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Effects of methandienone on some hematological parameters, kidney and liver functions tests in male mice

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ABSTRACT

Methandienone is widely used to treat many health problems such as aplastic anemia, wounds, burns, delayed puberty, early men climacterium, and has non-medical uses in fitness centres to increase physical activity and muscle volume. Abuse of methandienone was reported to impair its medical benefits and possibly to hepatic toxicity — the present study aimed at detecting the cytotoxic effects of methandienone in albino mice *Mus musculus*. For this purpose, 55 adult male mice were used and divided into 5 groups: negative control and positive control (cyclophosphamide) consisting of 5 mice and 3 other treated groups each consisting of 15 mice, methandienone was orally administered for 35 days to the mice in low, intermediate, and high doses with concentrations of 0.125, 0.25, and 0.5 mg/Kg body weight, respectively. Effects of treatment were measured using blood tests, including total white blood cell (WBC) count, total red blood cell (RBC) count, hemoglobin level and blood platelets count. Effects on kidney and liver functions were also tested by measuring serum levels of urea, creatinine, aspartate aminotransferase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP). Results show an abnormal change in blood parameters and increase in liver-kidney functions tests, and the effect was dose and time-dependent. From these results, we conclude that methandienone displayed a change in blood parameters and an increase in the activities of diagnostic marker enzymes in liver and kidney which may reflect significant damage in the structural integrity of liver and kidney. So we may also suggest that the use of methandienone should be given to the patient or young men in gymnasiums in the proper pharmacological dose to avoid its toxic effects.

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INTRODUCTION

Methandienone is an anabolic androgenic steroid that was derived and synthesized from testosterone and includes the stimulators anabol and danabol (Adnan M. Jassim et al., 2015). It enhances protein synthesis through enhancing anabolism and used as an anti-anemia drug due to its enhancement of RBC synthesis. Methandienone effects in healing wounds were also evident, and it was used in the treatment of corneal ulcer (Pope et al., 2014). Other medical uses of this steroid include treatment of aplastic anaemia, delayed puberty, climacterium virile, bone fractures, burns, and negative nitrogen balance. It also has non-medical uses in sports and fitness practices to increase physical activity and muscle volume(Adnan M. Jassim et al., 2015).

After its discovery in 1955, methandienone was synthesised and produced in 1958 as a drug that was soon used in wide distribution as an oral anabolic steroid medicine. However, abuse of

methandienone through high doses or consumption for longer times was shown to cause various and serious side effects, including muscular water retention, increased fat levels, oily skin, acne, body/facial hair growth, hepatotoxicity, and liver damage. It also has damaging effects on blood cholesterol levels through its effects of lowering high-density lipoprotein (HDL) and increasing low-density lipoprotein (LDL), leading to arteriosclerosis, myocardial infarction and other cardiovascular diseases. Furthermore, a previous case study reported that the use of the drug for longer periods (20 months) in high concentration (65mg daily) caused liver cell adenoma(Hernandez-Nieto et al., 1977). In men, the side effects of methandienone include testicular atrophy and gynecomastia(Adnan M. Jassim et al., 2015). As a result for the increased abuse by youths and in sports, methandienone was included in the list of prohibited substances by the International Olympic Committee(Geyer et al., 2003). The present study aimed at detecting the cytotoxic effects of methandienone on haematological parameters, liver and kidney in albino mice.

MATERIAL AND METHODS

Chemicals: Methandienone supplied from LA Pharma S.r.l. (Thailand). Cyclophosphamide was purchased from Zydus (Germany). All the other chemicals and reagents were used at analytical grade level.

