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Research Article

# DECREASED XANTHINE OXIDASE ACTIVITY IN LIVER AND KIDNEY TISSUES BY THE INTRAMUSCULAR ADMINISTRATION OF NANDROLONE DECANOATE

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**ABSTRACT**

Anabolic steroids either increases or decreases the activities of monoxygenase. A lot of studies has been carried out to investigate the effect of anabolic steroids on cytochrome P-450s but still a very fragmentary study has been conducted on their effects on xanthine oxidase activity, so a study has been carried out to ascertain the effect of nandrolone decanoate, a anabolic steroid, on xanthine oxidase activity. It is found that intramuscular administration of nandrolone decanoate significantly ($p>0.01$) elevates the activities of xanthine oxidase in liver and kidney tissues in the initial phase of study which then follows declining trends. Decreased xanthine oxidase activity signifies the increased histo-pathological state of tissues which is of pharmaceutical and therapeutic importance.

**Keywords:** Xanthine oxidase, Nandrolone decanoate, Detoxification, Liver and Kidney tissues, Toxicity.

## INTRODUCTION

Variation in cellular or biochemical components or processes, structure or function i.e. measurable in a biological system or sample provide information on the amplitude of response of an organism in relation to the magnitude of chemical insult and on the relation between biological effect and environmental contamination. The biomarkers often used to evaluate xenobiotic toxicity are the mixed function oxygenase (MFO) system [1]. MFO includes a group of enzymes which plays an essential role in the metabolism of a broad range of xenobiotics including carcinogens and endogenous and exogenous substrates [2].

Mixed function oxygenase (MFO) enzymes mostly represented by triad- cytochrome P-450 (CYP), aryl hydrocarbon hydroxylase (AHH) and xanthine oxidase (XOD) play a crucial role in xenobiotic detoxification by carrying out a series of oxidation reactions whereby relatively insoluble organic compounds are converted into water soluble metabolites which may be further conjugated and excreted in urine or bile [3].

Although the majority of oxidative metabolic reactions are mediated by the CYP superfamily of enzymes, a non-CYP mediated oxidative reaction is also reported to play an important role in the metabolism of xenobiotics. The major oxidative enzymes, other than CYP, involved in the metabolism of drugs and other xenobiotics are - the flavin containing monoxygenase, molybdenum hydroxylase such as aldehyde oxidase and xanthine oxidase, prostaglandin H synthetase, the lipoxygenase, the amine oxidases, alcohol and aldehyde dehydrogenase. These enzymes are reported to produce therapeutically active metabolites and reactive or toxic metabolites and they modulate the efficacy of therapeutically active drugs or contribute to detoxification. Many of them have been shown to be important in endobiotic metabolism [4].

Xanthine oxidase, a metalloflavoproteins and an important non CYP enzyme , catalyze both oxidation and reduction of a broad range of drugs and other xenobiotics indicating the importance of this enzymes in drug oxidation, detoxification and activation [5]. Xanthine oxidase and xanthine dehydrogenase, collectively called Xanthine oxidoreductase (XOR), are known to be rate limiting enzymes in purine catabolism and investigations show that they also metabolize a number of other physiological compounds. Recent studies have also demonstrated its ability to metabolize xenobiotic, including a number of anti-cancer compounds to their active metabolites [6]. Oxidation via aldehyde oxidase and xanthine oxidase are reported to give different metabolites to those resulting from P-450 hydroxylation [7] .Decreased xanthine activity is of great pathological and pharmaceutical importance.

Although it is demonstrated that a number of anabolic steroid either increase or decreases the activities of many cytochrome P-450 mediated pathways but very little study has been carried out to ascertain the activity of xanthine oxidase, a important non CYP drug metabolism enzyme. It is well established fact that xenobiotics induces the activities of drug metabolism enzymes but induction of xanthine oxidase activity by anabolic steroid is still fragmentary, considering all this facts a study has been carried out to ascertain the activity of xanthine oxidase by the administration of Nandrolone decanoate, the most commonly abused anabolic steroid.

