# Effects of intraperitoneal administration of Citral on male reproductive organs in the rat

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## ABSTRACT

**Introduction:** The aliphatic monoterpene 3, 7-dimethyl-2, 6-octadienal (Citral) is a flavour additive widely used in the cosmetic and food industries. It has been shown to have a negative impact on female fertility acting via hormone receptors. No data is available about the effects of Citral on the male reproductive system.

**Objective:** This study was carried out to study the effects of Citral on body weight, weights of the testes, epididymis, seminal vesicles, prostate, liver and kidneys, hepatic enzyme levels, plasma testosterone level and haemoglobin concentration, red blood cell count, white blood cell count and packed cell volume.

**Materials and Methods:** Mature male rats (n = 12) were given Citral at a dose of 300 mg/kg body weight in 1 mL of olive oil, by single intraperitoneal injection for 14 consecutive days. The control group was treated similarly with 1ml of olive oil alone. On day 15, organ weights, plasma testosterone levels, hepatic enzyme levels and haematological parameters were assessed.

**Results:** There were no effects on the liver enzyme levels or on haematological parameters. Testicular and accessory reproductive organ weights and plasma testosterone levels were significantly reduced in the Citral treated group, compared with the controls ($P < L.05$).

**Conclusion:** Citral in a dose of 300mg/kg body weight in 1 ml of olive oil, by single intraperitoneal injection for 14 consecutive days in rats leads to reduction in weights of the testes, epididymis, seminal vesicles, prostate and plasma testosterone levels.

## Introduction

The aliphatic monoterpene 3, 7-dimethyl-2, 6-octadienal (Citral) is a flavour additive widely used in the cosmetic and food industries [1]. Aliphatic monoterpenes are known to induce production of sterile ova in some insects [2, 3] increased embryonic loss [4], oocyte degeneration and impaired fertility in female rats [5]. It has been suggested that these consequences are the result of competition between aliphatic monoterpenes and hormone receptors [6].

There are no data about the effects of Citral on the male reproductive system of mammals. Therefore, this study was carried out to study the effects of Citral on the male reproductive organs of rats.

## Materials and Methods

Twenty four healthy male Wistar strain rats, three months of age and weighing 100 - 105 g were used in this study. Rats were housed under standardized animal house conditions with free access to water and food. The animals were assigned randomly into two groups (the experiment group and the control group), each group comprising twelve rats.

The study group was given intraperitoneal injections of Citral at a dose of 300 mg/kg body weight / day, for 14 consecutive days. Citral was dissolved in 1ml of olive oil neutralized to pH 7 by boiling with sodium bicarbonate [5]. The control group was treated similarly with the vehicle (olive oil) alone.

All animals were observed daily for mortality, overt signs of toxicity, stress and aversive behaviour. Food and water intake was recorded.

Body weights were taken throughout the study period. On day 1 post-treatment, rats from both groups were weighed and sacrificed by ether anesthesia. Blood was collected by cardiac puncture, serum prepared and alanine amino transferase, aspartate amino transferase and alkaline phosphatase levels were determined using Randox enzyme kits and a spectrophotometer (Jasco V500, Jasco corporation, Tokyo, Japan). For hematology blood was collected into sample bottles containing sodium ethylene diamine tetraacetic acid (EDTA) and haemoglobin concentration, red blood cell (RBC) count, white blood cell (WBC) count and packed cell volume (PCV) were assayed. Plasma testosterone was assayed using chemiluminescent immuno assay analyzer (Immulite, U.S.A) according to the instructions. The gross morphology of the testes, epididymis, seminal vesicles, prostate, liver and kidneys were observed and wet weights were recorded. For histological assessment organs were fixed in formalin and stained with haematoxylin and eosin (H&E).

Data are expressed as mean (standard deviation (SD)). For statistical analysis the t-test was used to compare the means of body weights, weights of the testes, epididymis, seminal vesicles, prostate, liver and kidneys, hepatic enzyme levels, plasma testosterone level and haemoglobin concentration, red blood cell count, white blood cell count and packed cell volume. $P<0.05$ was considered as significant.

## Results

Pretreatment body weights were similar in the two groups. There were no treatment related deaths, overt signs of toxicity, stress or aversive behaviour. Food and water intake remained unaltered. Administration of Citral 300 mg/kg body weight/day, intraperitonealy for 14 days caused no apparent toxic effects. The body weights of treated rats were similar to those of the control rats.

The post treatment weights of the testes, epididymis, seminal vesicles and prostate were significantly decreased in the study group compared to the control group (Table 1). The weights of kidneys and liver were not significantly altered between the two groups (Table 1).

