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Protective and Therapeutic Efficacy of Sodium Butyrate on Tamoxifen-Induced Non-Alcoholic Fatty Liver Disease in Male Rats.

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ABSTRACT

This study aimed to investigate the protective and therapeutic efficacy of sodium butyrate (*NaBu)* to reduce lipid accumulation and liver steatosis induced by tamoxifen (TAM) in rats. Animals were divided mainly into protective and therapeutic groups. Each group were subdivided into four subgroups; (control) received saline either for 7 or 14 days, (NaBu) (300mg/kg) injected intraperitoneally for 7 days prior to or after saline administration for 14 days, (TAM) (40mg/kg) received orally for 14 days prior to or after saline administration for 7 days, (NaBu-TAM) received NaBu for 7 days then TAM for 14 days, (TAM-NaBu) received TAM orally for 14 days then NaBu for 7 days. NAFLD was assessed in TAM group by increasing the levels of total lipids, triglycerides and MDA. In addition to the reduction in AST, ALT, total protein, albumin, A/G ratio, ALP and some oxidative biomarkers. NaBu administration as a protective or therapeutic agent showed reduction in total lipids, triglycerides and MDA levels, with an increase in the tested endogenous scavengers accompanied with healthy hepatic histopathological examination. sodium butyrate exhibited protective and therapeutic effects in dissolving accumulated lipid and reducing steatosis induced by TAM in liver.

Keywords: Tamoxifen; Sodium butyrate; Steatosis; Liver function; Lipid profile.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most public chronic liver challenging which influences 25–30% of the world's population [1, 2]. NAFLD is the accumulation of fat and triglycerides in the hepatocytes without a history of alcohol. NAFLD is developing from simple steatosis through non-alcoholic Steatohepatitis (NASH). Simple steatosis is a benign disorder that can be reversed by some drugs while NASH is an irreversible form of the disease which leads to hepatocellular inflammation and causes progress to fibrosis, cirrhosis and Hepatocellular Carcinoma (HCC) [2]. NASH is associated with some medications, possibly including tamoxifen [3].

Tamoxifen (TAM) citrate, (1[4(2dimethylaminoethoxy) phenyl] 1,2 diphenyl-1-butene) was approved by the Food and Drug Administration of the USA in 1977 as an adjuvant hormonal therapy for estrogen-receptor-positive (ER positive) breast cancer [4]. It is also used for infertility treatment due to its stimulatory effect on the secretion of pituitary gonadotropin hormones [5, 6]. As soon as tamoxifen was approved, it became the first line of adjuvant hormonal therapy and can reduce the mortality rate by 31%.[7]. The mechanism of TAM to inhibit cancer cell growth is through competitive blocking of estrogen receptors. In addition, TAM inhibits the growth of estrogen receptor-negative breast cancer cells [8]. Taking tamoxifen may cause 30-40% risk of developing NAFLD, according to different diagnosis devices [9]. About 43% of women with breast cancer who are treated with TAM developed steatosis [10] within the first 2 years of treatment then steatohepatitis and cirrhosis [11]. The effect of TAM on hepatic lipid metabolism has been investigated. Both the inhibition of fatty acid oxidation and increased triacylglycerol (TG) biosynthesis have been associated in the process [12].

A type of hepatotoxicity has been described in association with tamoxifen; nevertheless, its association with fatty liver is the most commonly encountered. Mild to moderate steatohepatitis, macrovascular steatosis, and rarely cirrhosis are the common results in liver biopsies either by increasing the developed fatty liver or improving the previously mentioned fatty liver disorders and even delaying fatty liver improvement [13, 14].

TAM caused oxidative liver damage due to overproduction of oxygen radical during TAM metabolism, and its genotoxic and carcinogenic effects have been clarified in rodents [15, 16]. The mechanism of TAM-induced hepatotoxicity appears to include mitochondrial dysfunction that causes steatosis due to impaired β-oxidation of fatty acids and leads to the generation of reactive oxygen species (ROS) and ATP depletion [17].

Butyrate is a short-chain fatty acid (SCFA) produced during fermentation of fibers and other substrates by an anaerobic bacteria resident in the gastrointestinal tract [18]. The bioactivities of sodium butyrate are related to inhibition of class I and class II histone deacetylases. Histone deacetylases regulate gene transcription through modification of chromatin structure by deacetylation histone proteins and transcription factors [19]. Elevation of SCFA availability by increasing dietary fiber intake or diet supplementation with butyrate may prevent the development of metabolic disarrangements and the insulin resistance associated with obesity [20]. These effects have been related to the promotion of energy expenditure through enhanced mitochondrial expression of uncoupling protein 1 (UCP1) [21]. Moreover, Butyrate protects against high-fat-diet-induced metabolic changes [22] and has anti-inflammatory properties [23]. The hepatic utilization of butyrate is proportional to the digestive supply [24].The mechanism of butyrate is recognized by transcriptional upregulation of detoxifying enzymes, such as glutathione-S-transferase (GST) which may protect cells from genotoxic carcinogens, such as H_2O_2 [25]. Moreover, it has been mentioned that sodium butyrate attenuated organ injury by minimizing oxidative stress and inflammation [26]. In healthy humans, it has been demonstrated that locally administered butyrate in physiological concentrations increased the antioxidant GSH and possibly decreased ROS production [27].