## MATERIALS AND METHODS

Before the experimental procedure is started, healthy mice weighing 25 to 30 grams are acclimatized in the animal room for four weeks and fed on standard animal diet. Adequate measures were taken to minimize pain or discomfort to the mice and the experiments were conducted in accordance with international standards on animal welfare as well as being compliant with local (Ethical committee of animal welfare of Gauhati University, Guwahati, Assam, India) and national regulations .

As per plan of the study the targeted number of animals are randomly divided as follows-

**Group I (Normal control group)**: 10 healthy male albino mice without any sign of deficiencies are randomly selected for normal control group and maintained throughout the whole period of experiment in the same condition.

**Group II (Nandrolone decanoate treated group):** This group consists of randomly selected animals from the general normally healthy pool of already acclimatized mice for the study and each of them is injected with 2.5 mg Nandrolone decanoate intramuscularly at 15 days interval upto 90 days. Doses were given to mice according to their body weights. The amount of dose administered ( i.e 2.5 mg) is selected only to ascertain whether this particular dose effects the activity of enzyme concerned as no previous studies were conducted in this particular dose.

The mice are anaesthetized by diethyl ether and dissected to collect liver and kidney tissues. The tissues are dried over a filter paper and immediately weighted and recorded. The tissue homogenate is prepared in deionised water with the help of homogenizer. Tissues are collected from normal control as well as experimental mice on the desired days i.e. 15$^{th}$, 30$^{th}$, 45$^{th}$, 60$^{th}$, 75$^{th}$ and 90$^{th}$ days.

Liver and kidney tissues are collected from all the experimental groups of animals at different day interval as 15$^{th}$, 30$^{th}$, 45$^{th}$, 60$^{th}$, 75$^{th}$ and 90$^{th}$ days for the estimation of xanthine oxidase.

Xanthine oxidase activity in hepatic and kidney tissues is estimated by following the method of Fried and Fried [8]. Xanthine oxidase activity in Unit/ litre of diluted sample obtained from the calibration curve is converted to the XOD activity in mg/tissue.

**RESULTS**

**Xanthine oxidase in liver tissue**

The mean, SD and SEM values of xanthine oxidase (unit/mg) in liver tissue of different experimental animals are presented in table 1 and the percentage deviation of nandrolone treated group from the normal control mean values are presented in table 2 and the comparison of mean values with significance of variance are presented in table 5.

The mean xanthine oxidase activities in the normal control group of animals are found to be 0.247± 0.010 on 15th day, 0.236 ± 0.009 on 30th day, 0.238 ± 0.010 on 45th day, 0.231 ± 0.010 on 60th day, 0.233 ± 0.010 on 75th day and 0.238 ± 0.010 unit/mg on 90th day of treatment. In nandrolone decanoate treated group, the mean xanthine oxidase activities are 0.343 ± 0.020 on 15th day, 0.382 ± 0.030 on 30th day and 0.384 ± 0.006 unit/mg on 45th day of treatment. It declines to 0.360 ± 0.022 on 60th day, 0.343 ± 0.024 on 75th day and 0.261 ± 0.003 unit/mg on 90th day of treatment (Table 1). It is observed that xanthine oxidase activity in nandrolone decanoate treated group increases significantly ($p<0.01$) from the normal control group throughout the investigation period expect the 90th days treatment where the trends declines and comes close to the normal control Table 5.

**Xanthine oxidase in kidney tissue**

The mean, SD and SEM values of xanthine oxidase in kidney tissue of different experimental animals are presented in table 3 and the percentage deviation of nandrolone treated group from the normal control mean values are presented in table 4 and the comparison of mean values with significance of variance are presented in table 5.

The mean xanthine oxidase activities in the normal control group of animals are found to be 0.210 ± 0.008 on 15th day, 0.207 ± 0.009 on 30th day, 0.198 ± 0.009 on 45th day, 0.200 ± 0.009 on 60th day, 0.204 ± 0.008 on 75th day and 0.203 ± 0.008 unit/mg on 90th day of treatment (Table 3).

