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Saccharomyces cerevisiae intervention for relieving flutamide-induced hepatotoxicity in male rats

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The aim of this work was to investigate the protective role of baker's yeast *Saccharomyces cerevisiae* against the hepatotoxic effect of the drug flutamide that is widely used for treatment of metastatic prostate adenocarcinoma. Administration of flutamide to adult male rats in a dose of 100 mg/kg.b.w. daily for 15 days resulted in serious hepatic injury. Highly significant increase in each of serum ALT, ALP, bilirubin, bile acids and cholesterol level, relative to the control group, was observed. Also, a highly significant increase in the serum glutathione-S-transferase isoforms: α-GST and π-GST and each of TNF-α and NO levels was recorded. Moreover, highly significant decrease in hepatic glutathione peroxidase and superoxide dismutase activities was observed. In addition, the authors noticed a significant increase in serum testosterone levels with concomitant highly significant increase in serum acid phosphatase activity. Prophylactic treatment of male rats with baker's yeast in a dose of 4.8 mg/kg b.w. daily for 15 days, followed by a combination of flutamide (100 mg/kg b.w.) and yeast (4.8 mg/kg b.w.) daily for other 15 days resulted in marked improvement in rat's liver function, whereas the serum testosterone and acid phosphatase levels retained values parallel to those recorded for the flutamide-treated rats. Histological examination of liver tissues showed that flutamide caused hydropic degeneration, necrotic areas and marked increase in Kupffer cells. The central vein is congested with blood and signs of apoptosis appeared in the hepatocytes in the form of fragmentation of the nuclei and blebbing of the cytoplasm. On the other hand, in the rats treated with both yeast and flutamide, the hepatic cords were more regularly arranged, signs of degeneration or apoptosis were less pronounced and some hepatocytes appeared binucleated. The authors postulate that each one of the powerful antioxidative components in *S. cerevisiae* effectively participated in attenuation of the oxidative stress caused by flutamide metabolites, and in promoting regeneration of new hepatocytes and meanwhile could restore liver function beyond normal status.

1. Introduction

Flutamide is α non-steroidal antiandrogenic anilide compound which is devoid of estrogenic, progestational or androgenic activities. This drug is used for treatment of metastatic prostate adenocarcinoma as a single therapy or in combination with luteinizing hormone releasing analogue (Dourakis et al. 1994; Chu et al. 1998). This antiandrogen drug is also used in combination with oral contraceptives for the treatment of hirsutism (Cusan et al. 1990) and benign prostate hyperplasia (Gomez et al. 1992).

Flutamide is mainly metabolized in the liver and its metabolites are excreted in the urine (Dourakis et al. 1994). After oral administration, flutamide is well absorbed and its circulating form is almost exclusively the active compound 2-hydroxyflutamide (Shet et al. 1997).

Since it was approved by FDA in 1989, hepatic toxicities including mild liver function impairment, hepatitis with cholestasis or bridging necrosis, fulminant hepatitis and even mortality have been reported in Western countries (Chu et al. 1998). Other side effects of flutamide include diarrhea, vomiting, dyspepsia, insomnia, muscle cramps, galactorrhea, gyneocomastia and breast tenderness (Chu et al. 1998).

Also of interest is the observation that flutamide can influence the metabolism of estradiol in particular the 2- and 4-hydroxylation of this essential hormone in patients with prostate cancer (Shet et al. 1997). Flutamide also inhibited mitochondrial respiration and adenosine triphosphate formation (Fau et al. 1994; Wysowski and Fourcroy 1996).

The metabolism of flutamide by the cytochrome P-450 system or by other microsomal enzymes results in the formation of reactive metabolites which can lead to lipid peroxidation and consequently to hepatocytic injury. However, the possibility of an immunological mechanism cannot be excluded despite the absence of fever or rash (Dourakis et al. 1994). Also, a number of reports of hematologic toxicity and hepatotoxicity including cases of fatal hepatotoxicity have appeared in the literature (Rosenthal et al. 1996). Flutamide-induced liver injury was diagnosed

clinically and histologically (Chu et al. 1998). Noteworthy, a recent review included flutamide on a list of newer agents associated with hepatotoxic reactions but did not include what the pathogenesis of the hepatotoxicity might be (Crownover et al. 1996; Rosenthal et al. 1996).