In the present study, we investigated the protective and therapeutic roles of sodium butyrate to suppress the side effects of tamoxifen-induced hepatotoxicity as well as NAFLD.

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MATERIALS AND METHODS

Experimental Animals:

The experimental animals used in this study were male albino rats (*Rattus norvegicus*) weighing 250-300 ±5 g. The animals were purchased from National Research Center (NRC, Giza, Egypt). Rats were grouped and housed in polyacrylic cages (eight animals per cage) in the well-ventilated animal house of the Department of Zoology, Faculty of Science, Cairo University. Animals were given food and water *ad libitum*. Rats were maintained in a friendly environment with a 12 h/12 h light-dark cycle at room temperature (22°C–25°C). Rats were acclimatized to laboratory conditions for 7 days before commencement of the experiment.

Chemicals and Reagents

Nolvadex (tamoxifen citrate 20 mg) was obtained from AstraZeneca (Egypt). Sodium butyrate was purchased from Sigma-Aldrich (St. Louis, MO, USA). Kits for all biochemical parameters were purchased from Biodiagnostic Company (Giza, Egypt).

Ethical Consideration

Experimental protocols and procedures used in this study were approved by the Cairo University, Faculty of Science, Institutional Animal Care and Use Committee (IACUC) (Egypt) (CUFS/F/PHY/45/14). All the experimental procedures were carried out in accordance with international guidelines for the care and use of laboratory animals.

Experimental Design

The study will include forty-eight male Wistar rats. Animals were divided into two main groups (28 rats/group): the protective and the therapeutic groups.

Protective Group animals were divided into four subgroups (7 rats/group) as follows.
Subgroup I: (control) rats received saline intraperitoneally (i.p.) for 7 days and then received saline orally for 14 days
Subgroup II: (NaBu) rats received sodium butyrate (300mg/kg) (i.p.) for 7 days and then received saline orally for 14 days
Subgroup III: (TAM) rats received saline (i.p.) for 7 days and then received TAM (40mg/kg) dissolved in saline orally for 14 days.
Subgroup IV: (NaBu-TAM) rats received sodium butyrate (300mg/kg) (i.p.) for 7 days and then received TAM (40mg/kg) dissolved in saline orally for 14 days.

Therapeutic Group animals of this group were divided into four subgroups (7 rats/group) as follows.
Subgroup I: (control) rats received saline orally for 14 days and then received saline (i.p.) for 14 days
Subgroup II: *(NaBu)* rats received saline orally for 14 days and then received sodium butyrate (300mg/kg) (i.p.) for 7 days
Subgroup III: (TAM) rats received TAM orally for 14 days and then received saline (i.p.) for 7 days.
Subgroup IV (TAM-NaBu) rats received TAM orally for 14 days and then received sodium butyrate (i.p.) for 7 days.

At the end of the experiment, rats were euthanized and sacrificed after being fasted overnight. A blood sample from each group was collected without anticoagulant in centrifuge tubes. The liver was removed and immediately blotted using filter paper to remove traces of blood and stored at -80°C for biochemical analyzes while the second part was suspended in 10% formal saline for fixation in preparation for the histopathological examination.

Biochemical Analyses

The collected blood samples were centrifuged at 4000 rpm for 20 minutes. The collected serum was stored at -20°C until used for biochemical assays. The levels of aspartate aminotransferase (AST), alanine

aminotransferase (ALT) [28], serum total protein [29], serum albumin [30], alkaline phosphatase activity (ALP) [31], serum total bilirubin and direct bilirubin [32], total lipids [33], triglycerides [34], serum total cholesterol and high-density lipoprotein (HDL) [35] were determined using Bio-diagnostic assay kits according to the manufacturer's instructions (Giza, Egypt).). Serum globulin concentration was calculated from the formula. Serum globulin concentration (g/dl) = Serum total protein - Serum albumin. Indirect bilirubin was calculated as follows: Indirect bilirubin = Total bilirubin−direct bilirubin. A/G ratio = Albumin/Globulin. The concentration of LDL- cholesterol can be calculated according to Friedewaldes formula [36]. LDL-cholesterol (mg/dl) = Total cholesterol– (triglycerides/5) – HDL Cholesterol. Very low density lipoprotein (VLDL) was calculated from this formula: VLDL=Triglycerides/5.

Hepatic Oxidative Stress Analysis

Malondialdehyde (MDA) [37], reduced glutathione (GSH) [38], glutathione-S-transferase (GST) [39], glutathione peroxidase (GPX) [40], catalase (CAT) [41], superoxide dismutase (SOD) [42], nitric oxide (NO) [43], and total antioxidant capacity [44] were determined using Bio-diagnostic assay kits according to the manufacturer's instructions (Giza, Egypt

Histopathological Examination

Histological sections (4 µm thick) were prepared from paraffin blocks of hepatic tissues fixed in 10% formal saline. Sections were stained with hematoxylin and eosin (H&E) [45].