Drug doses: Daily doses of 0.1 ml methandienone were given to the mice for 35 days with 3 different concentrations of 0.125, 0.25, and 0.5 mg/Kg body weight; Dosages were used within the human exposure dose-levels in the experiment

Laboratory Animals: Albino mice, *Mus musculus*, were obtained from the National Center for Drug Control and Research, Baghdad, Iraq. The mice were 8-12 weeks old with an average weight of 25.00± 2.00 gm and were kept in standard cages under controlled condition (25±1) °C with standard mice feeding that were provided *ad libitum* and water in the animal house, Biotechnology Branch, Applied Sciences Division, University of Technology, Baghdad, Iraq**.** The animals were processed, maintained, and used in accordance with "guide for the care and Use of Laboratory Animals," which was approved by the University of Technology (Baghdad, Iraq), Animal Ethical Committee.

Experimental Design: Fifty-five mice were used and divided into 5 experimental groups. Group 1, the negative control group consisted of 5 mice injected with 0.1 ml of distilled water. Group 2, the positive control group consisted of 5 mice injected with 20 mg/kg of Cyclophosphamide under the intraperitoneal membrane, twice a week for two weeks. Group 3, consisted of 15 mice orally injected with 0.5 mg/kg body weight of methandienone for 35 days. Group 4, consisted of 15 mice orally injected with 0.25 mg/kg body weight of methandienone for 35 days. Group 5, consisted of 15 mice orally injected with 0.125 mg/kg body weight of methandienone for 35 days. Blood samples were collected on days 7, 21 and 35 after treatment from 5 mice of each treated group.

Blood tests

Total WBC count

Blood cells were counted according to a Brown method using(Brown, 1984), Cells were counted according to the following formula**:**

$$\text{Total WBCs count per mm}^3\text{ or micro liter} = \frac{no\ of\ WBC\ counted\ \times blood\ diluting\ \ factors}{chamber\ \ dept\,h \times area\ \ of\ \ counted\ \ \ chamber}$$

$$\text{WBC per mm}^3 = \frac{white\ cells \times 20}{4 \times 0.1\ mm^3}$$

WBC per mm^3 = white cells × 50

Total RBC count

Blood (0.02 mL) was added to 4 mL of Hymes fluid in a clean test tube, the mixture was shaken well, and one drop was placed on the counting slide, covered with a slip and left for 2 minutes for cell settlement. The cells were examined under 40X magnification, and RBCs were counted in 5 small squares within the large middle one(Maiti, 1995).

$$total\ count(\frac{cell}{L}) = (cells\ counted\ \times dilution\ factor \times 10^6)/volume$$

Blood platelets count

Formalin citrate method was followed to calculate a number of platelets. The platelets were counted within the counting squares for RBCs, and the number was calculated as in the following formula(Nayak et al., 2011).

Total number of platelets = number of platelets counted in 5 squares X 1000

Hemoglobin measurement

Hemoglobin was measured using the method Makarem as hemoglobin is oxidized to methochlorubine with the presence of basal iron potassium cyanide, then methochlorbine is combined with potassium cyanide to be sianothmoclubin, which is absorbed at wavelength (540) nanometers, is used for total blood in this examination and preserved In a vial containing a coagulation blocker (EDTA)(Henry et al., 1974).

Biochemical tests of blood serum

Measurement of urea and creatinine: Serum urea level was measured according to the method of Schubert & Talk(Tiffany et al., 1972) simplified by Tiffany & Al (TIETZ, 2006). The method is based

on the enzymatic reactions of urea as indicated by the manufacturer (Biolabo),

Serum creatinine was measured using the test kit provided by the manufacturer (Biolabo) and based on Jaffe color reaction under a wavelength of 490 nm(TIETZ, 2006).

Measurement of ALP activity: ALP activity was measured according to the standard method of the German Society of Clinical Chemistry(TIETZ, 2006) and the Scandinavian Society of Clinical Chemistry using the test kit provided by Biolabo (France).

Measurement of AST activity: The test kit provided by Biolabo (France) was used to measure AST activity according to the method of Henry et al. and the recommendations of the International Union of Clinical Chemistry(TIETZ, 2006, Bergmeyer et al., 1986a).

Measurement of ALT activity: The test kit provided by Biolabo (France) was used to measure ALT activity according to the method of Henry et al. and the recommendations of the International Union of Clinical Chemistry (TIETZ, 2006, Bergmeyer et al., 1986b).

