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Effects of Crocin and resistance training on liver enzymes and PG axis hormones in toxicated male rats by Nandrolone Decanoate

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Abstract: Introduction: The purpose of this study was to investigate the effects of crocin and resistance training on liver enzymes and Hypothalamic - pituitary-gonadal axis hormones in toxicated male rats by Nandrolone decanoate.
Method & materials: Fifty six male Wistar rats (age: 9 weeks, weight: 190±10g) were randomly divided into eight groups: control group (Co, n=7), sham group (Sh), 10 mg/kg of Nandrolone decanoate group (Nd), resistance training group (RT), 12.5 mg/kg of crocin group (C1), 25 mg/kg of crocin group (C2), RT and 12.5 mg/kg of crocin group (RT-C1) , RT and 25 mg/kg of crocin group (RT-C2).All the groups were injected by Nandrolone decanoate (10mlg/kg of body) except (Co and Sh)groups. Resistance training was progressively and consisted of climbing the ladder and started with carrying a load of 50% of body weight. Twenty four hours after the last injection, the animals were sacrificed. Blood samples were obtained from left ventricle immediately and analyzed for liver enzymes (AST, ALT, ALP and GGT), albumin, total protein and PG axis hormones (LH, FSH, testosterone, DHT). Testicular and liver were brought out of their body for analyzing. Data were analyzed using two way analysis of variance.
Results: Data analysis revealed that the interaction effect of crocin and resistance training cause to significant changes on ALT, GGT, Albumin, Total protein, FSH levels($P<0.05$). The main effect of crocin on LH, testosterone, DHT, albumin and total protein were significant ($P<0.05$). Also, there were not any significant changes in number of spermatogonia, primary spermatocytes, spermatids and sertoli cells ($P>0.05$).
Conclusion: In general, resistance training and crocin consumption result in eliminate or improve of complication of Nandrolone decanoate in liver and testis organ.
Key words: Resistance training, Nandrolone decanoate, Crocin

INTRODUCTION

Anabolic-androgenic steroids (AAS) are compound derivatives of the male testosterone hormone (Joo and Sone, 2003). Nandrolone decanoate (ND) has been indicated to improve physical and muscular performance (Johansen et al, 1998), and resistance exercise training enhances strength (Headley et al, 2002). The long term use of steroids causes heart attack, liver toxicity, suppression of endocrine nervous glands (Kanayama et al, 2006), change in sexual tendencies and test is atrophy (Padersen et al, 2001). Disorder in axis pituitary–gonadal axis (PG axis hormones) function induces to change in its hormones such as LH, FSH and testosterone.The hypothalamus produces peptide hormone gonadotropin releasing hormone (GnRH) which it

provokes the synthesis and release of gonadotropic hormones: luteinizing hormone (LH) and follicle stimulating hormone (FSH) in adenohypophysis. They released into the systemic blood circulation and in testis, FSH regulates the spermatogenesis via sertoli cells and LH stimulates testicular androgens secretion such as testosterone and its activated form dehydrotestosterone (DHT) by effecting the leydig cells. The testosterone and DHT causes the development of male secondary sexual characteristics and inhibiting the pituitary secretion of LH and FSH (Campo et al, 2006). The liver is the largest organ of the human body and by help of difference enzymes has important roles in regulation of hormones activity and metabolism during resting, training and recovery. The main role of liver is detoxification of steroid hormones which long term consumption of them by people causes to accumulate theses steroids and result in liver toxicity (Singh et al, 2011).The best way for clinical assessing of liver is via measuring the liver enzymes activity (AST[1], ALT[2], ALP[3]and GGT[4]), because if the liver cells get injured, these transaminases would increase in blood and would finally damage the liver(Chung et al, 2007). Silva and Beneto reported that testosterone undecanoate (TU) treatment along with moderate physical training increased liver damage.Nowdays, inside the chemical drug treatments, try to use physical activity and herbs in treatment of liver cells and testis cells damages. One of these herbalsisCrocus sativus L, which is commonly known as saffron. The effective components of saffron arecrocin, crocetin and safranal. Crocin is a carotenoid and is responsible for the red color of saffron (Mohamadpour et al, 2013). Crocinhas different pharmacological effects such asprotective effects againstcardiovasculardiseases, nervous system protection, liver protection and inhibition of tumor cells proliferation. However, among the different protective mechanisms, the antioxidant activity of crocin is responsible for the pharmacological effects. With due attention to hypolipidemic andantioxidant activity effects of crocin, probably it can protect the liver fromSteatosis and oxidative stress (Sheng et al, 2006). Also, compound of saffron stigma acts as a stimulant to produce LH, FSH and testosterone hormones, proliferatethe possibility of seminiferous tubules epithelial cells and increase of leydig cells activity , and thus it causes to significant increase inSpermatocytes and Spermatogenesis levels (Modaresi et al,2008).With due attention to protective and impressive effects of physical activity and crocin on liver and testis and prevalence of consumption of Nandrolone decanoate between athletics and adults, this study is investigate the effects of crocin and resistance training and interaction of them on liver enzymes and PG axis hormones in toxicated male rats by Nandrolone decanoate.

2. Methods

2.1. Subjects

Fifty six male Wistar rats (age: 9 weeks, weight: 190±10g) were randomly divided into eight groups. Control group were fed standard rodent chow (Co, n=7), sham group were injected by 0.2 CC of saline(SH, n=7), 10 mg/kg of Nandrolone decanoate (ND,=7), resistance training (RT, n=7), 12.5 mg/kg of crocin (C1, n=7), 25 mg/kg of crocin (C2, n=7), RT and 12.5 mg/kg of crocin (RT-C1, n=7) , RT and 25 mg/kg of crocin (RT-C2, n=7). Animals were kept in a cage under a 12/12 h light/dark cycle at 22 ± 2°C, humidity of 50-60% had free access to water and food. Animals were acclimatized and familiarized to the laboratory conditions and exercised for the duration of fourteen days before the beginning of the protocol

2.2.Experimental design

1. Aspartate aminotransferase
2. Alanine aminotransferase
3. Alkaline Phosphatase
4. Gamma Glutamyl transferase

After obtaining the approval of the Institutional Review Board of our medical school, all experiments were carried out in accordance with the Guidelines of the Animal Care and use ethics committee of Baqiyatallah University of medical sciences.

Training exercise protocol was progressive resistance training form including 3 sessions per week for 8 weeks with three sets of five repetitions. The rest among sets and repetition were 90 s and 60 s, respectively. The resistance training consisted of climbing the ladder (1 meter with 26 escalators inclined at 85 degrees) carrying a load suspended from the tail. The initial load in the first week was 50% of their body weight and increased10%of their bodyweight gradually each week so that at 8th week arrived to 120% of their body weight (Secular and Roger, 2003). The rats were familiarized with the climbing the ladder two weeks ago. All injections were done between 12 AM to2 PM. The supplementation groups received crocin daily. The control group presented in training place but did not have any activities, consumption of any crocin or ND. All the groups received intramuscular injections, every week, in the quadriceps muscles using insulin syringe containing 10 mg / kg of ND except control and sham groups. The weight of animals were measured in initial and the end of protocol.

2.3.Crocin supplement

Crocin was powdered and then was packed in 1.5gr packs. Crocin was the product of Sigma company made in Germany. They were kept at +4°C.Both doses of crocin diluted in 0.2 CC of saline and was intraperitoneally injected (IP) daily at 2 PM.

2.4.Blood sampling and analysis

Twenty four hours after the last injection, the animals were anaesthetized by ether (Merk, Germany) and sacrificed. Blood samples were obtained from left ventricle immediately. Plasma was separated by centrifugation (3000g, 15min at 20°C) immediately after blood sampling and was frozen and stored at −20°C for subsequent analyses. The livers and testises were removed. The samples were analyzed for liver enzymes (AST, ALT , ALP and GGT), total protein, albumin and PG axis hormones (LH, FSH, DHT and testosterone). Serum AST, ALT , ALP,GGT, total protein, albumin, LH , FSH,DHT and testosterone were measured by radioimmunoassay (RIA) using kits (Monobind, USA).

2.5.Histological examination of testis tissue

The testises immediately were fixed in 10% formalin solution. Five micron thickness of testises sections were stained by hematoxylin-eosin (H& E) and examined by Japanese light microscopy.

2.6.Histological examination of liver tissue

The livers were fixed in 10% formalin. After fixing the tissue, it was thoroughly washed under running water and standard dehydration. Sections of 5 μm were cut in a microtome and were
evaluated by light microscopy

2.7. Statistical analysis

All statistical analyses were performed using the software statistical package SPSS version 23.0 (SPSS, Inc., Chicago, IL, USA). Data were expressed as mean ± standard deviation. All data were normalized by a Kolmogorov-Smirnov test. Levene test was used to assess the homogeneity of variances for a calculable variable. Data changes before and after training protocol were analyzed using the two way analysis of

variance (ANOVA), if any significant differences had been observed Bonferroni post hoc test had to be used to determine this differences. Statistical significance was set at $P<0.05$.