Statistical Analysis

Statistical analysis was performed with the use of SPSS (USA, version 19.0). The results represent samples of 7 rats per experiment. Values were expressed as means ± SE. Statistical comparisons between the groups were performed by using Student's t test. Probability (P) values of < 0.05 were considered significant.

To calculate the % of improvement, we used this formula:

$$\% \text{ of improvement} = \frac{\text{Treated group} - \text{TAM group}}{\text{Control group}} \text{X } 100$$

RESULTS

Serum biomarkers for Liver Function

Tamoxifen group (TAM) showed a significant decrease ($P < 0.05$) in AST, ALT, total protein, albumin and A/G ratio, while serum globulin concentration was elevated as compared to the corresponding control group in both the protective and therapeutic groups (Table 1). However, the present study indicated that sodium butyrate possesses protective and therapeutic activities. In the protective group a significant increase ($P < 0.05$) in serum AST, ALT, albumin, serum total protein and A/G ratio and a reduction in serum globulin were recorded as compared to TAM group. In the therapeutic group a significant increase ($P < 0.05$) was noticed in the levels of AST, ALT, protein, albumin and A/G ratio while a non-significant reduction in the serum globulin concentration was recorded as compared to the TAM group. The protection group recorded an amelioration in serum globulin and A/G ratio with percentage of improvement 49.64 and 67.18 respectively, the therapeutic group showed amelioration in serum AST, ALT, total protein and albumin with percent of improvement 20.83, 31.24 and 26.54 and 27.27 respectively. These results reflected the therapeutic efficacy of sodium butyrate when used after administration of TAM.

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Table 1. Protective and therapeutic effects of sodium butyrate on liver function tests and serum protein level in male rats received Tamoxifen

	Groups	AST (U/L)	ALT (U/L)	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	A/G ratio
Protective Group	Control	122.00 ± 1.85	33.45 ± 1.88	6.70 ± 0.63	3.81 ± 0.08	2.82 ± 0.91	2.59 ± 0.74
	NaBu.	118.11 ± 1.34	29.75 ± 1.14	5.99 ± 0.65	3.77 ± 0.02	2.73 ± 0.91	2.53 ± 0.65
	TAM	90.18 ± 1.47*	19.20 ± 1.27$^{*\varphi}$	5.06 ± 0.23*	3.00 ± 0.04*	3.62 ± 0.77	0.71 ± 0.16*
	NaBu-TAM	100.51 ± 1.98$^{*\#\varphi}$	25.81 ± 2.24$^{*\#}$	5.73 ± 0.37	3.93 ± 0.07$^{\#,}$	2.22 ± 0.53	2.45 ± 0.75$^{\#}$
	% improvement	8.46	19.76	10.00	24.40	-49.64	67.18
Therapeutic Group	Control	125.04 ± 1.95	32.20 ± 0.88	6.33 ± 0.13	3.74 ± 0.01	2.90 ± 0.71	2.47 ± 0.54
	NaBu.	120.14 ± 1.53	30.14 ± 1.11	5.91 ± 0.23	3.71 ± 0.05	2.81 ± 0.53	2.51 ± 0.46
	TAM	91.12 ± 0.9$^{*\varphi}$	18.25 ± 1.91$^{*\varphi}$	4.39 ± 0.21$^{*\varphi}$	2.89 ± 0.03$^{*\varphi}$	3.55 ± 0.75	0.73 ± 0.15*
	TAM-NaBu	117.17 ± 5.19$^{\#}$	28.31 ± 1.94$^{\#}$	6.27 ± 0.54$^{\#}$	3.91 ± 0.07$^{\#}$	2.85 ± 0.51	1.97 ± 0.36$^{\#}$
	% improvement	20.83	31.24	26.54	27.27	-24.13	50.20

Values are given as mean±SE. Protective and therapeutic groups are compared with control, NaBu and TAM groups where:

*P < 0.05, when compared to the control.

$^{\varphi}$P < 0.05, when compared to the NaBu group.

$^{\#}$P < 0.05, when compared to the TAM group.

Serum Cholestatic Indices

Although, serum ALP concentration showed highly significant decreased subsequent to Tamoxifen administration (Table 2), it declared a significant increase in total bilirubin, direct bilirubin, and indirect bilirubin was recorded in Tamoxifen group as compared to the corresponding control group in both protective and therapeutic groups. On the other hand, an elevation in serum ALP and a slight reduction in total bilirubin, direct bilirubin and indirect bilirubin have returned nearly to the normal range as compared to the TAM group in both protection and therapeutic groups (Table 2). The percentage of improvement in the therapeutic group is higher than that observed in the protection group.