Statistical Analysis: The obtained data were shown as mean ± standard error (SE) for all groups and subgroups. The differences were estimated between the control- group and the mean values of each group by extracting the value of the t-test. $P < 0.05$ was statistically significant.

RESULTS AND DISCUSSION

Total WBC count: Table 1, Shows the mean WBC number for mice treated with the three concentrations of Methandienone as well as for positive and negative control groups. Positive control treatment with 20mg/kg of CFA caused a significant decline ($P<0.01$) in total WBC number (4.14 cell/cubic mm) as compared to the negative control (8.18 cell/cubic mm). This result reflects abnormal changes related to treatment with CFA that is known to cause DNA damage. CFA is metabolized in the liver into phosphoramide and acrolein that might inhibit DNA polymerase and block the cytogenetic repair systems, affecting most of the intracellular metabolic activities. Therefore, CFA is considered a cytotoxic drug with potential mutagenic effects(Zarei and Shivanandappa, 2013).

Treatment with Methandienone showed a slight difference in WBC mean numbers in mice treated with 0.125 and 0.25 mg/kg, whereas treatment with 0.5 mg/kg causes a significant difference ($p<0.05$) as compared to the negative control. Treatments for 3 and 5 weeks caused significant declines ($P<0.01$) in total WBC mean numbers after treatment with each of the three concentrations of Methandienone as compared to the negative control. This decline might be due to the effects of the stimulant drugs Anabol and Dianabol, contained in Methandienone, known to be synthetic androgenic steroids that lead to increasing the concentration of the hormone Erythropoietin which can affect WBCs production through its damaging effects on bone marrow cells. Consumption of low amounts of Methandienone might not affect the total number of WBC, whereas taking high amounts for long periods might cause abnormal WBC numbers due to the accumulative effects of the drug and its metabolic compounds. The lower effect in the lower doses of the drug is possibly due to the removal of the compound and its toxic products through metabolic activities leading to their excretion from the body. These results are of interest during the follow up of cases of youths consuming these drugs.

Total RBC count: Table 1 demonstrates a significant decrease ($p<0.01$) in the mean number of RBCs as a result of the treatment with CFA (5.23 cell/cubic mm) as compared to the negative control (6.85 cell/cubic mm). RBCs mean number showed a slight increase ($P>0.05$) after one week of treatment with each of the 3 concentrations of methandienone. A different trend was observed after 3 weeks of treatment, with a significant increase in treatment with 0.125 or 0.25 mg/kg ($p<0.01$) and with 0.5 mg/kg ($p<0.05$) of methandienone, as compared to the negative control. After 5 weeks of treatment, the doses of 0.25 and 0.5 mg/kg showed significant increases ($p<0.01$) in RBC numbers as compared to the negative control.

These results indicate an increase in RBC number with the increase in the period and the dose of treatment. This increase might be attributed to the interaction of the drug with blood cell production and differentiation in the bone marrow, which is in agreement with previously reported results(Hassan and AL-Awaidy, 2014). Another reason might be the effects of the synthetic androgenic steroids on the hormone Erythropoietin which stimulates RBC production in the bone marrow.

Hemoglobin: The results in table 1 show a significant decrease ($p<0.05$) in Hb value in the CFA treated mice as compared to the negative control group. Treatments with methandienone showed no significant differences after 1 week, a significant increase ($p<0.05$) only with a dose of 0.5 mg/kg after 3 weeks, and a significant increase ($p<0.01$) Only with a dose of 0.5 mg/kg after 5 weeks.