3. Results

All obtained data from the variables, based on mean and standard deviation (Mean±SD) are presented in tables 1, 2 and 3.The results of this study showed that there was significant difference among the body weight of groups ($F_{7,46}=4.099$, P=0.001). The highest and the lowest Body weight (67.46% and 35.58% , respectively) among the groups were in (Co)group and C1 group, respectively (table 1).

The main effect of resistance training and crocin significantly reduced the LH levels ($F_{1,34}=22.470$, P=0.000 and $F_{2,34}=30.134$, P=0.000, respectively)and to increase testosterone($F_{1,34}=19.028$, P=0.000 and$F_{2,34}=33.691$, P=0.000, respectively) and DHT ($F_{1,34}=14.120$, P=0.001, $F_{2,34}=36.235$, P=0.000)levels in all the groups. The main effect of crocin on FSH was significant ($F_{2,34}=5.237$, P=0.010) (table3). The main effect of crocin on albumin ($F_{2,34}=19.730$, P=0.000) and total protein ($F_{2,34}=19.741$, P=0.005) were significant (table2). Also, The main effect of resistance training and crocin and interaction of them on weight of liver and testis, were not significant ($p>0.05$) (table 2 and 3).

The interaction effect of crocin and resistance training significantly decreased ALT ($F_{2,34}=3.298$, P=0.049) (fig 1), albumin ($F_{2,34}=12.590$, P=0.000)(fig 4)and total protein ($F_{2,34}=6.091$, P=0.005)(fig 3) levels in all the groups but this reduction in RT-C1 group was more (table 2).The interaction effect of crocin and resistance training significantly decreased FSH levels($F_{2,34}=10.642$, P=0.000)in all the groups but this reduction in RT-C2 group was more (fig 5 and table 3). The interaction effect of crocin and resistance training significantly increase GGT levels ($F_{2,34}=0.174$, P=0.039) in all the groups but this increase in RT-C1 group was more (fig.2 and table 2). Also, the interaction effect of crocin and resistance training did not cause any significant changes in ALP, AST, LH, testosterone and DHT levels in all the groups ($P>0.05$).

The results of testis histological analysis showed that no pathologic changes were seen in the groups that had received ND. In general, there were not any certain changes in number of spermatogonia, primary spermatocytes, spermatids and sertoli cells in experimental groups to control but leydig cells increased dramatically in experimental groups that had received crocin (fig6). Also, decrease of leydig cells in Nandrolone decanoate group (ND group) was seen.

4. Discussion

The purpose of this study was to investigate the effects of crocin and resistance training on liver enzymes and PG axis hormones in toxiciated male rats by Nandrolone decanoate. Based on the protocol, all the groups except the control and sham groups were confronted with high amount of ND (10 mg / kg of Nandrolone decanoate) every week. Body weight of all the groups increased. The highest and the lowest Body weight (increase of 67.46% and 35.58% respectively) among groups were in control group and C1 group, respectively. The results demonstrated that ALT levels were at least amount in resistance training group and this reduction along with crocin supplement especially dose of 12.5 mg/kg of crocin was significant and more clear. Interaction effect of resistance training and crocin decreased AST and ALP activity but were not significant but it is important clinically aspect and should not be unseen. Although, exercise that itself is one of the damaging factors on liver, it had made it possible that with reducing the complication of ND and the mediation of crocin, eliminate the harmful effects of using ND in high amount. It is interesting that training groups had the lowest albumin levels and the groups which had not use crocin had the highest albumin levels. This result showed that probably the groups which ND complications of them had been seen lower or been reduced by resistance training and crocin , had lower osmosis stress. Another match in results was repetition

of similar findings about total protein. This means, there was less total protein in resistance group and crocin supplement groupsbut it was higher in Nandrolone group and the groups without crocin. Crocin and Safranal singly have strong immediate influences on and against high toxic drug and can keep the amounts of albumin and total protein in normal range (Hariri et al,2010). Perhaps, as it was mentioned the presence and enzymes activity were decreased by one day recovery of physical activity or training adaptation. However, significant differences among interaction groups with other groups were seen. Also , changes in body weights of groups confirmed other findings. This result even in patients that used blood dialysis treatment along with combination of strength training and ND were seen(*Johansen et al,1998*). Although, there were significant statistical differences among body weight of groups before present study, the most change weight occurs in groups that consumed dose of 12.5 ml/kg crocin. Because of utilization of similar food for all the groups to control of pesky variable, probably having been doing resistance training had cause to enhance energy consumption and lower increase body weight in interaction groups. Likely,the use of high dose of crocin because of impression on catabolism specially lipids induce to lesser body weight changes (Xu et al,2005). It is indicated that crocine not only increase the cholesterol and free fat acid catalysis but also causes to enhance HDLin laboratory rats (Xu et al,2005). The relation among reduction of PG activity, mobility and aging with body weight enhancement and change in compound and fat percent, indicates some of unknown effects PG axis function on lipid catabolism. It is reported that in laboratory rats that had high fat diet, crocin consumption caused to increase of lipid metabolism(Xu et al,2005).This finding can be indicative of improvement of liver function.The less liver weight in interaction effect of resistance training and crocin supplement groups (dose of 25mg/kg) could be due to fatty liver catabolism (Hallsworthet al,2011). Also, it could be because of the changes in ions and blood flow or decrease of body water that result in Shrinkage of the liver (Latour et al,1999). Toxicology studies showed that consumption of 15 mg/kg ND as ananabolic steroid induced to damage specially genetics in blood, kidney, liver and heart of rats (Pozzi et al,2012). Also, ND caused to increase in AST, ALT activity and the ratio of them in albino mice (Chowdhury andMahanta, 2014).Physical activity in accordance with duration and intensity could be from different mechanisms specially ischemic reperfusion and invasion of free radicals in to liver, cause kinds of damage in this organ (Fojt et al,1976,Praphatsorna et al,2010). This report suggested that even oxygen and blood flow were decreased after high intensity physical activity (Fojt et al,1976). Although, these findings were not repeat about the liver enzymes changes after one bout aerobic training in untrained persons (Ajami Nezhad et al,2014). The various adaptation to a regular training such as enzymatic and non enzymatic antioxidants defense reinforcement and anti- inflammatory andantioxidant properties of crocin decrease the effects of liver damage or improve them (Bakhtiary et al, 2014, Altinoz et al, 2015). Pay attention to it that anabolic steroids due to steroid structure do not need to membrane receptors and easily result in severe oxidative stress in all the body cells. In usual, additional steroids for treatment of patient, excrete via the liver and kidney (Silva et al,2010) but exercise trainings cause the consumption of them. Because steroids have anabolic and androgenic impressions. In treatment of patient, its androgenic property was cleared but exercise trainings induces using both of steroids effects (Silva et al,2010). Our result showed that interaction effects of resistance training and crocin (doses of 1 and2) on PG axial function was not statistically significant. Only, this interaction effect caused significant reduction in FSH levels but each of them separately induced to decrease in LH, FSH levels and increase in testosterone and DHT levels. If Concurrently with the reduced LH and FSH,increaseof testosterone and DHT has been seen too, likely it is assumed that these interventions result in decrease PGfunction. But decrease in LH, FSH and enhance in testosterone and DHT, likely indicatesthe consumption of these hormones in tissues not inhibitor of PG axis. Its reason is this point that theoretically DHT is more active than testosterone. DHT, 2 to 3 fold desire to stronger bind to receptors than testosterone and 15 to 30 fold more desire than adrenal androgens (Hemat, 2004). Therefore, probably crocin has inhibited the complication of ND.It is clear that ND caused to increase negative feedback and decline in PG axis function. Resistance training in turn could be the eventual factor in enhance of testosterone and DHT levels, too. The

studies reported that crocin increases secretion of testosterone and DHT. Crocin mediated signaling pathway causes to increase LH and FSH secretion. Also, it enhances their tissue consumption by gonads. This occurrence will result in increase secretion of androgens. Tissue consumption of LH and FSH in turn will decrease these hormones levels. On the other hand, increase of testosterone induced to create negative feedback on hypothalamus and declines LH and FSH secretion (Asadi et al, 2013, Sakr et al, 2014 ,Hesari et al, 2015). The results of testis histological analysis showed that no pathologic changes were seen in the groups that had received ND. In general, there were not any certain changes in numberof spermatogonia, primary spermatocytes, spermatids and sertoli cells in experimental groups to control but leydig cells increased dramatically in experimental groups that had received crocin. Also, decrease of leydig cells in Nandrolone decanoategroup (ND group*)* was seen. Our results showed that the C1 group (dose of 12.5 mg/kg of crocin) had the greatest testicle weight and C2 group (dose of 25 mg/kg of crocin) had the lowest testicle weight among groups. Khayatnouri et al (2011) reported that consumption of low dosage (50-100mg/kg) of saffron insignificantly stimulated the testis tissue and spermatogenesis procedure while high dosage of 200mg/kg of saffron had inhibitory and toxic effects on testis tissue and spermatogenesis (Khayatnouri et al,2011). Foletto et al suggested that AAS directly affects the testis and their functions. In experimental animals, AAS caused to decrease testicular size and weight. It appears that probably Two factors LH and FSH impair the spermatogenesis (de Paivaet al, 2010). According to our result probably high dose of crocin inhibited the increase of testis.