Table 2. Protective and therapeutic effects of sodium butyrate on serum cholestatic markers in male rats received Tamoxifen

	Groups	ALP (U/L)	Total Bilirubin (mg/dL)	Direct Bilirubin (mg/dL)	Indirect Bilirubin (mg/dL)
Protective Group	Control	232.01 ± 12.75	0.68 ± 0.04	0.66 ± 0.04	0.02 ± 0.02
	NaBu	230.11 ± 10.34	0.66 ± 0.14	0.59 ± 0.05	0.07 ± 0.01*
	TAM	144.84 ± 9.82$^{*\varphi}$	0.79 ± 0.03*	0.96 ± 0.02$^{*\varphi}$	0.11 ± 0.03*
	NaBu-TAM	189.97 ± 6.40$^{*\varphi\#}$	0.74 ± 0.03	0.67 ± 0.06$^{\#}$	0.07 ± 0.01*
	% improvement	19.02	-7.35	-43.93	-200.00

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Therapeutic Group	**Control**	229.01 ± 11.51	0.61 ± 0.03	0.65 ± 0.02	0.04 ± 0.01
	NaBu	220.15 ± 10.43	0.69 ± 0.11	0.59 ± 0.03	0.02 ± 0.01
	TAM	139.53 ± 8.89$^{*\varphi}$	0.81 ± 0.01*	0.98 ± 0.21$^{*\varphi}$	0.17 ± 0.01$^{*\varphi}$
	TAM-NaBu	226.14 ± 4.24$^{\#}$	0.74 ± 0.03$^{*\#}$	0.66 ± 0.01$^{\#}$	0.08 ± 0.02$^{\#}$
	% improvement	37.81	-11.47	-49.23	-225.00

Values are given as mean±SE . Protective and therapeutic groups are compared with control, NaBu and TAM groups where:

*P < 0.05, when compared to the control.

$^{\varphi}$P < 0.05, when compared to the NaBu group.

$^{\#}$P < 0.05, when compared to the TAM group.

Serum Lipid Profile

Serum total lipids, triglycerides, HDL-cholesterol and VLDL levels were significantly higher in TAM group as compared with the corresponding controls, while total cholesterol and LDL-cholesterol was decreased significantly in both protective and therapeutic groups (Table 3). However, a significant reduction in serum total lipids, triglycerides, and LDL-cholesterol and VLDL levels with an elevation in total cholesterol and LDL-cholesterol were observed after pre-treatment with sodium butyrate as compared to their TAM groups. Post-treatment with sodium butyrate caused a significant decrease in total lipids, triglycerides, HDL-cholesterol and VLDL and a significant increase in total cholesterol and LDL-cholesterol levels were noticed as compared to the TAM group. Pretreatment and post-treatment with sodium butyrate in male rats administered with TAM have returned the value of HDL-cholesterol to nearly the control level (Table 3).

Table 3. Protective and therapeutic effects of sodium butyrate on serum liver lipid profile in in male rats received Tamoxifen

	Groups	Total Lipids (mg/dL)	Triglycerides (mg/dL)	Total cholesterol (mg/dL)	HDL-cholesterol (mg/dL)	LDL-cholesterol (mg/dL)	VLDL-(mg/dL)
Protective Group	**Control**	776.69 ± 60.26	83.07± 8.16	79.91 ± 2.93	37.51 ± 1.34	26.57 ± 2.44	16.61 ± 1.63
	NaBu	769.51 ± 50.98	79.13 ± 6.13	70.88 ± 2.88	35.53 ± 1.31	25.56 ± 2.56	15.99 ± 1.72
	TAM	1369.12 ± 70.50$^{*\varphi}$	117.87 ± 6.35$^{*\varphi}$	64.43 ± 3.27*	51.96 ± 5.62$^{*\varphi}$	12.79± 1.82$^{*\varphi}$	23.57 ± 1.27$^{*\varphi}$
	NaBu-TAM	568.86 ± 23.71$^{*\varphi\#}$	72.48 ± 3.52$^{\#}$	73.52 ± 2.14$^{\#}$	36.07 ± 0.77$^{\#}$	12.84± 0.71$^{*\varphi}$	14.49 ± 0.71$^{\#}$
	% improvement	-103.03	-54.60	11.30	-42.36	0.188	-54.66
Therapeutic Group	**Control**	781.65 ± 53.21	80.11 ± 8.20	87.14 ± 6.54	36.40 ± 1.58	33.73 ± 7.22	16.02 ± 1.03
	NaBu	797.6571 ± 49.11	78.15 ± 5.28	85.15 ± 5.59	34.49 ± 1.67	32.66 ± 6.25	15.09 ±1.35
	TAM	1380.23 ± 66.45$^{*\varphi}$	113.91 ± 5.32$^{*\varphi}$	65.22 ± 2.89$^{*\varphi}$	50.12 ± 4.45$^{*\varphi}$	15.11 ± 1.72$^{*\varphi}$	22.78 ± 152$^{*\varphi}$
	TAM-NaBu	561.39 ± 37.61$^{*\varphi\#}$	61.67 ± 3.67$^{\#}$	73.39 ± 2.18$^{\#}$	37.92 ± 1.68$^{\#}$	23.35 ± 2.36$^{\#}$	12.33 ± 1.98$^{\#}$
	% improvement	-104.75	-65.21	9.37	-33.51	24.42	-65.23

Values are given as mean±SE. Protective and therapeutic groups are compared with control, NaBu and TAM groups where:

*P < 0.05, when compared to the control

$^{\varphi}$P < 0.05, when compared to the NaBu group

$^{\#}$P < 0.05, when compared to the TAM group

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Hepatic Oxidative stress

An increase in the level of liver MDA subsequent to TAM administration either in protective or in therapeutic groups, as compared to the corresponding control groups was recorded in table 4. On the other hand, liver GSH, GPX, SOD, CAT, GST, and NO levels decreased significantly ($P < 0.05$) subsequent to TAM group either in protective or in therapeutic groups, as compared to the corresponding control groups. Sodium butyrate administration before or after TAM administration caused a significant decrease ($P < 0.05$) in MDA concentration either in protective and therapeutic groups. On the other hand, a significant increase ($P < 0.05$) in liver GSH, GPX, SOD, CAT, GST, and NO levels in both protective and therapeutic groups with % change in the therapeutic group is better than that in the protective group (Table 4).

Table 4. Protective and therapeutic effects of sodium butyrate on some liver antioxidant activities in male rats received Tamoxifen

	Groups	MDA (nmol/gtissue)	GSH (mg/gtissue)	GPX (U/g tissue)	SOD (U/gtissue)	Catalase (U/Min)	GST (nmol/min/g. tissue)	NO (umol/L)
Protective Group	**Control**	45.90 ± 1.10	5.35 ± 0.25	13945 ± 52.62	254.87± 13.84	1.35 ± 0.03	2.42 ± 0.02	17.63 ± 0.58
	NaBu	43.85 ± 1.25	5.99 ± 0.13	14956 ± 43.59	260.13 ± 12.78	1.46 ± 0.08	2.94 ± 0.03*	18.97 ± 0.61
	TAM	84.75 ± 1.93$^{*\varphi}$	3.30 ± 0.25$^{*\varphi}$	11895 ± 58.67$^{*\varphi}$	180.15 ± 7.54$^{*\varphi}$	0.86 ± 0.07$^{*\varphi}$	0.63 ± 0.01$^{*\varphi}$	7.20 ± 0.39$^{*\varphi}$
	NaBu-TAM	46.78 ± 1.57$^{*\#}$	7.14 ± 0.96$^{\#}$	14045 ± 54.68$^{*\varphi\#}$	212.54 ± 10.66$^{*\#}$	1.30 ± 0.02$^{\#}$	2.39 ± 0.02$^{\varphi\#}$	14.84 ± 0.63$^{*\varphi\#}$
	% improvement	-82.72	71.77	15.41	12.70	32.59	72.72	43.33
Therapeutic Group	**Control**	42.55 ± 1.33	5.24 ± 0.23	13822 ± 50.11	275.24 ± 11.25	1.29 ± 0.05	2.61 ± 0.05	16.15 ± 0.24
	NaBu	40.49 ± 1.29	5.98 ± 0.36	14799 ± 41.19	281.13 ± 10.17	1.35 ± 0.07	2.69 ± 0.07	17.01 ± 0.33
	TAM	91.75 ± 1.11*$^{\varphi}$	3.150 ± 0.41*$^{\varphi}$	11895 ± 58.67*$^{\varphi}$	165.13 ± 13.55$^{*\varphi}$	0.91 ± 0.05$^{*\varphi}$	0.71 ± 0.05$^{*\varphi}$	6.99 ± 0.25$^{*\varphi}$
	TAM-NaBu	52.97 ± 1.04*$^{\varphi\#}$	7.73 ± 1.46$^{\#}$	14240 ± 46.67$^{*\varphi\#}$	265.44 ± 12.33$^{\#}$	1.84 ± 0.02$^{*\varphi\#}$	3.01 ± 0.03$^{*\varphi\#}$	17.04 ± 0.68$^{\#}$
	% improvement	-91.13	87.40	16.96	36.44	72.09	88.12	62.22

Values are given as mean±SE. Protective and therapeutic groups are compared with control, NaBu and TAM groups where:

*P < 0.05, when compared to the control.

$^{\varphi}$P < 0.05, when compared to the NaBu group.

#P < 0.05, when compared to the TAM group.

Histopathological Examination

The liver of the control and NaBu male rats revealed normal hepatic architecture and showing normal hepatic parenchyma (Fig. 1, A, B). The liver of rats received TAM showed fatty changes, vacuolar degeneration, fatty cells infiltrations with bounded vacuoles and signet ring appearance (arrows) (Fig. 1, C). The liver of the protection group showed healthy architecture with less dilated blood sinusoids (Fig. 1, D). Liver of the therapeutic group showed apparently healthy hepatic cords and blood sinusoids (Fig. 1, E) (H&E X 400).