It was observed that Hb levels also increase with increasing the dose and the period. This might be due to the effect of the drug on the protein components of Hemoglobin in the bone marrow. Hb increases due to erythropoietin stimulation by the

Table 1: Effects of methandienone on blood components

Groups	7days	21 days	35 days
	Mean ± SE	Mean ± SE	Mean ± SE
		Total WBCs count (X 10^3/ml)	
Control -ve	8.18 ± 0.08**	-	-
Control +ve (CFA)	4.14 ± 0.30	-	-
0.125 mg/kg	8.00 ± 0.76	6.49 ± 0.11**	5.89 ± 0.36**
0.25 mg/kg	7.57 ± 0.25	6.36 ± 0.13**	5.27 ± 0.33**
0.5 mg/kg	7.22 ± 0.29*	6.17 ± 0.14**	5.13 ± 0.60**
		Total RBCs count (X 10^3/ml)	
Control -ve	6.85 ± 0.06**	-	-
Control +ve (CFA)	5.23 ± 0.20	-	-
0.125 mg/kg	6.89 ± 0.22	7.32 ± 0.13**	7.72 ± 0.24*
0.25 mg/kg	6.91 ± 0.14	7.47 ± 0.16**	7.75 ± 0.13**
0.5 mg/kg	7.15 ± 0.18	7.60 ± 0.27*	7.83 ± 0.18**
		Hb (g/dl)	
Control -ve	11.30 ± 0.16*	-	-
Control +ve (CFA)	8.79 ± 1.07	-	-
0.125 mg/kg	11.35 ± 0.15	11.42 ± 0.28	11.96 ± 0.36
0.25 mg/kg	11.46 ± 0.14	11.71 ± 0.23	12.42 ± 0.71
0.5 mg/kg	11.62 ± 0.22	12.23 ± 0.12*	12.85 ± 0.10**
		Platelets count (X 10^3/ml)	
Control -ve	794.71 ± 16.65**	-	-
Control +ve (CFA)	487.83 ± 15.15	-	-
0.125 mg/kg	879.00 ± 22.21*	821.35 ± 11.54	943.54 ± 7.91**
0.25 mg/kg	872.67 ± 16.63**	1088.50 ± 8.66**	1221.55 ± 22.17**
0.5 mg/kg	936.40 ± 17.91**	1127.67 ± 6.52**	1353.51 ± 13.56**

P<0.01=**, P<0.05=*

Table 2: Effects of methandienone on urea and creatinine activity

Groups	7days	21 days	35 days
	Mean ± SE	Mean ± SE	Mean ± SE
		Urea (m.Mol/L)	
Control -ve	40.11 ± 0.65**	-	-
Control +ve (CFA)	82.85 ± 0.95	-	-
0.125 mg/kg	42.42 ± 0.84	42.80 ± 0.58*	51.23 ± 1.50**
0.25 mg/kg	37.69 ± 0.45*	43.86 ± 0.50*	57.20 ± 2.39**
0.5 mg/kg	43.88 ± 1.78	44.65 ± 0.88*	60.10 ± 1.46**
		Creatinine (U.Mol/L)	
Control –ve	0.30 ± 0.02*	-	-
Control +ve (CFA)	0.47 ± 0.04	-	-
0.125 mg/kg	0.30 ± 0.15	0.33 ± 0.009	0.35 ± 0.02
0.25 mg/kg	0.33 ± 0.04	0.35 ± 0.01	0.42 ± 0.04*
0.5 mg/kg	0.34 ± 0.02	0.38 ± 0.08	0.51 ± 0.06*

P<0.01=**, P<0.05=*

Synthetic androgenic steroids, which was proved by Bachman *et al.* who showed that the increase in Hb and hematocrit as a result of testosterone uptake was associated with a remarkable increase in erythropoietin and decrease in ferritin and hepcidin levels(Pamela and Richard, 2005). In a study designed to evaluate methandienone effects on some blood and immune parameters of albino male rats that were orally administered for 8 weeks with 3 concentrations of the drug's suspension, there were significant increases in RBC and platelets number, hemoglobin concentration, PCV, and lymphocyte percentage, as well as significant decreases in total WBC count, percentages of granulocytes and monocytes, and total protein in the blood of treated animals(Kinson and Lubek, 1981).