Recent studies suggest that most of the potentially serious drug interactions of interest to physicians involve drug hepatic biotransformation pathways catalyzed by the cytochrome P-450 group of enzymes. Nevertheless, the exact mechanism of flutamide-induced hepatocyte injury remains unclear (Crownover et al. 1996; Chu et al. 1998). Moreover, at present there is no effective therapy for flutamide-induced hepatitis (Chu et al. 1998).

Hartwell et al. (1997) have pioneered a novel approach in which the yeast *Saccharomyces cerevisiae* is used to discover compounds with powerful chemotherapeutic potential. Remarkably, studies with the yeast *S. cerevisiae* as a model system have provided invaluable insights into the action of many drugs and compounds with quite specific activities in both mammals and fungi (Cardenas et al. 1999).

The aim of the present study was to elucidate the pathway of flutamide-induced hepatic injury through biochemical and histologic examinations and to investigate the chemoprotective function of the active antioxidant components in the yeast *S. cerevisiae* for relieving flutamide-induced hepatotoxicity in male rats and restoring liver functions beyond normal status.

2. Investigations, results and discussion

2.1. Biochemical study

The major function of flutamide is to inhibit the binding of circulating adrenal androgen by nuclear receptors in the prostate gland and malignant cells originating in the prostate (Crownover et al. 1996).

It has been reported that flutamide is rapidly metabolized in the liver by the cytochrome CYP 1 A2 to 2-hydroxyflutamide with subsequent hydrolysis to 3′-trifluoromethyl-4′-nitroaniline or glucuronidation of the hydroxylated aromatic ring and excretion in the urine (Shet et al. 1997). In addition, under certain physiological conditions, two minor metabolites are formed during the metabolism of flutamide by the cytochrome CYP 3 A4. However, the structure of these two minor metabolites remains unknown (Shet et al. 1997). Within hepatocytes, biotransformation occurs at the endoplasmic reticulum. A study has shown that the radiolabeled 2-hydroxyflutamide is capable to covalently bind to microsomal membrane fragments (Berson et al. 1993; Shet et al. 1997).

In the present work, oral administration of flutamide to male rats in a dose of 100 mg/kg.bw. daily for a period of 15 days resulted in serious degeneration in their livers. Highly significant increase ($P < 0.01$) in each of serum ALT, ALP, bilirubin, bile acids and cholesterol level, relative to the control group, was observed (Table 1, group II).

The authors postulate that metabolization of flutamide by the cytochrome P-450 enzymes to the reactive electrophilic 2-hydroxy-flutamide induces activation of Kupffer cell phagocytes. Activated Kupffer cells release the superoxide anion radical and reactive oxygen and nitrogen species and chemotactic cytokines which consequently induce neutrophil extravasation and activation (Jaeschke et al. 2002; Spolarics and Wu, 1997) and impose depletion of antioxidant activity (Sivakomar et al. 2001). Also the generated reactive oxygen species (ROS) by the 2-hydroxyflutamide-protein adducts in Kupffer cells are covalently bound to proteins and form DNA mutagenic adducts in the liver which extend to cells of the immune systems.

Gomez et al. (1992) and Raiford and Thigpen (1994) reported that the severity of hepatic injury correlated well with alterations in serum ALT level. Rosenthal et al. (1996) and Chu et al. (1998) found that flutamide aggressively affected liver functions reflected in elevated serum ALT and AST levels. Raiford and Thigpen (1994) reported that flutamide significantly elevated serum total bilirubin concentration. Also, cholestatic hepatitis with or without eosinophilia and cholestatic juandice have been reported (Crownover et al. 1996). Increased concentrations of the hydrophobic bile acids are especially hepatotoxic, and they accumulate in the liver in cholestatic disorders (Jaeschke et al. 2002). Toxic bile acids facilitate Fas and tumor necrosis factor-associated apoptosis (Higuchi and Gores 2003).

Table 2 group (II) shows highly significant increase in the serum glutathione-S-transferase isoforms: α-GST and π-GST levels as compared to control group. Whereas α-GST is primarily found in hepatocytes and π-GST is located on the bile duct epithelium reflecting bile duct injury (Platz

Table 1: Effect of yeast (*S. cerevisiae*) on serum parameters of liver functions of flutamide-treated rats