The present study, however, has some limitations. One of the limitations of our study relies on the absence of amount of variables such as liver enzymes and PG hormone axis before initiate of protocol in order to comparison of data to precise determination of variations. It is recommended that future studies measure some of these enzymes and hormones before protocol.

6. Conclusions

Totally the result of present study has showed that interaction effect of resistance training and both dose of crocin resulted in decrease of the complication of ND in liver and testis. Probably, antioxidants properties of crocin is one of the important factor in improvement of liver and testis organs. None of the groups had significant changes in number of spermatogonia, primary spermatocytes ,spermatids and sertoli cells.

Acknowledgment

The authors wish to thank volunteers for their enthusiastic participation in this study.

Table legends

Table 1.Mean and standard deviation (Mean±SD) of body weight

Table 2.Mean and standard deviation (Mean±SD) of liver enzymes, albumin, total protein

Table 3.Mean and standard deviation (Mean±SD) of PG axis hormones

Figure legends

Fig.1The mean of ALT in different groups

Fig.2The mean of GGT in different groups

Fig.3The mean of total protein in different groups

Fig.4The mean of albumin in different groups

Fig.5The mean of FSH in different groups

Fig.6The mean of leydig cells in different groups

Fig7: Normal seminiferous tbule testis

Fig8: seminiferous tbule of testis sham group

Fig9: seminiferous tbule of testis Nandrolone Decanoate group

fig10: seminiferous tbule of testis CR1 + ND group

Fig11: seminiferous tbule of testis CR2 + ND group

Fig12: seminiferous tbule of testis CR1 + ND + TR group

Fig13: seminiferous tbule of testis CR2 + ND + TR group

Fig14: abnormal liver of tissue TR and SH groups

Fig15: abnormal liver of tissue ND + CR2 group

Fig16:abnormal liver of tissue ND + CR1 group

Fig17: normal liver of tissue CO group

Fig18: abnormal liver of tissue ND + CR1 + TR group

Fig19: abnormal liver of tissue ND + CR2 + TR group

Table.1

Group	Weight (gr)		Percentage of increase
	Pre test	Post test	
C	180.00±3.83	301.43±19.03	67.46
SH	184.43±1.90	288.71±13.11	56.54
ND	190.43±8.20	283.14±33.38	47.68
RT	196.00±8.06	281.28±21.48	43.51
C1	200.83±5.00	285.67±15.65	42.24
C2	199.14±7.36	270.00±30.17	35.58
RT+C1	197.33±6.22	277.00±20.27	40.37
RT+C2	199.71±8.03	272.71±23.63	36.55

C: Control, **SH**: sham, **ND**: 10 mg/kg of Nandrolone Decanoate, **RT**: Resistance Training, **C1**: 12.5 mg/kg of crocin, **C2**: 25 mg/kg of crocin, **RT+C1**: Resistance training and 12.5 mg/kg of crocin, **RT+C2**: Resistance training and 25 mg/kg of crocin

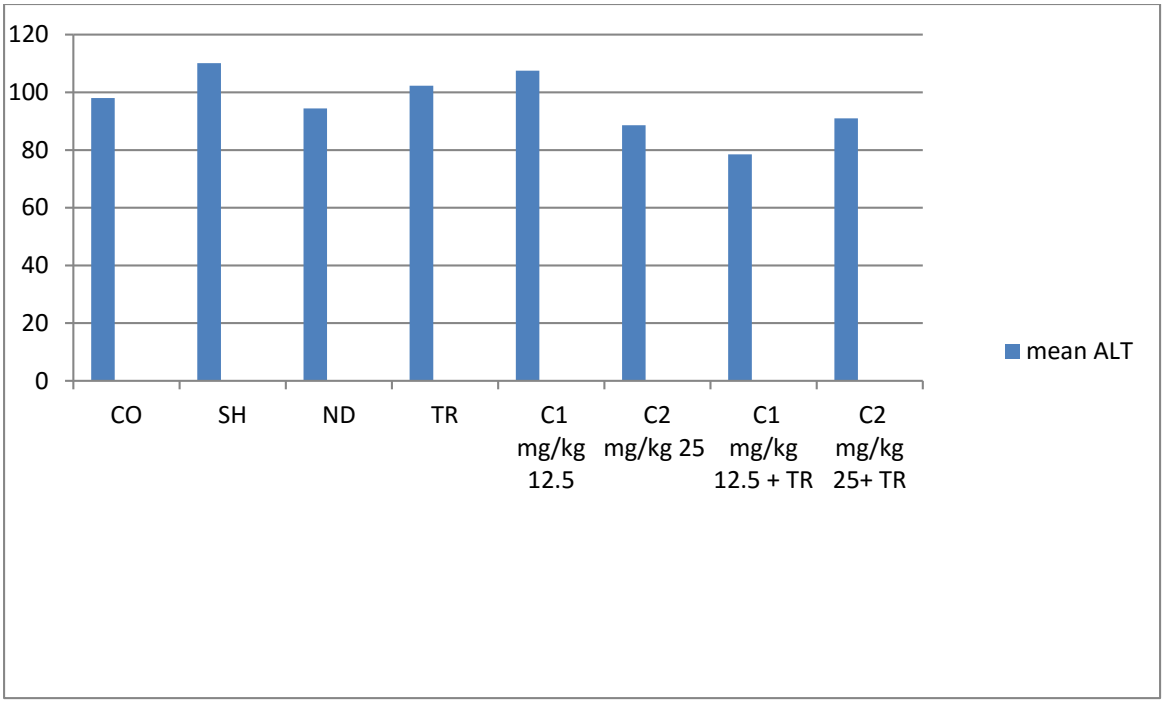
Table.2

group	Liver weight (g)	AST(u/L)	ALT(u/L)	ALP(Iu/L)	GGT(Iu/L)	Total protein(g/dl)	Albumin
C	12.22±1.41	299.14±45.60	98.00±25.88	949.57±423.84	1.43±0.53	9.86±0.43	**5.34±0.36**
SH	11.11±1.83	262.86±80.78	110.14±21/00	914.71±105.10	1.86±0.86	10.51±0.48	**5.63±0.22**
ND	10.26±1.11	293.28±82.17	94.43±14.03	968.14±253.26	2.43±0.78	10.36±0.46	**5.48±0.21**
RT	9.74±1.77	294.00±50.43	102.28±12.26	898.57226.89	1.86±0.69	9.03±1.32	**4.81±0.46**
C1	9.91±1.50	292.00±67.30	107.50±11.73	882.17±187.78	2/00±0.63	8.22±0.20	**4.52±0.15**
C2	9.64±1.06	202.57±51.83	88.57±20.70	801.14±276/07	2.14±0.90	8.13±0.39	**4.54±0.13**
RT+C1	9.13±1.03	207.67±52.25	78.50±12.04	665.67±228.10	3/00±0.89	8.37±0.22	**4.70±0.14**
RT+C2	9.90±0.85	198.28±55.20	91.00±33.70	669.43±166.78	2.71±0/75	8.43±0.63	**4.71±0.24**

Table.3

group	testicle weight (g)		LH(mlu/mL)	FSH(mlu/mL)	Testosterone**(Nmo/L)**	DHT(ng/dL)
	Right	Left				
C	1.42±0.06	1.40±0.11	7.07±0.23	4.03±0.48	5.70±0.44	45.86±3.62
SH	1.60±0.06	1.56±0.06	7.08±0.36	4.08±0.30	5.71±0.43	47.28±3.66
ND	1.44±0.18	1.47±0.19	6.50±0.42	3.53±0.31	6.06±0.31	48.27±4.63
RT	1.46±0.18	1.50±0.16	6.13±0.30	3.98±0.25	6.30±0.53	51.41±4.85
C1	1.60±0.21	1.68±0.19	5.98±0.35	4.47±0.32	6.70±0.47	55.87±2.23
C2	1.37±0.13	1.33±0.10	5.71±0.33	3.61±0.32	7.08±0.58	58.40±4.63
RT+C1	1.43±0.09	1.48±0.13	5.60±0.33	3.72±0.28	7.33±0.34	58.12±3.56
RT+C2	1.46±0.10	1.50±0.09	4.98±0.21	3.74±0.45	8.13±0.46	67.68±3.80