Administration of Citral for 14 days at the given dosage had no effect on the liver enzyme or on haemoglobin concentration, PCV and RBC and WBC counts (Table 2).

The Citral treatment at the given dosage had no significant effect on the serum alanine amino transferase, aspartate amino transferase and alkaline phosphatase levels (Table 3).

Plasma testosterone level was significantly reduced in the Citral treated group as compared with the controls (Table 4).

No significant alteration in the macroscopic appearance of the organs was detected. H & E stained histological sections of the organs (liver, kidney, seminal vesicles, prostate) revealed no differences when compared with those of the control group. However, testicular sections showed diminution of the seminiferous tubular diameter compared to that of control rats though this was not quantified.

## Discussion

It has been shown previously that the Lethal Dose 50% ($LD_{50}$) for Citral when administered intraperitoneally is 460 mg/kg body weight [5]. We have selected the dose of 300 mg/kg body weight because it was the largest dose that caused no apparent toxic effects [5].

Citral did not exert any toxic effect on body growth. Furthermore, there were no significant alterations in the hepatic and renal weights nor in the liver enzyme levels. This suggests that this dose of Citral given for 14 days had no metabolic toxicity.

The decreased weights of the testes and secondary sex organs observed in the Citral treated group may be the result of pituitary inhibition [7]. This finding is in accordance with the fact that the plasma testosterone levels are decreased in the Citral treated group. This acute response observed was similar to that observed with deoxyribonucleic acid (DNA) alkelating agents such as bisulphan[8] and ionizing radiation[9].

**Table 1 - Effects of Citral on mean body weight in g (SD) and mean organ weights (g/body weight) (SD).**

| Group | Citral group | Control group |
|---|---|---|
| Initial body wt | 120.00 (3.02) | 122.00 (4.88) |
| Final body wt | 132.00 (3.00) | 137.00 (8.00) |
| Testis wt | 1.03 (0.02)* | 1.78 (0.04) |
| Seminal vesicle wt | 0.03 (0.01)* | 0.68 (0.02) |
| Prostate wt | 0.07 (0.01)* | 0.20 (0.001) |
| Epididymis wt | 0.29 (0.03)* | 0.62 (0.04) |
| Liver wt | 3.50 (0.69) | 3.40 (0.46) |
| Kidney wt | 0.60 (0.02) | 0.60 (0.01) |

** P<0.05, compared with the control group.*

**Table 2 - Effects of Citral on mean haematological parameters (SD).**

| Group | Citral group | Control group |
|---|---|---|
| Hb concentration g/dL | 12.04 (0.40) | 11.98 (0.20) |
| RBC count ($10^6$/$mm^3$) | 8.20 (0.21) | 7.80 (0.34 ) |
| WBC count ($10^3$/$mm^3$) | 5.46 (0.28) | 5.50 (0.21) |
| PCV% | 52.0 (1.00) | 51.00 (1.90) |

**Table 3 - Effects of Citral on mean hepatic enzyme levels (SD).**

| Group | Citral group | Control group |
|---|---|---|
| Alanine amino transferase (IU/L) | 23.20 (2.1) | 24.28 (1.2) |
| Asparate amino transferase (IU/L) | 50.87 (2.2) | 52.13 (1.6) |
| Alkaline phosphatase (K.A. units / 100 mL) | 53.14 (2.0) | 53.47 (1.6) |

**Table 4 - Effects of Citral on mean plasma testosterone level (SD).**

| Group | Citral group | Control group |
|---|---|---|
| Plasma testosterone | 20.24 (0.63)* | 28.04 (0.28) |

** P<0.05, compared with the control group.*

The decreased plasma testosterone level by day 14 suggests a toxic effect of Citral on the Leydig cells. It has been shown that organophosphoric compounds inhibit non specific esterase activity in Leydig cells decreasing testosterone production [10].

The diminution of seminiferous tubular diameter observed in this study is also in agreement with the reduced plasma testosterone levels observed [10].

A great deal of evidence has accumulated in recent years to suggest that there has been a gradual increase in male reproductive pathology over the past 30 - 40 years, as evidenced by increased rates of testicular cancer and declining sperm quality. A number of factors such as pesticides, exogenous estrogens, and heavy metals have been shown to have a negative impact on male reproduction [11]. In the light of these considerations, identification of substances that have adverse effects on male fertility is important.

## Conclusion

Aliphatic monoterpenes, like Citral, are in widespread use in the cosmetic and food industry. Therefore, the results of this study raise some serious questions regarding the impact of these compounds on human reproduction. Although the dose of Citral used in this study is quite large, similar effects on human reproduction may well be obtained with prolonged exposure to smaller doses of aliphatic monoterpenes. Further research is underway to study its effects on spermatogenesis and other reproductive parameters.

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