Blood platelets

The results in table 1 show a significant decrease (p<0.01) of mean platelets number in the CFA treated mice as compared to the negative control group. After 1 week of treatment with methandienone, the dose of 0.125 mg/kg showed a significant increase (P<0.05) as well as the doses

Table 3: Effects of methandienone on ALT, AST and ALP activity

Groups	7days	21 days	35 days
	Mean ± SE	Mean ± SE	Mean ± SE
		ALT (U/L)	
Control -ve	26.20 ± 0.63**	-	-
Control +ve (CFA)	91.75 ± 6.71	-	-
0.125 mg/kg	26.33 ± 1.85	39.00 ± 0.63**	42.30 ± 1.30**
0.25 mg/kg	29.33 ± 0.97	39.93 ± 0.84**	50.00 ± 1.58**
0.5 mg/kg	31.75 ± 2.95	55.00 ± 0.95**	64.50 ± 1.11**
		AST (U/L)	
Control –ve	114.00 ± 7.26**	-	-
Control +ve (CFA)	484.50 ± 26.94	-	-
0.125 mg/kg	190.50 ± 19.56*	219.00 ± 5.01**	303.00 ± 7.02**
0.25 mg/kg	215.50 ± 25.58*	235.50 ± 3.31**	343.50 ± 0.50**
0.5 mg/kg	236.00 ± 6.02**	272.67 ± 7.70**	281.00 ± 46.14*
		ALP (U/L)	
Control –ve	146.28 ± 8.15**	-	-
Control +ve (CFA)	514.50 ± 25.09	-	-
0.125 mg/kg	148.47 ± 4.35	153.00 ± 4.10	183.50 ± 2.21*
0.25 mg/kg	156.00 ± 9.13	158.23 ± 1.27	248.00 ± 31.58*
0.5 mg/kg	237.33 ± 15.62*	252.67 ± 28.06*	258.27 ± 1.28**

P<0.01=**, P<0.05=*

0.25 and 0.5 mg/kg showed a highly significant increase (P<0.01) in platelets number as compared to the negative control.

After 3 weeks of treatment, only the doses of 0.25 and 0.5 mg/kg showed a significant increase (p<0.01), whereas after 5 weeks of treatment all the 3 doses caused a significant increase (p<0.01). It was previously reported that oral uptake of androgens is associated with decreasing prostacyclin levels, which is an inhibitor of thrombosis, and increasing fibrinogen levels(Hossain, 2018). Consumption of synthetic androgenic stimulants affects thrombocytosis and platelet aggregation, leading to increasing the threat of coagulation(Kennedy and Thursby-Pelham, 1956).

Kidney functions (effects on the urinary system)

Blood urea concentration

The results in table 2 show a significant increase (p<0.01) in mean urea concentration of the positive control group as compared to the negative control. Treatment for 1 week indicated a slight and insignificant increase, while a significant increase (p<0.05) was recorded with all the 3 concentrations after 3 weeks and 5 weeks of treatment as compared to the negative control.

The reason behind such an increase in urea concentration after 5 weeks of treatment might be damage in the collecting urinary tubules caused by precipitation of the drug or its metabolites, leading to reducing its efficiency in urea filtration(Shahad F Obeid et al., 2018). This is in agreement with previous reports on the effects of cortisone on the permeability of renal tissues and reduction of renal efficiency in uric acid filtration, which is reflected through its high concentration in the blood(Vieira et al., 2008).

Blood creatinine concentration

The results in table 2 show a significant increase (p<0.05) in mean urea concentration of the positive control group as compared to the negative control. The results also indicated significant increases only with treatments of 0.25 and 0.5 mg/kg of methandienone after 5 weeks of treatment. Changes in serum creatinine levels might be due to impaired production or excretion mechanisms.