In nandrolone treated group, the mean xanthine oxidase activities is observed as 0.289 ± 0.006 on 15th day which increases to 0.356 ± 0.032 on 30th day, 0.362 ± 0.006 on 45th day, 0.367 ± 0.022 unit/mg on 60th day of treatment. The enzyme activity is found to be declining as 0.336 ± 0.014 on 75th day and 0.249 ± 0.009 unit/mg on 90th day of treatment (Table 3).

**DISCUSSION**

Xenobiotic metabolizing enzymes play central role in the biotransformation, metabolism and detoxification of xenobiotics or foreign compounds that are introduced to the human body. These enzymes, represented by cytochrome P-450s (CYP), aryl hydrocarbon hydroxylase (AHH) and xanthine oxidase (XOD), protect or defend the body against the potential harmful insults from the environment [9]. Xanthine oxidase, an important non CYP enzyme, is reported to take part in metabolism of drugs and other xenobiotics indicating the importance of this enzyme in drug oxidation, detoxification and activation [5].

Anabolic steroids are one of the xenochemicals that are reported to induce the activities of drug metabolizing enzymes. Short and long term side effects of anabolic steroids have been demonstrated in many organs, but the liver adverse effects are the most common [10] .Much more works were stressed on the ability of CYP enzymes in the metabolism and detoxification of xenobiotics but very little work is emphasized on the ability of non CYP enzymes in drug metabolism. Recent studies have demonstrated the ability of xanthine oxidase to metabolize xenobiotics including drugs [4] and a number of anti-cancer compounds to their active metabolites [6]. Xanthine oxidase (XO) is a critical source of reactive oxygen species (ROS) in inflammatory disease [11].It is also demonstrated that xanthine oxidase activity generates free-radicals and cause cellular damage which is of therapeutic significance [12].

In the present investigation, xanthine oxidase activity in liver and kidney tissues under different experimental condition maintains similarity in their trends in both the tissues. In these organs the xanthine oxidase activity is observed to be elevated within the range from 30 percent in the initial period to about 25 percent above the normal base line at the terminal part of the experiment with peak values of 80 percent elevation during the intermediate part covering 45th to 60th days. It is observed that xanthine oxidase activity decreases in the liver tissue in the terminal phase of the study which may be due to increased pathophysical condition of liver tissues as contributed by higher doses of nandrolone decanoate. Similar decreased activity of xanthine oxidase is observed in kidney tissues. Illicit uses of anabolic steroids are one of the potent causes of cellular adenomas and adenocarcinomas [13].Decreased xanthine activity is reported with advanced stage deep tumour penetration, large tumour size and cellular aneuploidy [14]. A correlation between enhanced growth of tumour, enzymatic protein degradation and dropping of xanthine oxidase activity has also been demonstrated [15].

Decreased xanthine oxidase activity is also linked to various types of cancer. Patients with breast cancer and other cancers showed a decreased xanthine oxidoreductase expression[14,16]. Xanthine oxidoreductase expression is used as a new marker for gastric cancer [14].

In the present investigation, it is observed that xanthine oxidase activity declines in the terminal phase of study after a good initial peak; however the enzymatic fluctuation in different day's intervals varied in both liver and kidneytissues. The potential role of selective presence of enzymes in different tissues can be derived from many forms, which generate reactive metabolites *in situ* from a number of xenobiotics. Selective expression of the enzyme forms possibly contributes to tissue specific damage caused by xenobiotics [17] .

From the above findings, it can be concluded by suggesting that Nandrolone decanoate is one of the potential inducer of xanthine oxidase activity in liver and kidney tissues. The decreased xanthine oxidase activity in both the tissues can be of potential importance in knowing the pathological state of tissues. However, a much more investigations have to be carried to ascertain the activity of xanthine oxidase by other anabolic steroids which will be of pharmaceutical and therapeutic importance.