Fig.1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80
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40
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SH
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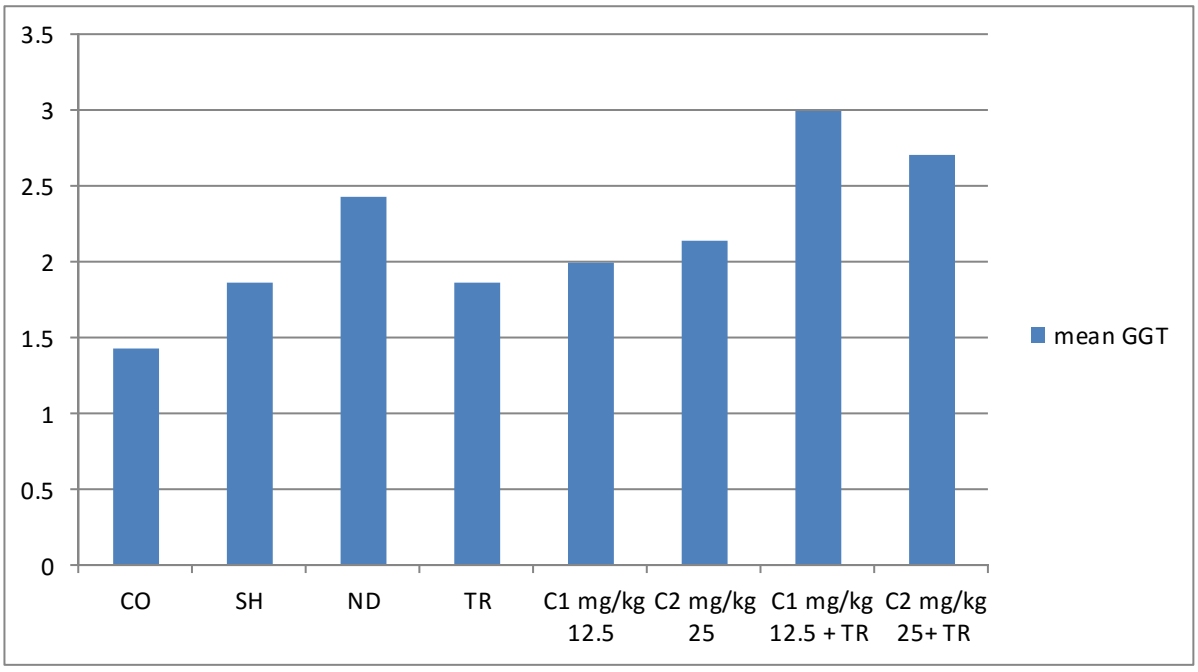
C1 mg/kg 12.5 + TR
C2 mg/kg 25+ TR
mean ALT

Fig.2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C2 mg/kg 25
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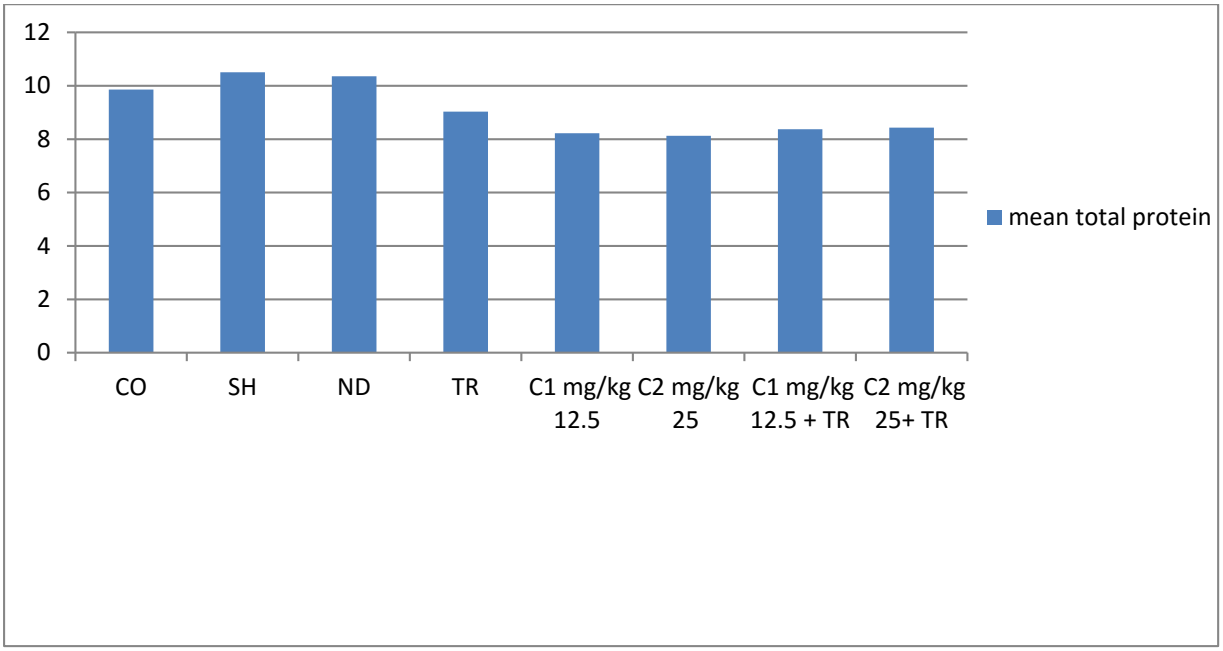
C2 mg/kg 25+ TR
mean GGT

Fig.3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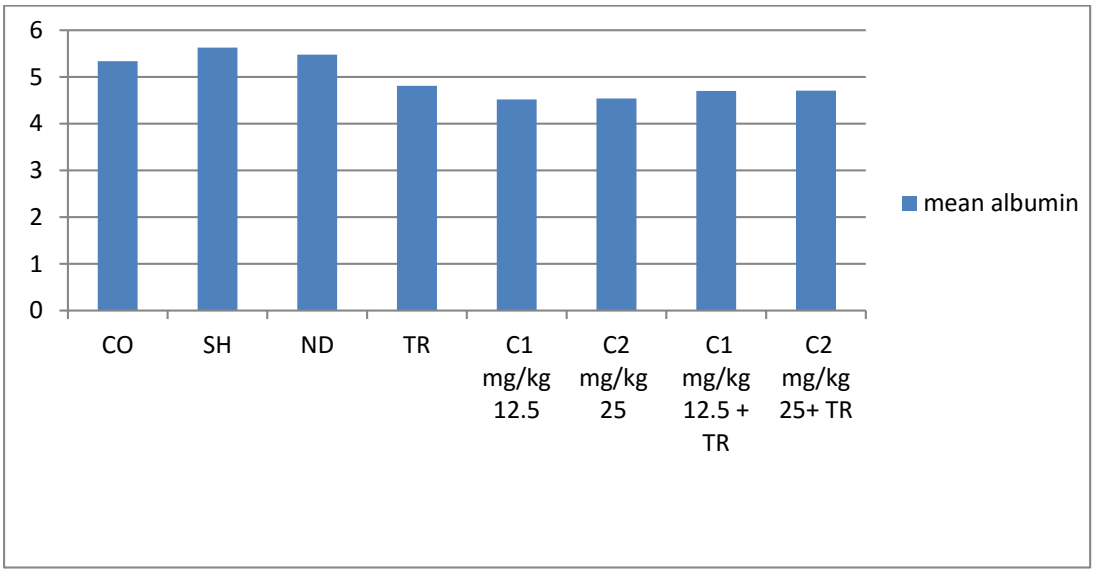
C2 mg/kg 25+ TR
mean total protein