Groups	ALT (U/L)	ALP (U/L)	Bilirubin (mg/dL)	Bile acids (μmol/L)	Cholesterol (mg/dL)
Control (Group I)	31.5 ± 1.57	190.0 ± 13.2	0.65 ± 0.24	4.5± 0.4	64.5 ± 6.38
Flutamide (Group II)	$66.75 \pm 5.85^{**a}$	$427.2 \pm 45.1^{**a}$	$2.15 \pm 0.21^{**a}$	$7.4 \pm 0.5^{**a}$	$112.2 \pm 11.2^{**a}$
Yeast (Group III)	35.25 ± 5.88^{a} ns	201.0 ± 21.1^{a} ns	0.71 ± 0.32^{a} ns	4.8 ± 0.5^{a} ns	$48.37 \pm 3.31^{*a}$
Yeast + Flutamide (Group IV)	$42.12 \pm 4.0^{**b}$	$279.0 \pm 32.1^{*b}$	$1.61 \pm 0.13^{*b}$	$6.0\pm 0.4^{*b}$	$84.4 \pm 5.52^{*b}$

Values are mean ± SE for 8 rats per group.
[a] = Differences in relation to control group (group I). [b] = Differences in relation to flutamide-treated group (group II)
* $P < 0.05$ ** $P < 0.01$ ns = Differences are insignificant at $P > 0.05$

Table 2: Effect of yeast (*S. cerevisiae*) on damaged liver of flutamide-treated rats

Groups	S.π-GST (μg/L)	S.α-GST (μg/L)	S.TNF-α (pg/ml)	S. NO (μmol/L)
Control (Group I)	12.9 ± 1.4	170.0 ± 6.0	51.56 ± 1.29	36.4 ± 1.0
Flutamide (Group II)	$18.8 \pm 1.8^{*a}$	$289.6 \pm 34^{**a}$	$65.08 \pm 0.52^{**a}$	$50.0 \pm 0.5^{**a}$
Yeast (Group III)	13.2 ± 1.6^{a} ns	188.0 ± 29.9^{a} ns	$47.73 \pm 0.69^{*a}$	$32.9 \pm 1.2^{*a}$
Yeast + Flutamide (Group IV)	15.6 ± 1.2^{b} ns	227.3 ± 24.3^{b} ns	$55.11 \pm 0.73^{**b}$	$40.1 \pm 0.5^{**b}$

Values are mean ± SE for 8 rats per group S = serum
[a] = Differences in relation to control group (group I) [b] = Differences in relation to flutamide-treated group (group II)
* $P < 0.05$ ** $P < 0.01$ ns = Differences are insignificant at $P > 0.05$

et al. 1997), α-GST is readily released into the bloodstream in the event of an hepatocyte injury (Flendrig et al. 1999) and therefore, it is a more sensitive indicator of hepatotoxicity than transaminases (Clarke et al. 1997). Quantitation of π-GST levels in the bile may be of value to assess the risk from toxin-induced hepatic damage. Sipowicz et al. (1997) observed striking increase in both α-GST and π-GST as a result of a marked increase in the levels of cytochrome P-450 (CYP) isoforms: 1A2 and 2A5 in hepatocytes, evidencing major oxidative stress in the mice livers due to increased generation of reactive oxygen species (ROS).

In the present study, a significant positive correlation was found between serum TNF-α and NO levels (Table 2 group II). This result suggests that the Kupffer cells, activated by 2-hydroxyflutamide, may stimulate NO formation directly or indirectly via TNF-α (or other cytokines) and consequently participate in the development of the hyperdynamic circulation. Increased production of TNF-α results in coagulation, thrombosis and local infarcts compromising local tissue perfusion and massive liver damage (Leist et al. 1995). Strong synergy for induction of inducible nitric oxide synthase (i NOS) expression was seen between TNF-α, interleukin-1 β(IL-1β), interferon gama (INF-γ) and liposaccharide (Geller et al. 1993). Also, NO can react with superoxide anion radical to generate peroxynitrite (Radi et al. 1991) which causes protein nitration with further tissue injury by decomposing to the more toxic hydroxyl radical (Billiar 1995).

The data shown in Table 3 reveals a highly significant decrease ($P < 0.01$) in hepatic glutathione peroxidase (GPX) and superoxide dismutase (SOD) levels for the flutamide-treated group II as compared to the control group, a matter which reflects the severity of oxidative stress resulting from excessive generation of reactive oxygen species which impose depletion of the antioxidants activity (Sivakomar et al. 2001). Fau et al. (1994) found that flutamide decreased the reduced glutathione (GSH)/glutathione disulfide (GSSG) ratio and total protein thiols. This was associated with an early increase in phosphorylase – a activity and the release of lactate dehydrogenase with subsequent loss of cell viability.