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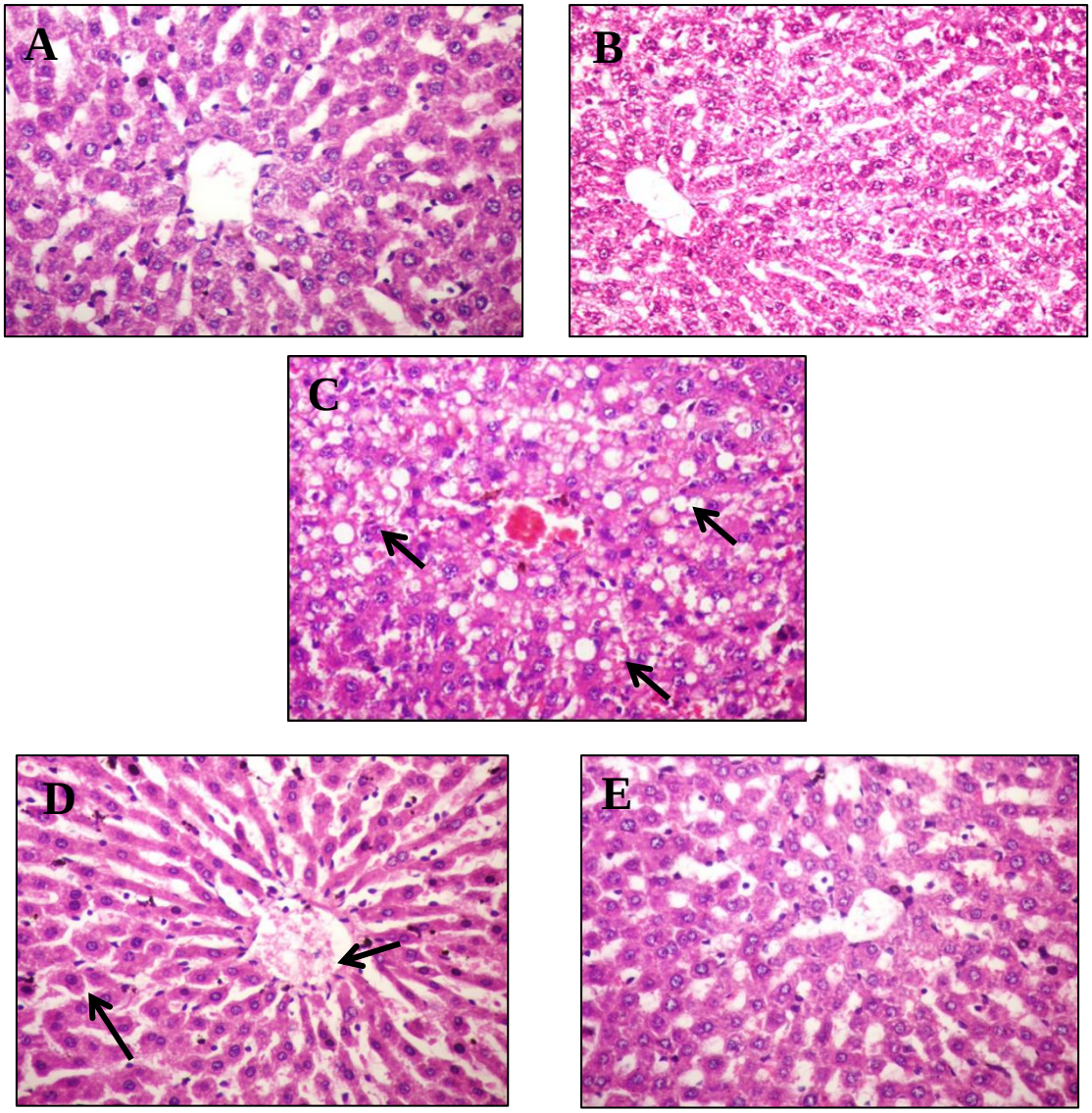

Figure 1. Photomicrograph of liver sections from (A, B) control and NaBu rats: showing normal structure of hepatic lobules and central vein. (C) TAM rats: showing variable degree of vacuolization (arrows), lipid accumulation and microvascular steatosis. (D) Protective rats: showing less dilated blood sinusoids (arrows). (E) Therapeutic rats: showing healthy hepatic cords and blood sinusoids (H&E X 400)

DISCUSSION

Tamoxifen (TAM), a synthetic non-steroidal anti-estrogen, has been widely used for many decades as the "gold standard" adjuvant treatment, which acts primarily as a competitive inhibitor of estrogen through binding to its receptors in breast cancer cells [46]. TAM toxicity has been studied in animals and linked with hepatotoxicity. TAM-induced hepatotoxicity was recorded by the elevation of the liver function parameters such as transaminases (AST, ALT) and alkaline phosphatase (ALP) which is in line with several published reports [47-49]. This elevation may be due to extensive hepatic damage, caused during long-term TAM treatment according to the dose concentration and the route of the administration. Tamoxifen administration to the male rats in the present study produced a significant decrease in the activity of AST, ALT, and ALP. These data are in agreement with Hemieda (2007) who demonstrated that female rats are more susceptible than males to TAM-induced hepatocellular injury [50]. This was confirmed by the histopathological alteration in the liver of the male rats in the present study.