Fig.4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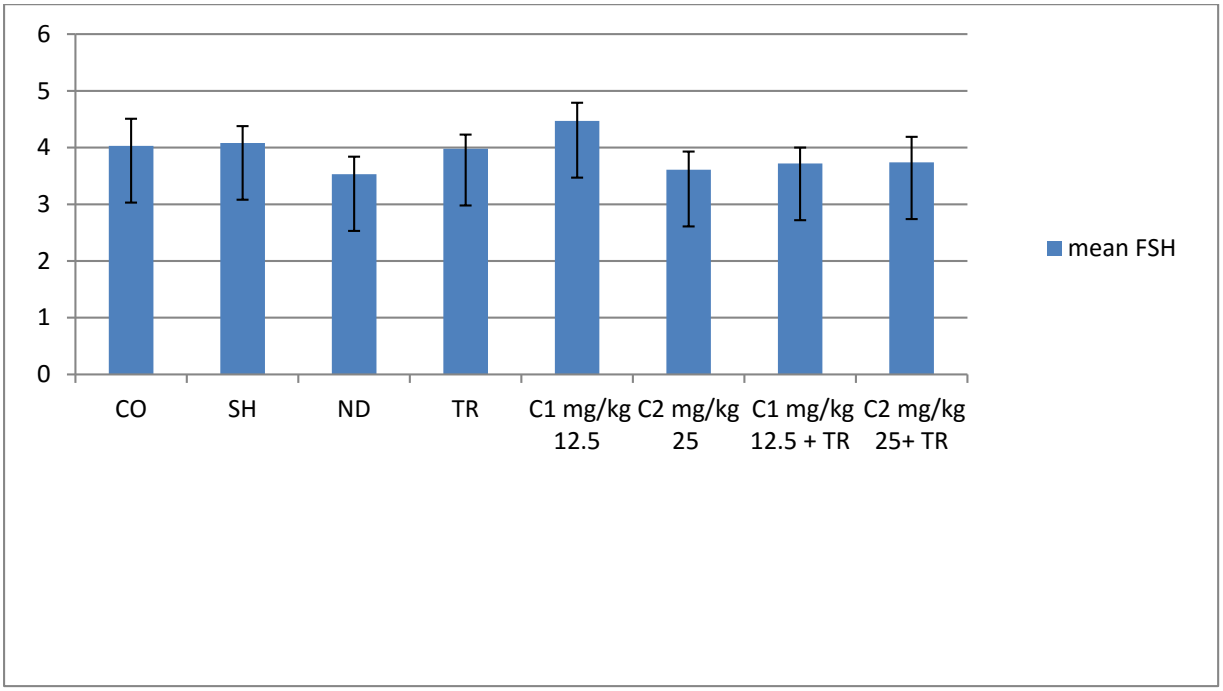
C2 mg/kg 25+ TR
mean albumin

Fig.5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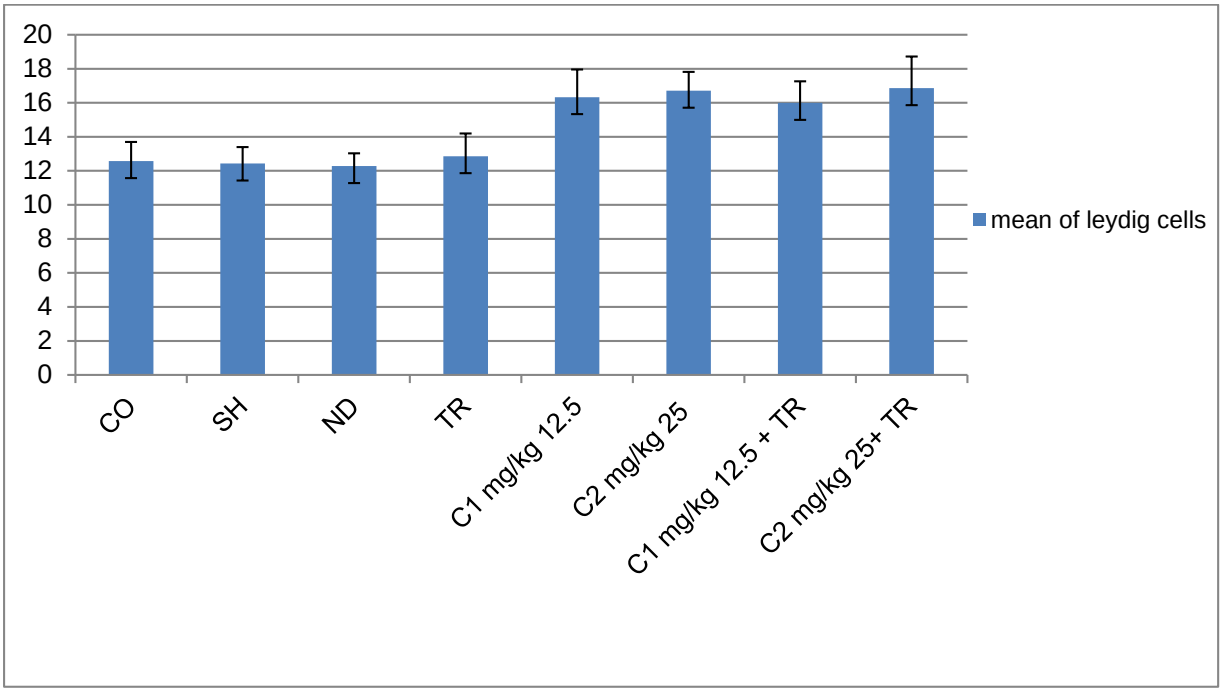
C1 mg/kg 12.5 + TR
C2 mg/kg 25+ TR
mean FSH

Fig.6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CO
SH
ND
TR
C1 mg/kg 12.5
C2 mg/kg 25
C1 mg/kg 12.5 + TR
C2 mg/kg 25+ TR
mean of leydig cells