Flutamide acts by blocking the binding of testosterone (and dihydrotestosterone) to its intracellular receptors. It also blocks the inhibitory effect of testosterone on gonadotropin secretion, and therefore serum luteinizing hormone and testosterone concentrations increase (Catalona 1994). Significant increase ($P < 0.05$) in serum testosterone levels with concomitant highly significant increase ($P < 0.01$) in serum acid phosphatase activity were observed for the flutamide-treated rats (Table 4, group II) as compared to control group. Marchetti and Labrie (1988) and Pazos et al. (2000) reported that blockade of the rat androgen receptors by flutamide led to a marked elevation in serum testosterone level associated with a significant increase in plasma gonadotropin concentration (Shin et al. 2002). Activity of the lysosomal enzymes, acid phosphatase and beta glucuronidase increased in the free state in flutamide-treated rats (Mathur and Chattopadhyay 1982). In addition, Mukherjee et al. (1992) observed an increased activity of kidney beta glucuronidase reflected on increased availability of testosterone. Noteworthy, You et al. (1998) reported that androgen receptor (AR) staining intensity was decreased in the testicular tissue in the flutamide-treated rats.

Table 3: Effect of yeast (*S. cerevisiae*) on liver antioxidant enzyme activities in flutamide-treated rats

Groups	L.GPX (Units/mg liver protein)	L.SOD (Units/mg liver protein)
Control (Group I)	0.99 ± 0.037	95.9 ± 6.2
Flutamide (Group II)	$0.72 \pm 0.011^{**a}$	$69.1 \pm 2.5^{**a}$
Yeast (Group III)	$1.31 \pm 0.045^{**a}$	$126.3 \pm 5.4^{**a}$
Yeast + Flutamide (Group IV)	$0.93 \pm 0.057^{**b}$	$86.3 \pm 2.8^{**b}$

Values are mean ± SE for 8 rats per group
L = liver
[a] = Differences in relation to control group (group I)
[b] = Differences in relation to flutamide-treated group (group II)
** $P < 0.01$

Table 4: Effect of yeast (*S. cerevisiae*) on testicular function of flutamide-treated rats

Groups	S. testosterone (ng/ml)	S. acid phosphatase (U/L)
Control (Group I)	7.90 ± 0.58	9.25 ± 0.86
Flutamide (Group II)	$9.26 \pm 0.46^{*a}$	$14.17 \pm 0.89^{**a}$
Yeast (Group III)	8.72 ± 0.26^{a} ns	9.60 ± 0.73^{a} ns
Yeast + Flutamide (Group IV)	9.46 ± 0.29^{b} ns	$11.75 \pm 0.43^{*b}$

Values are mean ± SE for 8 rats per group
S = serum
[a] = Differences in relation to control group (group I)
[b] = Differences in relation to flutamide-treated group (group II)
* $P < 0.05$ ** $P < 0.01$
ns = Differences are insignificant at $P > 0.05$

Programmed inhibition of Kupffer cells activity protects the liver from a number of toxicants that require biotransformation to elicit toxicity. Badger et al. (1997) suggested that the mechanism of this protection is thought to result from depleted secretion of inflammatory and cytotoxic products from Kupffer cells. Taking advantage of this approach, the authors propose a novel concept of hepatoprotection against Kupffer cell-induced injury, after clinical use of flutamide, through oral administration of the bakers yeast *Saccharomyces cerevisiae*, without paralyzing the vital function of Kupffer cells in host defence mechanism. This yeast contains a number of the essential antioxidant trace elements, mainly selenium and zinc in addition to cobalt (Table 5), together with yeast cell wall mannans (Krizkova et al. 2001 and Aizawa et al. 1989), the polysaccharide beta-glucan (Cross et al. 2001; Lee et al. 2001; Hunter et al. 2002; Kogan et al. 2002) and the IMP2 gene (Masson and Ramotar 1996). In addition, it contains the antioxidative cytosolic enzymes: glutathione peroxidase (GPX), superoxide dismutase (SOD) and catalase (CAT) together with mitochondrial Mn-SOD, seleno-DL-methionine, sodium selenite and vitamins A, B_{12} and E (Bronzetti et al. 2001).

