Slater T. (1982) Activation of carbon tetrachloride: chemical principles and biological significance. In: McBrien, DCH; Slater, TF; eds. *Free radicals, lipid peroxidation and cancer.* New York, NY: Academic Press, pp. 243–274.

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