Fig7: Normal seminiferous tbule testis

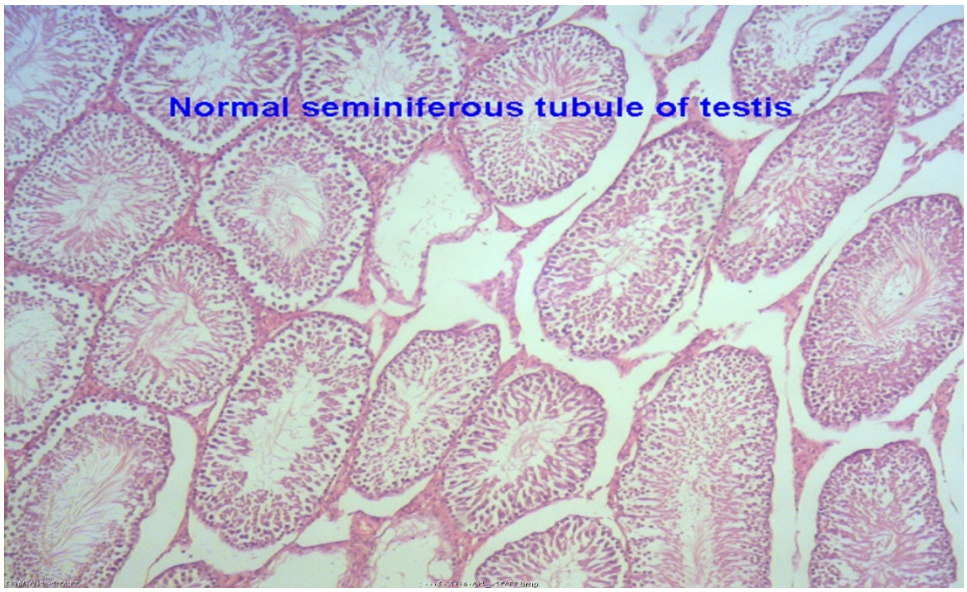


Fig8 : seminiferous tbule of testis sham group

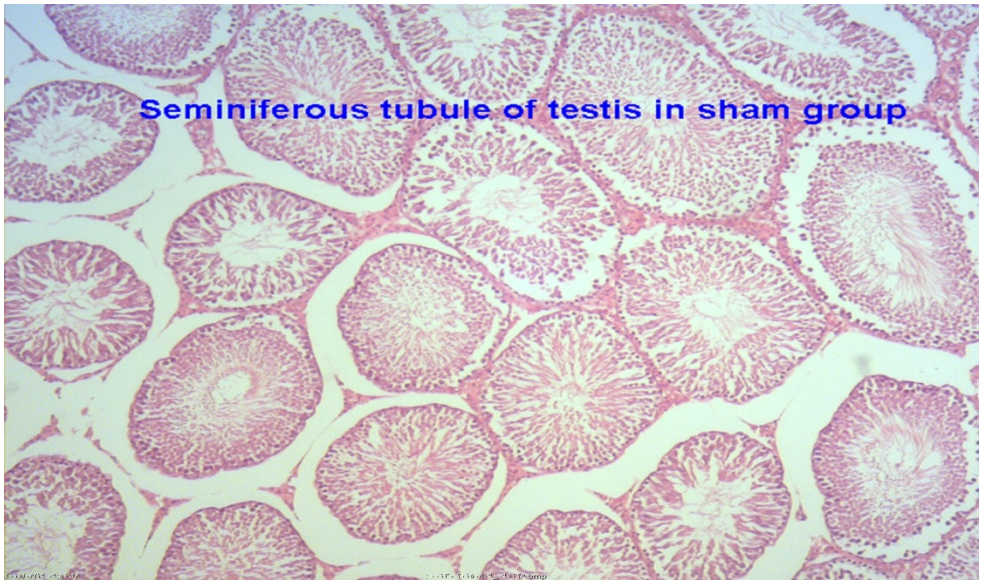


Fig9: seminiferous tbule of testis Nandrolone Decanoate group

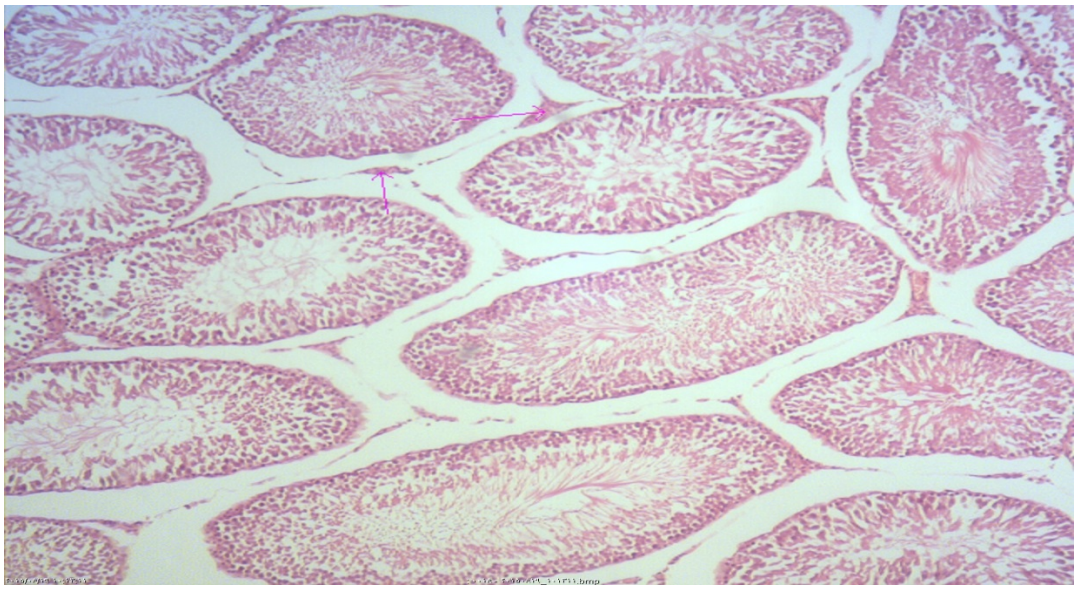


fig10: seminiferous tbule of testis CR1 + ND group

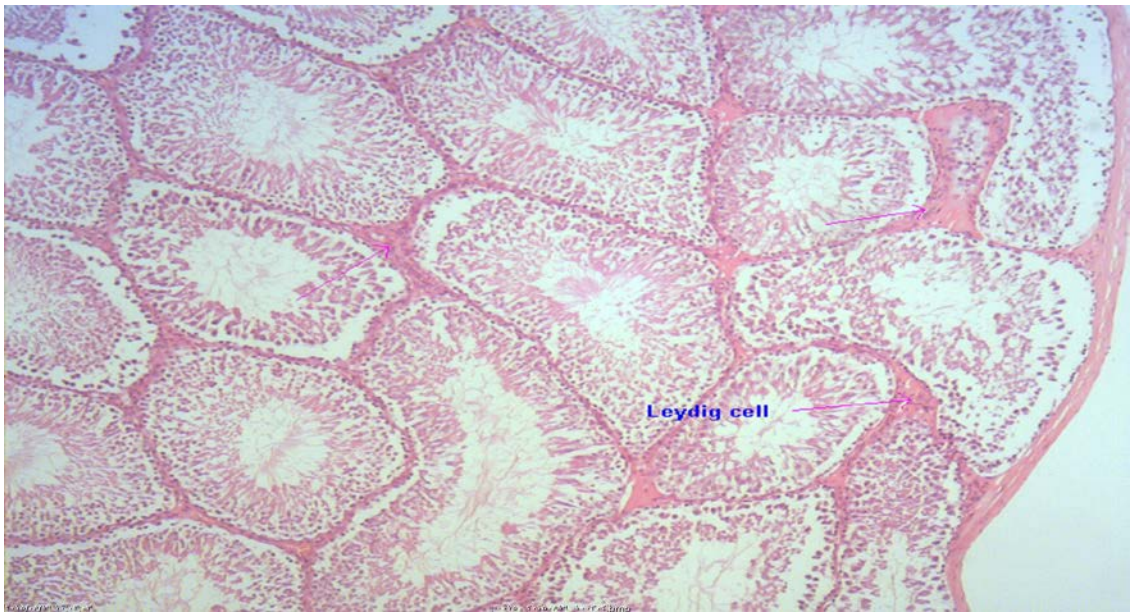


Fig11: seminiferous tbule of testis CR2 + ND group