Firstly, we assessed the direct effect of *S. cerevisiae* on normal rat's liver function (Table 1, group III) through oral administration of the yeast in a dose of 4.8 mg/kg b.w. daily for a period of 30 days. No marked adverse action of the yeast on the rat's liver could be observed. Intangible increase in serum ALT, ALP, bilirubin and bile acids levels was detected, with a significant drop in serum cholesterol level as compared to control group (Table 1, group III). The significant decline in serum cholesterol

Table 5: Detection of the essential trace elements in the yeast *S. cerevisiae*

Se (μg/g)	Zn (μg/g)	Co (μg/g)
8.58	5.48	0.19

levels may be mediated by the action of the beta-glucan moiety present in the yeast (Uusitupa et al. 1992).

S. cerevisiae could hardly affect serum α-GST and π-GST levels whereas it significantly decreased serum TNF-α and NO levels (Table 2, group III) as compared to control. On the other hand, the yeast could significantly increase liver GPX and SOD levels (Table 3, group III) as compared to control group. As a selenoprotein, GPX provides a useful tool for investigating the functional Se-status in animals (Malbe et al. 1995). For several years, GPX was thought to be the only known selenoprotein in mammalian tissues. *In vivo* studies with the radioactive ^{75}Se revealed detection of 13 Se-containing proteins of vital biological importance (Behne et al. 1991). A portion of the yeast selenomethionine is metabolized to selenocysteine which is then decomposed to selenide by selenocysteine β-lyase. This selenide-Se fraction is incorporated into the GPX molecule (Yoshida et al. 1999). In this way, we can interpret the marked increase observed in the liver GPX level of male rats (Table 3, group III). Field et al. (1988) reported that Se produced a highly significant and lasting increase in GPX in animals.

Serum testosterone levels slightly increased by the action of the antiandrogen moiety present in *S. cerevisiae* (Rana et al. 1998) on the androgen receptors, whereas serum acid phosphatase level retained a value comparable to that in the control group (group I) (Table 4, group III).

Prophylactic treatment with *S. cerevisiae*, prior to clinical use of flutamide (Table 1, group IV) was found to stimulate the rat's immune system and to modulate the hepatotoxic symptoms resulting from the reactive metabolites of flutamide. Meanwhile, oral administration of the yeast (4.8 mg/kg. b.w. daily for 15 days), followed by a combination of flutamide (100 mg/kg b.w.) and yeast (4.8 mg/kg b.w.) daily for other 15 days resulted in a marked improvement is rat's liver functions. Serum ALT level highly significant decreased ($P < 0.01$), whereas each of serum ALP, bilirubin, bile acids and cholesterol level significantly decreased ($P < 0.05$) (Table 1, group IV) as compared to the corresponding data for the flutamide-treated rats (Table 1, group II). These data show to what extent the powerful active components in *S. cerevisiae* could modulate the severe hepatotoxicity caused by the reactive electrophilic metabolite 2-hydroxyflutamide and counteract the hard oxidative stress. Table 2 (group IV) reflects marked decrease in both serum π-GST and α-GST levels whereas the serum TNF-α and NO levels were significantly decreased as compared to group II. On the other hand, both liver GPX and SOD levels were significantly increased ($P < 0.01$) (Group IV) as compared to the corresponding data for the flutamide-treated rats (Table 3, group II). However, the concentration of testosterone in the rat serum retained a value parallel to that for the flutamide-treated rats (Table 4, group IV), whereas serum acid phosphatase level significantly decreased as compared to the flutamide-treated rats (Table 4, group IV).

One possibility meriting consideration is that activation of Kupffer cells by 2-hydroxyflutamide induces a complex network of cytokines (Jaeschke et al. 2002) and reactive oxygen species which participate in sinusoidal accumulation of granulocytes and microcirculatory failure with subsequent vascular inflammation and impairment in liver functions (Bilzer et al. 1999). Therapeutically, *S. cerevisiae* represents an immunopotentiator and biological response mediator. The polysaccharide beta-glucan, one of the major cell wall components of *S. cerevisiae,* acts as nonspecific immune system stimulant. It stimulates cytotoxic T-lymphocytes, β-cells and activates macrophages (Cross et al. 2001; Kogan et al. 2002). Fullerton et al. (2000) reported that beta-glucan may have great potential as an alternative therapeutic modality for prostate cancer.

Krizkova et al. (2001) stated that the *S. cerevisiae* cell wall mannan has relatively good antioxidative and antimutagenic effects as it is capable to scavenge reactive oxygen radicals. Masson and Ramotar (1996) proposed that the *S. cerevisiae* c-IMP2 gene prevents oxidative damage by regulating the expression of genes that are directly required to repair DNA damage.