Although tamoxifen is an extremely effective treatment for breast cancer, it also exhibits many plasma alterations. A significant decrease in serum protein, albumin, and A/G ratio were documented in the tamoxifen-treated group which could be related to either damage of vital biological processes or to the change in the permeability of liver or kidney leading to the leakage of protein via kidney [51]. Moreover, a significant decrement of serum total protein concentration in serum of TAM-treated rats was the reason for liver dysfunction. As male rats treated with TAM showed an elevation in serum globulin and bilirubin level, thus the observed significant increase in globulin, may be due to the most severe impairment of albumin formation in the liver, which attempt by the body to compensate with increased output of beta and gamma globulin which, clarify the reduced level of A/G ratio [52]. Similar results exhibited a decreases in serum total protein and albumin in female rats received TAM [53, 54]. In addition, the non-significant increase in total bilirubin, direct bilirubin and indirect bilirubin in the TAM-treated group were in line with several publications [47, 48]. This elevation may be due to the higher affinity of TAM for hepatic tissues than other tissues.

Butyrate is a four-carbon fatty acid (SCFA) normally produced as a result of bacterial fermentation of fiber in mammalian intestines [55]. Butyrate is capable of maintaining epithelial cell differentiation [56] and inducing apoptosis of cells in a number of cancer cell lines [57]. In the present study, prophylactic and treatment with sodium butyrate significantly improve the organ functions and able to prevent liver inflammation and damage by the reduction of the inflammatory signaling in the liver cells. [58, 59]

Numerous clinical studies and animal experiments have demonstrated that TAM treatment was concomitant with the development of NAFLD [9, 10, 60] Moreover, TAM-induced fatty liver was observed in more than 30% of breast cancer patients. In addition, chronic administration of TAM is associated with various complications, including hypertriglyceridemia, changes in plasma cholesterol levels and NAFLD [61]. Approximately 60% of liver triglycerides (TGs) are derived from free fatty acids (FFA) influx from the adipose tissue; 25% are from *de novo* lipogenesis (DNL), and 15% are from the diet. In contrast, FFA may be utilized either through β-oxidation via re-esterification and stored as TGs in lipid droplets, or packaged then exported as a very low-density lipoprotein. Hence, the hepatic fat accumulation can occur as a result of increased fat synthesis, decreased fat oxidation and/or decreased fat transfer [62]. Epidemiological studies revealed that tamoxifen treatment could impair fatty acid β-oxidation and cause fatty liver [63].

The elevation of total lipids, triglycerides and VLDL concentrations in the present were recorded in Tamoxifen group. It was suggested that the elevated serum lipid may be due to an increased rate of fat absorption. In addition, the estrogen agonist action possessed by tamoxifen in the liver could be responsible for the increase in total lipid levels [64]. Tamoxifen is a cationic drug; electrophoretically, taken up by mitochondria to accomplish high concentrations that inhibit both oxidation and respiration [65, 66]. Moreover, Estrogen induces hyperlipidemia through its multiple effects on lipid metabolism, including increased synthesis of TG and VLDL. TAM is essentially antiestrogenic, but it has some estrogenic activities. The effects of TAM on lipid metabolism may be attributable to its complex combination of estrogenic and antiestrogenic activities. Therefore, plasma lipid levels should be tested periodically during tamoxifen treatment, even if the patients are normolipidemic during the pretreatment stage [67].

In the current study, we observed that the levels of total and LDL-cholesterol were decreased significantly, whereas triglyceride and HDL- cholesterol levels were increased in the experimental male rats received tamoxifen. Lipids and lipoprotein results were in agreement with those of previously reported by Song et al. (1970) [68], who mentioned that estrogens increase the concentrations of VLDL and HDL-cholesterol, lipids and reduce LDL- cholesterol concentrations in women receiving tamoxifen [68]. However, tamoxifen inhibits hepatic triglycerides (TG) secretion. These combined effects could decrease fat removal from the liver and cause steatosis, despite a secondary down-regulation of hepatic Fatty Acid Synthesis (FAS) expression [69]. Maor et al.(2013), recorded that Tamoxifen increased hepatic fat content, through blocking the role of estrogen in preserving hepatic lipid homeostasis by supporting the expression of genes involved in lipid β- oxidation [70]. Also, TAM promotes hepatic steatosis by increasing lipogenesis, this was confirmed by the histopathological structure of the liver [12].

Short-chain fatty acid (SCFA), butyrate could attenuate *de novo* lipogenesis through a mechanism of action that includes alterations in gene expression of regulatory enzymes for lipogenesis [71]. Additionally, SCFA by-products may promote lipogenesis [72, 73]. Moreover, butyrate could reduce hepatic triglyceride

contents and induce anti-oxidative enzymes that help to prevent the progression of NASH to hepatocellular carcinoma [74].

