

## EFFECTS OF TAMOXIFEN AND NAF ON SERUM AND HEPATIC ENZYMES

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**SUMMARY:** Male Wistar rats were given tamoxifen (2 or 4 mg/kg bw/day) and sodium fluoride (20 mg/kg bw/day), five days a week, for six months. Significant adverse changes were observed in the activities of the serum enzymes alanine and aspartate aminotransferase (AlAT and AspAT) and gamma-glutamyl transpeptidase ( $\gamma$ -GT), the liver content of cytochrome P-450, and the activity of hepatic NADPH-cytochrome c reductase.

Keywords: Cytochrome P-450; Food-borne fluoride; Rat intoxication; Serum enzymes; Tamoxifen.

### INTRODUCTION

Antagonists of steroid hormones are increasingly used for pharmacotherapy of estrogen-dependent tumors. The use of tamoxifen (a nonsteroidal estrogen antagonist) for this purpose is associated with a serious risk of stimulating estrogen receptors, thereby leading to hypertrophy of the endometrium and induction of neoplastic transformations in the uterine body.<sup>1-4</sup>

Osteoporosis is a chronic disease of bone tissue with an unclear etiology. This disease is usually managed with hormones, calcium and vitamin D preparations, and occasionally with sodium fluoride which is incorporated into the apatite structure of the bone. However, the effects of sodium fluoride are subject to much controversy owing to the inherent toxicity of NaF and its adverse effects on mineralization of bone tissue.<sup>5-7</sup> Like tamoxifen, sodium fluoride is chronically administered, whereby the toxic action of NaF on the organism may be potentiated.

The aim of this research was to examine the chronic effects of tamoxifen and sodium fluoride by measuring changes in the biochemical marker serum activities of alanine and aspartate transaminotransferase (AspAT and AlAT) and gamma-glutamyl transpeptidase ( $\gamma$ -GT), the liver content of cytochrome P-450, and the activity of hepatic NADPH-cytochrome c reductase.

### MATERIALS AND METHODS

The study was performed in 60 randomly selected young adult male Wistar rats weighing 260–360 g (mean 292 g). Both the control and study groups participated concurrently in the experiment. Rats were divided into six groups of ten animals each. Group I served as control, while the remaining groups received tamoxifen (Zemide, Wyeth, D) and/or NaF mixed with standard chow in the form of globules. Complete consumption of globules was ascertained before the animals were granted access to normal chow. The

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drugs were administered during six months, five days per week, at doses indicated in the table below.

**Table.** Protocol for drug ingestion (5 days per week) by rats for six months

Group I	Control (n = 10)
Group II	Tamoxifen 2 mg/kg bw orally (n = 10)
Group III	Tamoxifen 4 mg/kg bw orally (n = 10)
Group IV	NaF 20 mg/kg bw orally (n = 10)
Group V	Tamoxifen 2 mg + NaF 20 mg/kg bw orally (n = 10)
Group VI	Tamoxifen 4 mg + NaF 20 mg/kg bw orally (n = 10)

Animals were weighed every four weeks. Autopsy was performed after lethal ether anesthesia. Livers were excised, weighed, and examined visually. Blood for biochemical determinations was obtained by cardiac puncture. The hepatic microsomal fraction was prepared by ultracentrifugation of the post-mitochondrial supernatant (100,000 x g, 1 hr), frozen in liquid nitrogen, and stored for up to 5 weeks at  $-70^{\circ}\text{C}$  until assayed.

The activities of alanine (AlAT) and aspartate aminotransferase (AspAT)<sup>8</sup> and gamma-glutamyl transpeptidase ( $\gamma$ -GT)<sup>9</sup> in the serum, the liver content of cytochrome P-450,<sup>10</sup> and the activity of NADPH-cytochrome c reductase<sup>11</sup> in the hepatic microsomal fraction were determined.

Statistical analysis was performed with Student's t-test taking the level of significance as  $p < 0.05$ .

### RESULTS

The highest mean body weight (bw) at the end of the experiment was observed in the control group (321 g) with an average weight gain of 29 g. Significantly lower weight gains ( $p < 0.05$ ) occurred in group II (17 g) and group III (15 g). Weight gain was lowest in group VI (8.8 g;  $p < 0.01$ ). Weight gain in groups IV and V was 26 g and 23 g, respectively (not significant).