Glutathione peroxidase (GPX) together with superoxide dismutase (Cu/Zn-SOD) and catalase (CAT) form part of the cellular antioxidant defence system against reactive metabolites. They protect cells against damage caused by free radicals and hydroperoxides or lipoperoxides (Macpherson 1994). Pereira et al. (2001) explained the role of SOD1, SOD2 and TPS1 genes which encode the cytoplasmic Cu/Zn-SOD, mitochondrial Mn-SOD and trehalose-6-phosphate synthase TPS1, respectively, in the development of resistance to oxidative stress. In addition to its antioxidant activity, Cu/Zn-SOD plays vital role in zinc homeostasis (Wei et al. 2001).

In fact, the trace elements (Table 5) function as cofactors to antioxidant enzymes as they increase enzymes scavenging activities. It has been reported that cobalamin (B_{12}) deficiency induces elevated levels of both methylmalonic acid and total homocysteine (Stabler et al. 1990). Separate studies with two Se/Co supplemented products showed lasting responses to Se but transient responses to the Co in the form of increase in plasma vitamin B_{12} concentration. Benefits in terms of increased liver B_{12} concentration were more prolonged (Field et al. 1988; Suttle et al. 1990). Zinc (Table 5) has been shown to have an antioxidant role: it protects the sulfhydryl group against oxidation and prevents hydroxyl and superoxide anion radicals generation by transition metals (Gibbs et al. 1985; Bray and Bettzer 1990). However, the liver seems to play a special role in zinc metabolism (Hall et al. 1979). Beytut and Aksakal (2003) reported that Se and vitamin E are effective in reducing hepatic damage in glucocorticoid-treated rats.

Noteworthy, the antiandrogen moiety in *S. cerevisiae* (Rana et al. 1998) exerted synergistic action with flutamide in blocking the rat androgen receptor reflected on increase in serum testosterone level which retained a value parallel to that recorded for the flutamide-treated rats (Table 4, group IV). Blocking androgen receptors has primarily been considered an essential requisite in the pathway of treating prostate carcinoma.

Fortunately, the liver has enormous regenerative capacity for hepatocytes. We hypothesize that each one of the aforementioned powerful antioxidative components in *S. cerevisiae* effectively participates in attenuation of the oxidative stress and in modulation of the severe hepatotoxicity of flutamide metabolites and succeeded in promoting the regeneration of new hepatocytes and meanwhile could restore rat's liver functions beyond normal status.

However, these postulations were once more clarified throughout the histologic examinations of thin sections of the livers of all animal groups.

2.2. Histologic examination

Treatment with flutamide resulted in drastic changes in the histological features of the rat's liver. Unlike the regular

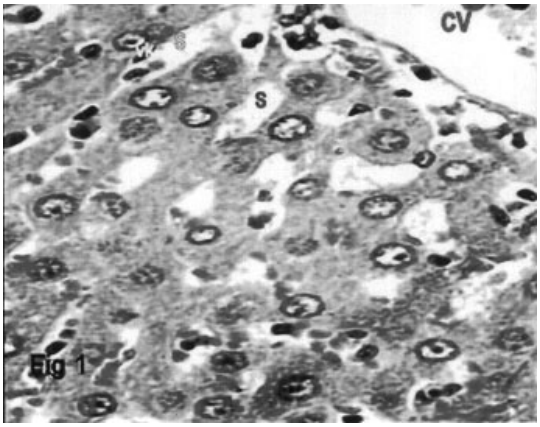

Fig. 1: Section in liver of control rat. CV: central vein, vk: Von Kupffer cells, S: sinusoid. (Hx, E. × 400)

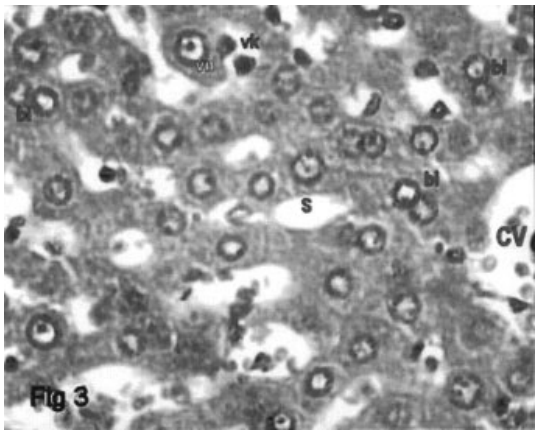

Fig. 3: Section in liver of rats treated with yeast alone. CV: central vein, S: sinusoid, vk: Von Kupffer cells, bn: binucleated cells. (Hx, E. × 400)