The mechanisms by which tamoxifen exert its effects on lipoproteins still unclear. Several reports suggest that tamoxifen may act as an estrogen agonist on the liver and as an antiestrogen on other tissues. Hepatic estrogenic effect of tamoxifen has an important role in changing the plasma lipoproteins. These mechanisms include (1) increased synthesis of very low density lipoprotein leading to increased triglyceride levels; (2) decreased level of apolipoprotein B synthesis possibly due to an estrogen-like increase in catabolism of apolipoprotein B-LDL, which leads to decreased LDL-cholesterol and (3) increased levels of apolipoprotein A-1 synthesis, resulting in high concentrations of HDL-cholesterol. Data of the current study, suggests that the reduction of cholesterol and LDL-cholesterol levels and the increasing of HDL-cholesterol level could predict reduction in risk of death by 40% from coronary heart disease in postmenopausal women [75]. Tamoxifen therapy for early-stage breast could reduce deaths from acute myocardial infarction by 67% in postmenopausal patients [76].

Adjuvant TAM was associated with higher risk of development of non-alcoholic fatty liver [11]. Fatty changes were observed in TAM group in the present study which may be due to impaired protein synthesis. According to Marzouk [77], mitochondria are known to contain many of the enzymes necessary for the metabolism of triglycerides (i.e. fatty acid oxidases). This led to another explanation that the fatty changes observed in the present study may be due to mitochondrial damage. TAM decreases hepatic triglyceride secretion, and it accumulates electrophoretically in mitochondria, where it impairs -oxidation and respiration. TAM also inhibits topoisomerases and mitochondrial DNA synthesis and progressively depletes hepatic mitochondrial DNA *in vivo*. These combined effects could decrease fat removal from the liver, thus causing hepatic steatosis despite the secondary down-regulation of hepatic fatty acid synthase expression [69].

Reactive oxygen Species (ROS) have been discovered to play a vital role in anticancer drug-induced toxicity [78]. TAM generates ROS production and thiol depletion in a dose-dependent manner. The balance between ROS and cellular thiol levels plays a pivotal role in regulating cell cycle progression and apoptosis. This oxidative burst, must be balanced and counteracted by endogenous antioxidants [79]. In accordance with the studies of El Beshbishy et al. (2005), the present study showed that TAM caused a significant increase in the malondialdehyde (MDA) concentration in liver tissues and H_2O_2 generation. These results indicated that the lipid peroxidation may be attributed to the hexose monophosphate shunt (HMP) in rat liver which is strongly inhibited by TAM, so that the NADPH levels inside cells were decreased leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals [80]. Some intracellular antioxidant enzymes decreased with the increase of lipid peroxidation [81]. The state of oxidative stress observed during TAM administration was accompanied by decreased hepatic glutathione content and increased peroxidation [82]. The depletion of GSH promotes the generation of reactive oxygen species and oxidative stress with a cascade of effects, thereby affecting the functional as well as the structural integrity of the cell [83].

Glutathione-S-transferase (GST) comprises a relatively high amount of total cytosolic protein, and responsible for the high-capacity metabolic inactivation of electrophilic compounds and toxic substrates [84]. Superoxide dismutase (SOD) and catalase (CAT) act as mutually supportive anti-oxidative enzymes, which provide protective defense against reactive oxygen species [85]. Oxidative stress noticed after TAM intoxication was associated with decreased activities of hepatic SOD, CAT, GPX after lipid peroxidation [86]. This may be due to oxidative stress-induced inactivation and /or exhaustion as the decreased hepatic GPX activity may lead to H_2O_2 accumulation in the liver which in turn inactivates SOD [87]. Also, TAM has been shown to potentiate nitrous oxide production in breast cancer patients through enhancement of nitric oxide synthase II expression [88]. The levels of antioxidant enzyme activities in liver homogenates (GST, GPX, SOD and CAT) and GSH were significantly improved upon treatment of TAM-intoxicated rats with sodium butyrate which upregulate the detoxifying enzymes such as GSH and GST. Moreover, through the epigenetic mechanism by the inhibition of HDACs, results in the regulation of gene expression and in the control of cell fate which were concomitant with the results achieved from this study [27].

Histopathological investigation supports the obtained results from the biochemical analysis. The treatment with butyrate in the present study was able to prevent liver inflammation, damage, and hepatic lipid accumulation by normalizing the hepatic markers of steatosis. This effect of butyrate was also associated

with a reduction of triglycerides content which was significantly enhanced by TAM. However, treatment with sodium butyrate can significantly improve the histopathological changes in the liver and could effectively protect liver tissue.

CONCLUSION

The present results indicated that tamoxifen inhibits hepatic triglycerides (TG) secretion and could decrease fat removal from the liver which causing steatosis. Butyrate, a metabolite of the natural fermentation of carbohydrate and dairy products exhibited protective and therapeutic effects in dissolving the accumulated lipid and reducing the steatosis induce by TAM. Moreover, this study confirmed that butyrate has a physiological action in fat absorption *in vivo*. In addition, sodium butyrate exhibits good hepatoprotective, therapeutic, and antioxidant potential against TAM intoxication. So we suggest that the administration of sodium butyrate in combination with TAM in breast cancer patients may attenuate and improve the efficacy and potency of the drug and reduced its side effects.

Competing Interest

The authors declare that they have no competing interests.

Author's Contributions

H Saleh conceived of the study, performed the statistical analysis and drafted the manuscript. B Mohamed participated in the methodology of the study. Asem S. Marie participated in its design and coordination and helped to draft the manuscript.

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