The liver in the control group weighed  $3.14 \pm 0.9$  g per 100 g bw. Liver weight was lowest in group IV ( $2.74 \pm 0.45$ ) but the difference was not significant.

At the end of the experiment, biochemical parameters in group II did not differ significantly from the control group I.

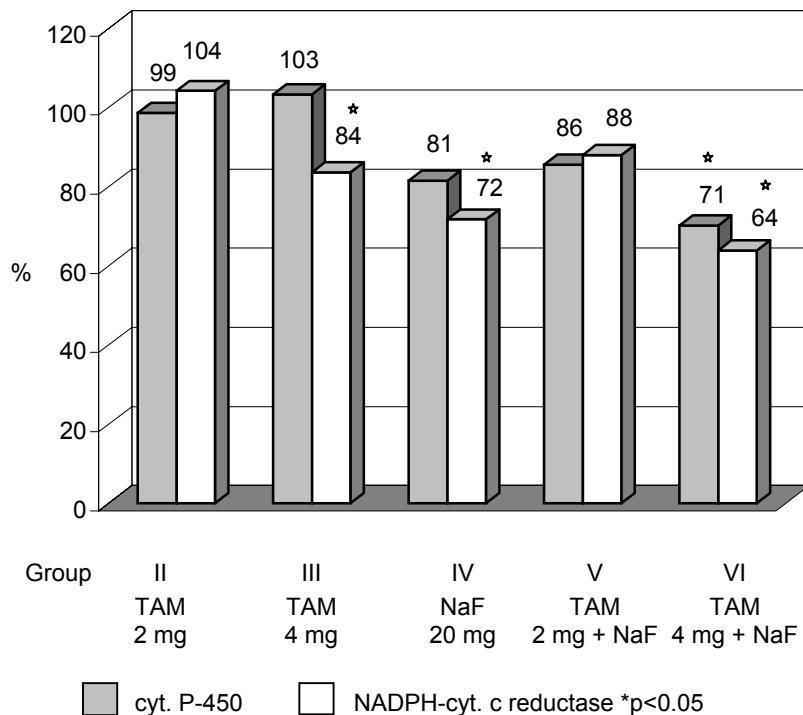
In contrast to the absence of significant differences in group II, group III had a significant 16% reduction in the activity of hepatic NADPH-cytochrome c reductase and a 24% increase in  $\gamma$ -GT activity in the serum ( $p < 0.05$ ).

In group IV the activity of hepatic NADPH-cytochrome c reductase was significantly reduced by 28%. The liver content of cytochrome P-450 was reduced by 19%, although not significantly. Serum activities of ALAT and  $\gamma$ -GT in this group increased by 40% and 39%, respectively.

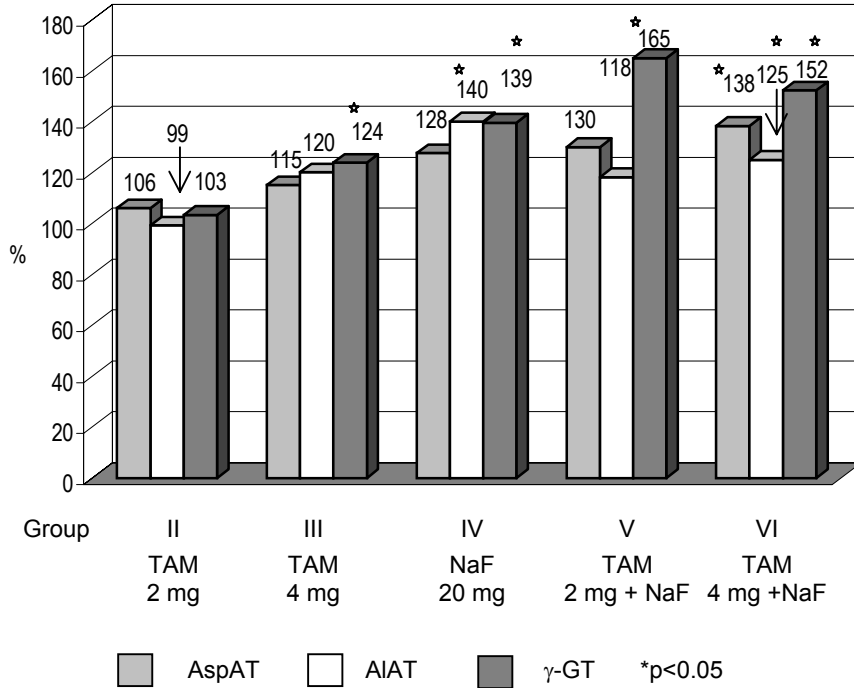
In group V, the liver showed a 14% decrease in cytochrome P-450 content and a 12% decrease in NADPH-cytochrome c reductase activity. Serum As-pAT and ALAT activities in this group increased by 30% and 18%, respectively, which were not significant. The serum  $\gamma$ -GT activity, however, increased significantly by 65%.