Creatinine is synthesized in the liver and the kidney and transported to other organs through its initial binding to phosphatase compounds that have high energy and later its re-conversion to creatinine in the organs other than its production sites. The slight increase in creatinine level was explained as a result of high blood urea level that causes primary toxics to damage body tissues, leading to increased creatinine secretion through the kidney. The failure of kidneys function in excreting creatinine increases its serum level(Abdulghaffar A. Abdulameer et al., 2017). It was previously reported that elevated urea levels in the serum caused a marked elevation in serum creatinine due to mechanical and chemical damages in kidney and liver tissue caused by urea.

Amine group-transferring enzymes

ALT activity: The increases in the activities of these two enzymes occur when the liver tissue is damaged as a result of some diseases such as liver cancer and cirrhosis or viral infections. The results in table 3 show a significant increase ($p<0.01$) in the enzyme activity of the positive control group as compared to the negative control.

Treatment with methandienone caused a slightly significant increase ($p<0.05$) in the enzymatic activity after 1 week of treatment, whereas the increase was highly significant ($p<0.01$) after 3 and 5 weeks of treatment. These results confirm the suggested correlation between drug consumption and the consequences of the accumulation of its chemical metabolites(Vieira et al., 2008). Several diseases are diagnosed through the increased activities of ALT and AST in the serum during damage in hepatic cells. These amine-transferring enzymes are of great importance in the non-basic amino acid synthesis and the process of energy release from proteins in the cells. Imbalance in the activity of these enzymes might cause an imbalance in protein formation.

AST activity: The results in table 3 show a significant increase ($p<0.01$) in mean AST activity of the positive control group as compared to the negative control. After 1 week of treatment with methandienone. AST serum activity showed a Slightly significant increase ($P<0.05$) with 0.125 and 0.25 doses as well as a highly significant increase ($p<0.01$) with 0.5 mg/kg dose of the drug. After 3 and 5 weeks of treatment, each of the 3 doses caused a highly significant increase ($p<0.01$) in AST activity as compared to the negative control. AST and ALT levels increase during liver tissue damage such as liver necrosis.

The results of the present study are in agreement with those of previous reports who aimed to clarify the effects of some stimulants used in sports on the liver and kidney functions in male rats(Jwad and Mohammed, 2017). The results indicated elevated AST, ALT and ALP levels as compared to the negative control, which might be attributed to the possibility of releasing these enzymes from the cytoplasm to the blood flow due to liver necrosis and hypertrophy as well as the disruption of the plasma membrane.

ALP activity: Enzymes in general and ALP, in particular, are considered as subsidiary materials with importance in intracellular biological activities that are resulted from their roles in enhancing biochemical reactions. Therefore, any damage in cells affects the activity of these enzymes and ultimately the reactions they are responsible for. The results of the present study (table 3) show a significant increase ($p<0.01$) in mean ALP activity of the positive control group as compared to the negative control.

After 1 and 3 weeks of treatment with methandienone, only the dose of 0.5 mg/kg caused a significant increase ($p<0.05$) in ALP activity as compared to the negative control. After 5 weeks of treatment, the doses of 0.125 and 0.25 caused a significant increase ($p<0.05$) whereas the dose of 0.5 mg/kg resulted in a highly significant increase ($p<0.01$) in ALP activity as compared to the control group. The results indicate that the increase in ALP activity is associated with the increase in dose and period of treatment, which is in agreement with the previously reported observations(Jwad and Mohammed, 2017). ALP is considered one of the most sensitive hepatic enzymes to any kind of liver damage, and it is released in high amounts into the blood when the liver tissue is under oxidative stress. Therefore, the present increase in ALP activity indicates a severe effect of the stimulant drug on the hepatic tissues that caused direct release of the enzyme from cells to blood.

CONCLUSION

Our results may suggest that methandienone displayed a change in blood parameters and an increase in the activities of diagnostic marker enzymes in liver and kidney which may reflect significant damage in the structural integrity of liver and kidney. So we may also suggest that the use of methandienone drug should be given to the patient or young men in gymnasiums in the proper pharmacological dose to avoid its toxic effects.

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Conflict of Interest Statement

Authors declare no conflict of interest.

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