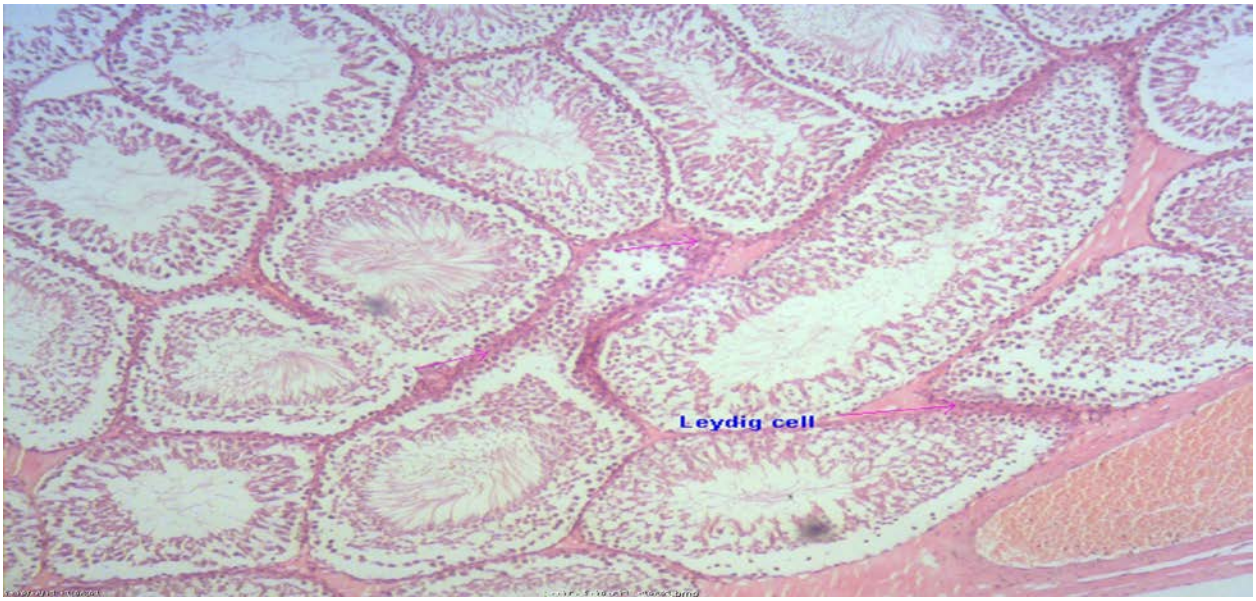


Fig12: seminiferous tbule of testis CR1 + ND + TR group

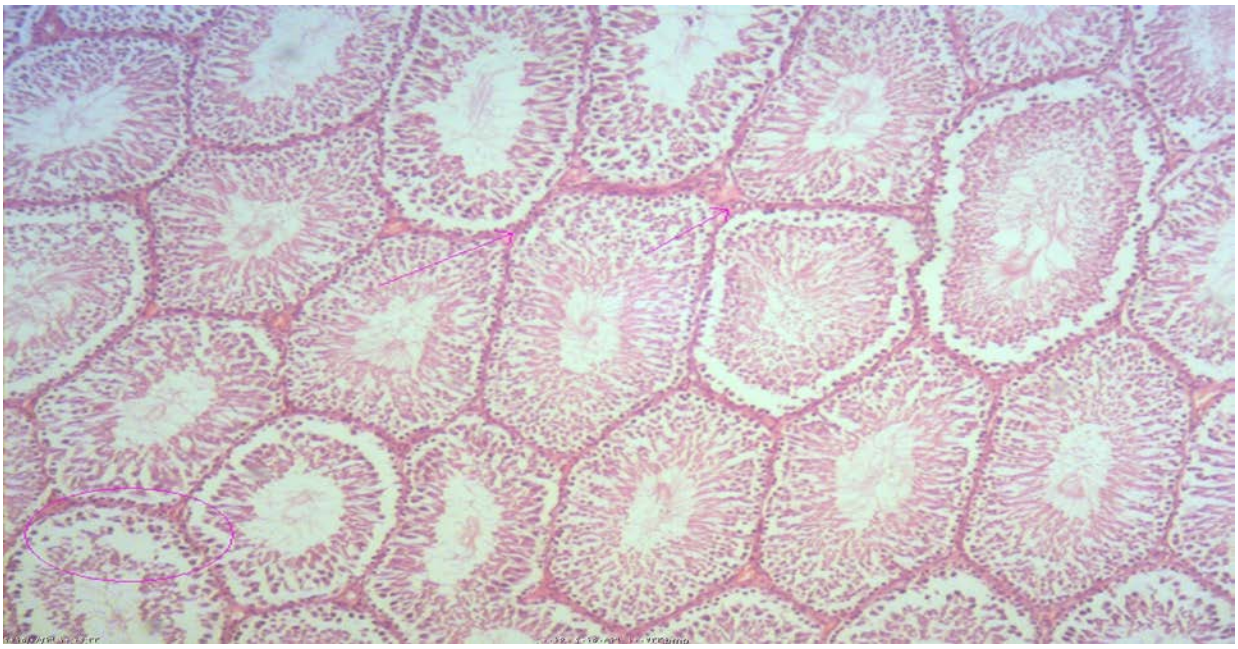


Fig13: seminiferous tbule of testis CR2 + ND + TR group

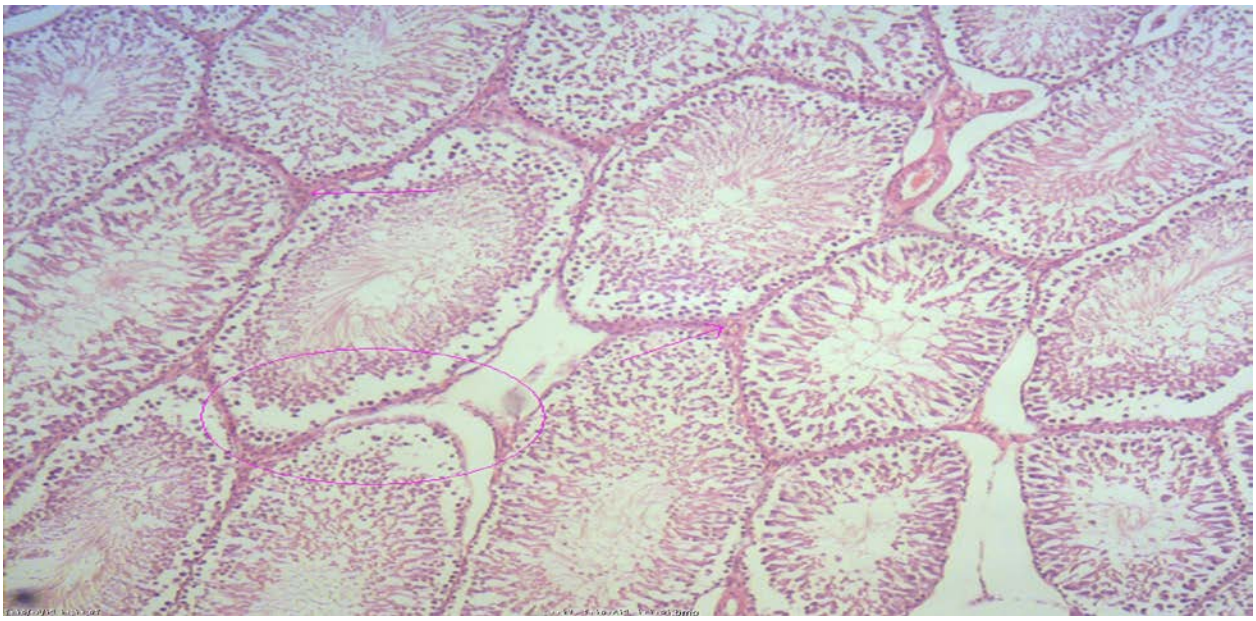


Fig14: abnormal liver of tissue TR and SH groups

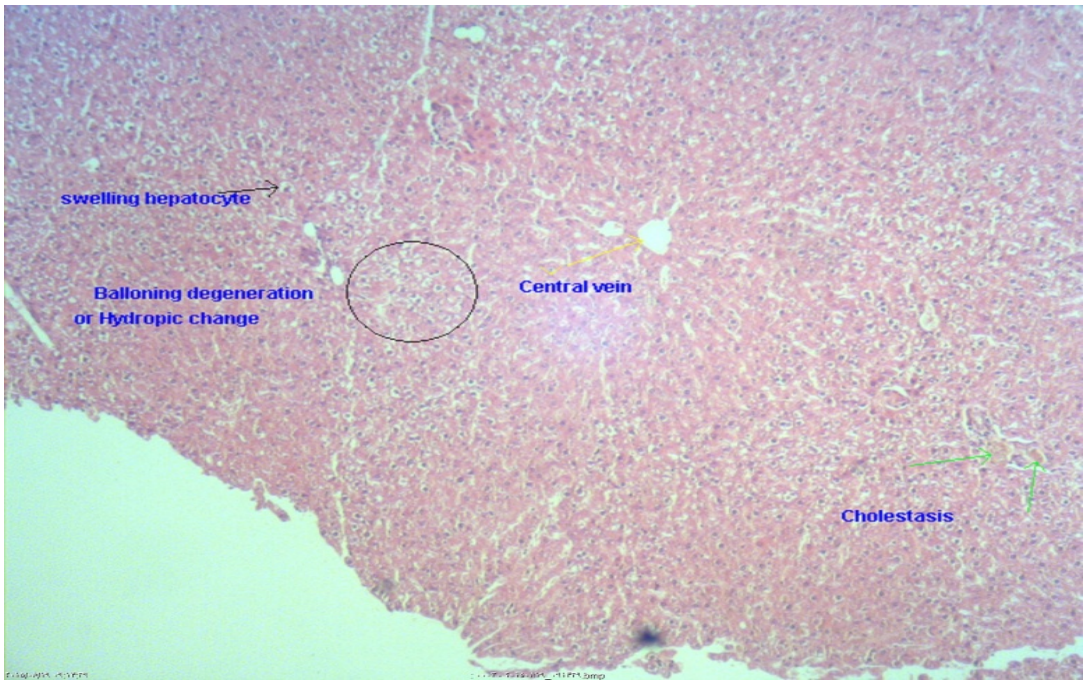


Fig15: abnormal liver of tissue ND + CR2 group

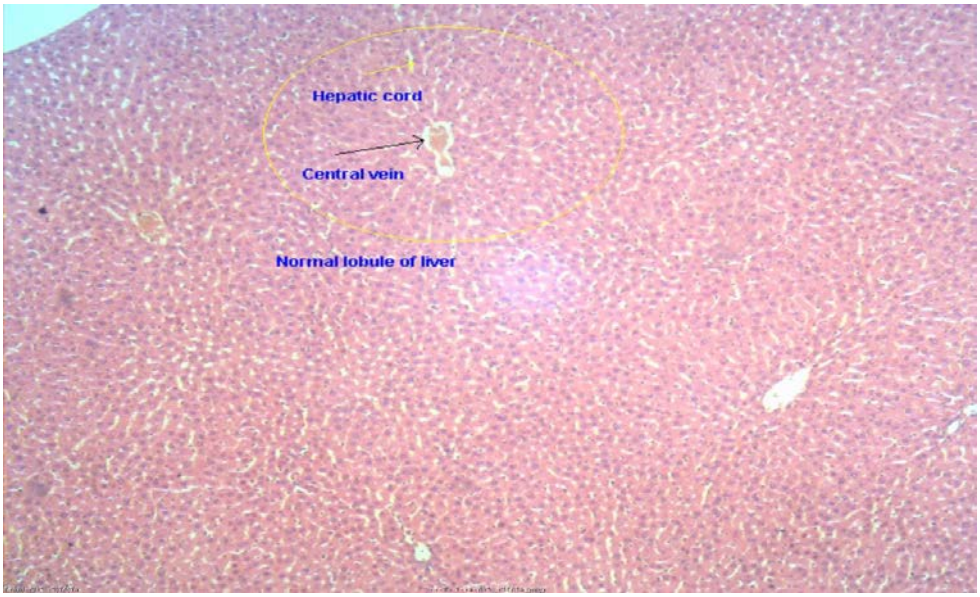