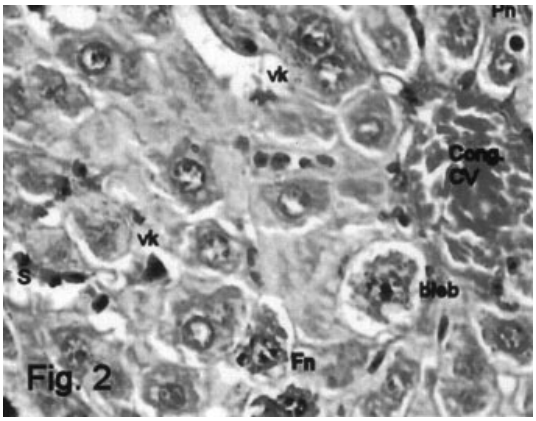

Fig. 2: Section in liver of flutamide treated rat. Cong. CV: congested central vein, S: sinusoid, vk: von Kupffer cells, bleb: hepatocyte with blebbing cytoplasm. Fn: hepatocyte with fragmented nucleus, Pn. Hepatocyte with pyknotic nucleus. (Hx, E. × 400)

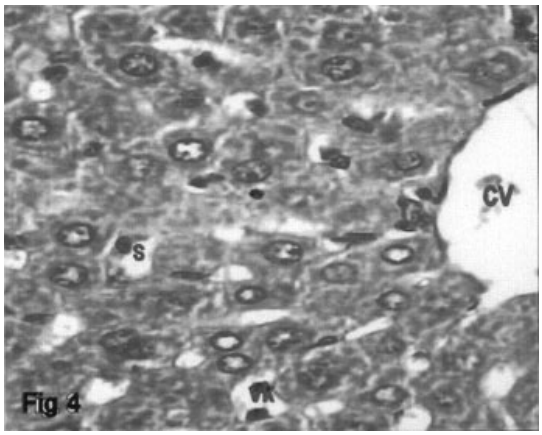

Fig. 4: Section in liver from rats treated with yeast and flutamide. CV: central vein, S: sinusoid, vk: Von Kupffer cells, bn: binclueated cells. (Hx, E. × 400)

arrangement of hepatic cords observed in sections from control rats (Fig. 1), the cords in the liver of flutamide-treated rats (Fig. 2) are not regularly arranged. Moreover, the hepatocytes encroach on the sinusoids resulting in their narrowing and the central vein is congested with blood. The Kupffer cells are large especially in dilated sinusoids. The hepatocytes have an eosinophilic cytoplasm. Signs of apoptosis appear in the hepatocytes in the form of fragmentation of the nuclei and blebbing of the cytoplasm. Some hepatocytes appear completely devoid of nuclei. In conformity with these results, histologic examination of liver biopsy for two patients treated with flutamide showed massive hepatic necrosis (Dourakis et al. 1994). Gomez et al. (1992) observed histologic signs of acute cholestatic hepatitis, also areas of spotty necrosis along with acidophilic bodies with nuclear remnants were also seen for the needle biopsy of the liver of one patient showing juandice after treatment with flutamide.

In yeast-treated animals, regular arrangement of hepatic cords is clearly observed. The sinusoids are of normal arrangement and size. Binucleated hepatocytes are more abundant (Fig. 3).

In rats treated with both yeast and flutamide (Fig. 4) the hepatic cords are more regularly arranged as compared to the flutamide-treated rats. Signs of degeneration or apoptosis are less pronounced, and some hepatocytes appeared binucleated.

In conclusion, we stress the need for a better knowledge of the potential hepatic side-effects of flutamide in clinical practice and for cautiously monitoring administration of this drug. The authors recommend that flutamide administration should be restricted only to advanced stages of metastatic prostate carcinoma. Prophylactic treatment of patients with *S. cerevisiae* should precede, then go in parallel with the clinical use of flutamide with repeated liver function tests. Administration of flutamide must be immediately discontinued if the hepatic transaminases exceed four-fold normal laboratory values.

3. Experimental

3.1. Materials

Yeast was produced by The Egyptian Starch, Yeast and Detergents Co., Alexandria, Egypt. Flutamide was produced by Schering Plough Co., U.S.A.