In group VI the liver cytochrome P-450 content and NADPH-cytochrome c reductase activity decreased by 29% and 36%, respectively. Serum As-pAT, ALAT and  $\gamma$ -GT activities increased by 38%, 25%, and 52% as compared with controls.

All these results are displayed in Figures 1 and 2.



**Figure 1.** Content of hepatic microsomal cytochrome P-450 and activity of NADPH-cytochrome c reductase (control = 100%).



**Figure 2.** Activities of serum AspAT, AIAT, and γ-GT (control = 100%).

**DISCUSSION**

The aim of this study was to examine the chronic toxicity of tamoxifen (2 or 4 mg/kg bw/day) and sodium fluoride (20 mg/kg bw/day). After six months, significant changes in the activities of some serum enzymes, the liver content of cytochrome P-450, and the activity of hepatic NADPH-cytochrome c reductase were noted. Additional information on toxicity was obtained by weighing the animals and inspecting the liver at autopsy.

The lowest weight gain was noted in animals given 4 mg tamoxifen and 20 mg NaF. Animals given tamoxifen only (2 or 4 mg) also demonstrated lower weight gain as compared with controls. These findings suggest that tamoxifen and NaF exerted a toxic effect on the rats. Fluoride is known to inhibit weight gain in experimental animals.<sup>12,13</sup> A similar reaction to tamoxifen does not appear to have been reported.

Among known toxic effects of fluorides in the living organism are suppression of basic reactions of the energy metabolism, including inhibitory action on components of the respiratory chain, as well as interference with mineralization processes in hard tissues.<sup>14-16</sup> The hepatotoxicity and nephrotoxicity of fluorides were investigated by us previously.<sup>17-22</sup>

Osteoporosis is a chronic disease of bone tissue with an unclear etiology. Risk factors of osteoporosis include hormonal disorders, estrogen deficiency related to menopause, calcium and vitamin D deficiencies, sedentary life style, and unhealthy diets.

The use of sodium fluoride for the management of osteoporosis has frequently been questioned owing to its toxicity, as well as associated disorders of mineralization in the form of increased bone fragility. The present results corroborate the toxicity of sodium fluoride.<sup>5,7,23</sup> As noted in the Results, rats administered NaF at a dose of 20 mg/kg bw/day exhibited significantly higher activities of ALAT and  $\gamma$ -GT, while the activity of NADPH-cytochrome c reductase was significantly reduced in this group.

Tamoxifen, a derivative of triphenylethylene, is a synthetic non-steroid drug used for the management of estrogen-dependent tumors, especially breast cancer. The drug is metabolized in the liver with the involvement of cytochrome P-450 to N-oxide, N-desmethyl, and 4-hydroxy metabolites.<sup>24</sup> Adverse effects of tamoxifen have been demonstrated, the most serious one being risk of fatal endometrial cancer.<sup>2-4</sup> In experimental animals, tamoxifen has been shown to induce hepatic cancer, raising the specter of the same effect in women.<sup>1,25-28</sup> The present work provides further evidence for the toxicity of tamoxifen. Changes were most pronounced in animals given tamoxifen (4 mg) in combination with NaF (20 mg) (group VI), indicating an additive effect for the toxicity of tamoxifen and NaF.

The present findings point to the possibility of similar action of both substances in women treated with tamoxifen and in patients given fluoride preparations for osteoporosis.

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