**Table 1: Presenting the mean values of xanthine oxidase (unit/mg) in liver tissue of different experimental groups at different days of interval.**

| Groups | | Days of treatment | | | | | |
|---|---|---|---|---|---|---|---|
| | | 15th day | 30th day | 45th day | 60th day | 75th day | 90th day |
| **Normal Control Group (n=10)** | Mean | 0.247 | 0.236 | 0.238 | 0.231 | 0.233 | 0.238 |
| | SD± | 0.032 | 0.031 | 0.032 | 0.033 | 0.031 | 0.034 |
| | SEM± | 0.010 | 0.009 | 0.010 | 0.010 | 0.010 | 0.010 |
| **Nandrolone Treated Group (n=10)** | Mean | 0.343 | 0.382 | 0.384 | 0.360 | 0.343 | 0.261 |
| | SD± | 0.065 | 0.095 | 0.020 | 0.071 | 0.075 | 0.011 |
| | SEM± | 0.020 | 0.030 | 0.006 | 0.022 | 0.024 | 0.003 |

SD=standard deviation , SEM=standard error of mean.

**Table 2: Presenting percentage deviation of xanthine oxidase (unit/mg) in liver tissue of nandrolone treated group from the mean values of normal control group.**

| Groups | Mean % deviation | Days of treatment | | | | | |
|---|---|---|---|---|---|---|---|
| | | 15th day | 30th day | 45th day | 60th day | 75th day | 90th day |
| **Normal control group** | Mean | 0.247 | 0.236 | 0.238 | 0.231 | 0.233 | 0.238 |
| **Nandrolone treated group** | % deviation | 38.86 | 61.86 | 61.34 | 57.14 | 47.21 | 9.66 |

**Table 3: Presenting the mean values of xanthine oxidase (unit/mg) in kidney tissue of different experimental groups at different days of interval.**

| Groups | | Days of treatment | | | | | |
|---|---|---|---|---|---|---|---|
| | | 15th day | 30th day | 45th day | 60th day | 75th day | 90th day |
| **Normal Control Group (n=10)** | Mean | 0.210 | 0.207 | 0.198 | 0.200 | 0.204 | 0.203 |
| | SD± | 0.024 | 0.028 | 0.027 | 0.028 | 0.025 | 0.025 |
| | SEM± | 0.008 | 0.009 | 0.009 | 0.009 | 0.008 | 0.008 |
| **Nandrolone treated Group (n=10)** | Mean | 0.289 | 0.356 | 0.362 | 0.367 | 0.336 | 0.249 |
| | SD± | 0.021 | 0.103 | 0.020 | 0.069 | 0.045 | 0.031 |
| | SEM± | 0.006 | 0.032 | 0.006 | 0.022 | 0.014 | 0.009 |

SD=standard deviation, SEM=standard error of mean.

**Table 4: Presenting percentage deviation of xanthine oxidase (unit/mg) in kidney tissue of nandrolone treated group from the mean values of normal control group.**

| Groups | Mean % deviation | Days of treatment | | | | | |
|---|---|---|---|---|---|---|---|
| | | 15th day | 30th day | 45th day | 60th day | 75th day | 90th day |
| **Normal control group** | Mean | **0.210** | **0.207** | **0.198** | 0.200 | 0.204 | 0.203 |
| **Nandrolone treated group** | % deviation | 37.61 | 71.98 | 82.82 | 83.50 | 64.70 | 22.66 |

**Table 5: Presenting significance of difference in the mean values of xanthine oxidase (Unit/mg) in liver and kidney tissue between normal and nandrolone treated group at different day's interval**.

| Tissues | Group | | Days of treatment | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 15th | 30th | 45th | 60th | 75th | 90th |
| **Liver** | **Between** | t | -15.02 | -4.65 | -12.41 | -5.44 | -4.21 | -2.2 |
| | **Normal and** | p | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | >0.01 |
| | **Nandrolone** | df | 18 | 18 | 18 | 18 | 18 | 18 |
| **Kidney** | **treated** | t | -2.48 | -1.49 | -4.88 | -2.24 | -2.56 | -1.16 |
| | | p | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | >0.01 |
| | | df | 18 | 18 | 18 | 18 | 18 | 18 |

t= test of significan, p= level of significance, df=degree of freedom

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