3.2. Animals and drug administration

32 adult Sprague Dawley male rats weighing 170–200 g were used in this study. The animals fed standard laboratory pellet diet and tap water *ad libitum*. The environmental conditions were standardized with respect to temperature, humidity and light. The animals were divided into four groups (8 rats each). The first group served as control group. The second group was orally given flutamide, dissolved in water, in a dose of 100 mg/kg b.wt. (Clos et al. 1988) daily for 15 days. The third group was orally given yeast, dissolved in water (4.8 mg/kg b.wt.) (Bogye et al. 1998), daily for 30 days. The fourth group was pre-treated with yeast (4.8 mg/kg b.wt.)

daily for 15 days then received flutamide (100mg/kg b.wt.) in combination with yeast (4.8 mg/kg b.wt.) daily for other 15 days.

3.3. Sample collection

The fasting blood samples were collected from the retroorbital plexus of rats (Schermer 1967) and were left to clot, then centrifuged at 5000 r.p.m. for 10 min to separate sera which in turn were used for the determination of different biochemical parameters. After collection of blood samples, all animals were sacrificed and liver tissues were removed and immediately homogenized in 50 mM ice-cold phosphate buffer (pH 7.4) (Lin et al. 1998) to give 2% homogenate (W/V). The homogenate was centrifuged at 1000 r.p.m. for 10 min. The supernatant (2%) was used for the determination of glutathione peroxidase activity. The supernatant (2%) was further diluted with phosphate buffer solution to give 0.5% dilution for the determination of superoxide dismutase activity and protein content.

3.4. Biochemical analyses

Serum alanine aminotransferase (ALT) activity and serum bilirubin levels were determined by colorimetric methods according to Reitman and Frankel (1957) and Scherlock (1951) respectively, using Randox Laboratories kits. Serum alkaline phosphatase (ALP) activity was determined by kinetic spectroscopy using Randox Laboratories kit according to the method of Bowers and McComb (1966). Serum bile acids and cholesterol levels were measured according to the methods described by Mashige et al. (1981) and Richmond (1973), respectively, using kits purchased from Randox Laboratories. Quantitative estimation of rat Pi-glutathione-s-transferase (π-GST) and alpha glutathione-s-transferase (α-GST) in serum were carried out by enzyme linked immunosorbent assay (ELISA) according to the methods of Platz et al. (1997) and Flendrig (1999), respectively, using kits purchased from Biotrin International LTD.

Quantitative determination of TNF-α was carried out by an ELISA procedure according to the method of Corti et al. (1992) using a kit purchased from Diaclone Research Company. Plasma nitrate and nitrite concentrations as an indicator of nitric oxide generation were analysed using Griess reaction after quantitative conversion of nitrate to nitrite by nitrate reductase according to the method of Moshage et al. (1995) using ELISA technique. Liver glutathione peroxidase activity was determined using reduced glutathione and cumene hydroperoxide as substrates, using 20 µl homogenate supernatant, by the modified method of Paglia and Valentine (1967). Hepatic superoxide dismutase activity was assayed by a red formazan dye reduction procedure (Suttle 1986) using 50 µl diluted homogenate supernatant. The specific activities of superoxide dismutase and glutathione peroxidase were expressed as unit of activity/mg liver protein. The protein content of liver tissue was measured applying the method of Lowry et al. (1951).

Quantitative estimation of serum testosterone levels was carried out by the ELISA procedure of Parker (1981) using a kit purchased from Biochem ImmunoSystems Co. Serum total acid phosphatase activity was determined by the method of Fishman and Lerner (1953) using a kit purchased from Randox Laboratories.

3.5. Determination of zinc, cobalt and selenium content in yeast sample

The concentration of each of zinc and cobalt in yeast solution (40 g yeast in 100 ml distilled water) was detected using Flame Atomic Absorption (Varian Spectr AA 220) and selenium concentration in yeast solution (10 g yeast in 100 ml distilled water) was determined using a fully automated Atomic Absorption Spectrometer (Varian Spectr AA220) attached with a graphite furnace (GTA 110).

3.6. Histopathological techniques

At the time of termination of the experiment, the liver was excised from each animal and fixed in 10% formaline saline solution for 24 h. More washing in tap water overnight was followed by dehydration in graded alcohol, clearing in xylene for 20 min and embedded in paraffin wax. Transverse sections were then cut at 5 µm thickness and mounted on albuminized slide paraffin sections and were used for demonstration of the general pathological structures with haematoxylin and eosin stain (Drury and Wallington 1980).

3.7. Statistical analysis

The results were expressed as means ± SE. Statistical analysis of the differences between means was performed using Student's "t" test (Snedecor and Cochran 1967).

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