Fig16:abnormal liver of tissue ND + CR1 group

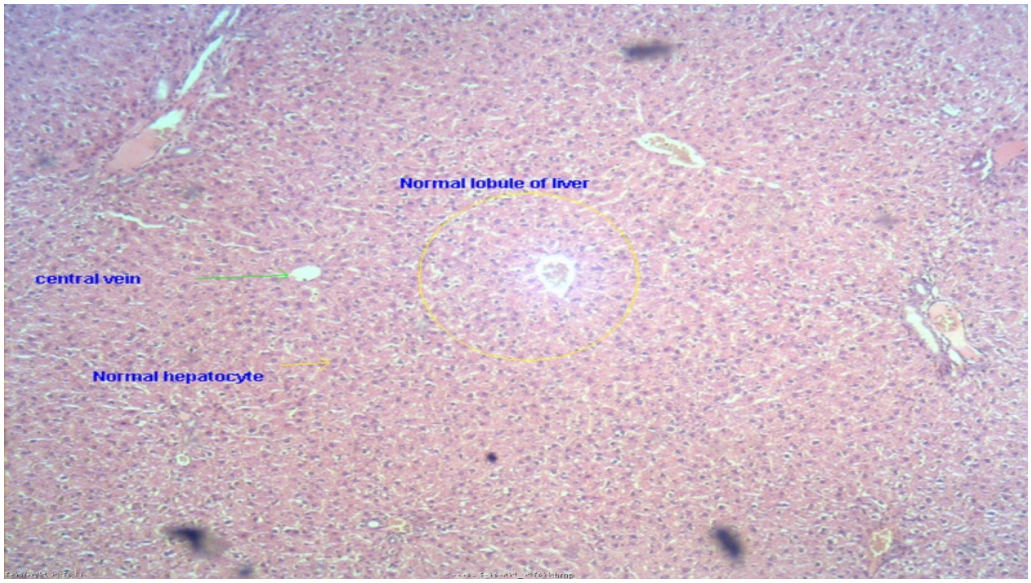


Fig17: normal liver of tissue CO group

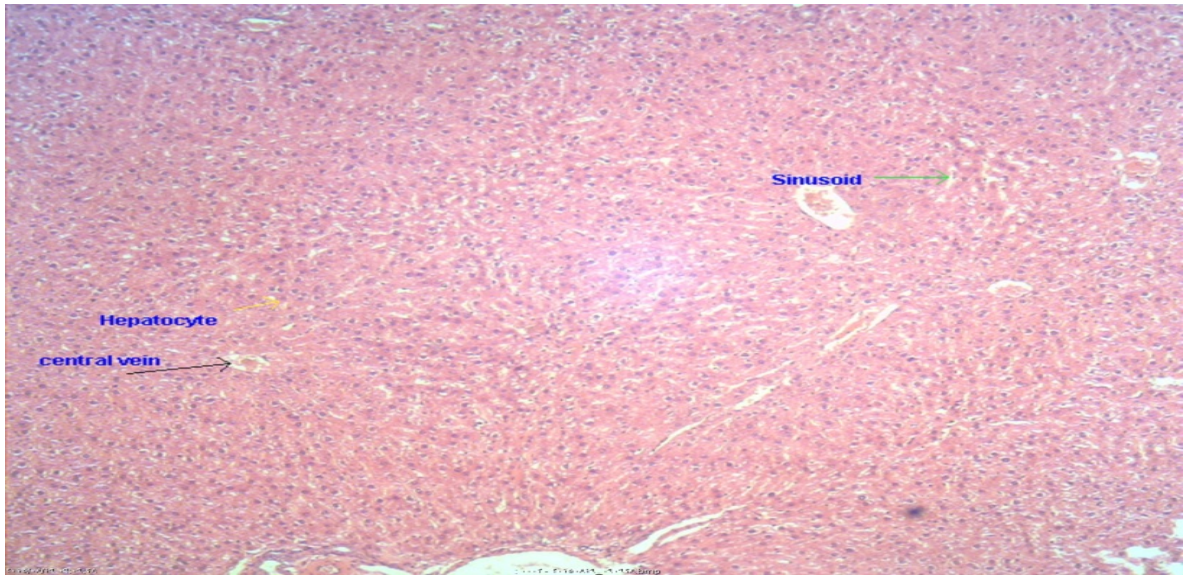


Fig18: abnormal liver of tissue ND + CR1 + TR group

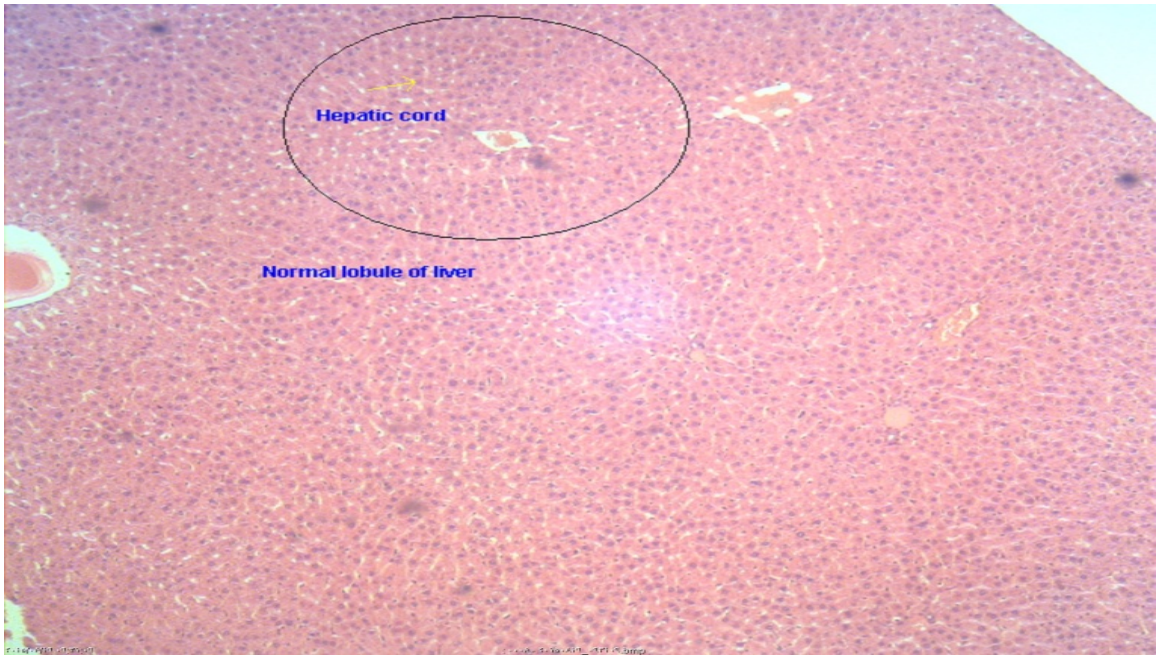
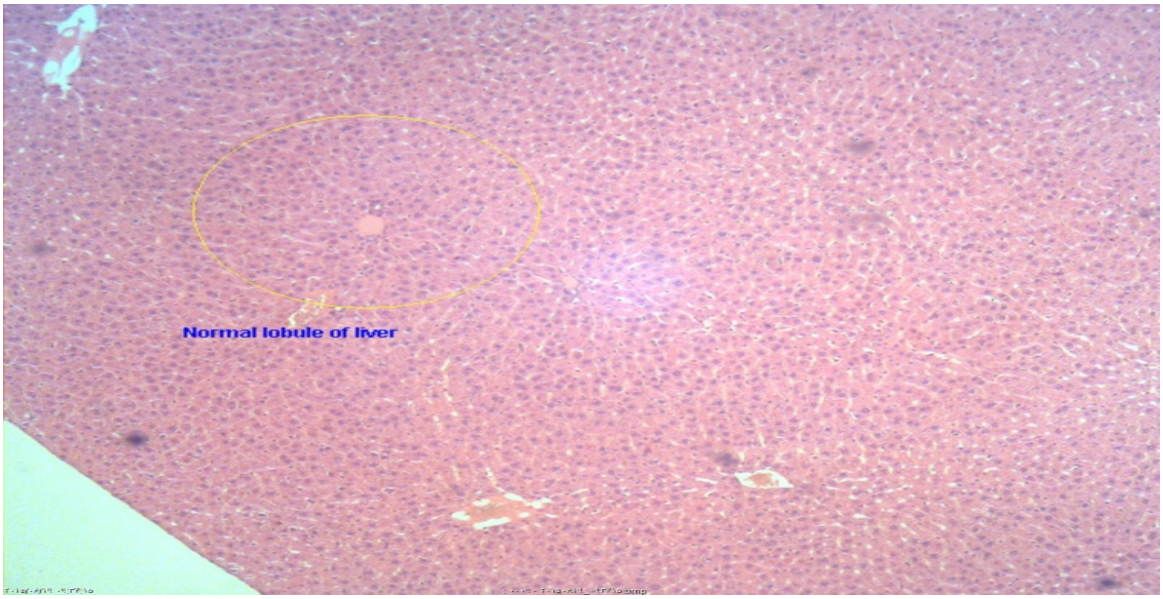


Fig19: abnormal liver of tissue ND + CR2 + TR group



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