

MORPHOLOGICAL AND BIOCHEMICAL CHANGES IN WISTAR RAT LIVERS AFTER CLOFIBRATE TREATMENT

Bogdanska Jasna¹, Toseska- Trajkovska K¹, Labudovic D¹, Cekovska S¹, Topuzovska S¹, Petrusevska G²

¹Department of Biochemistry, School of Medicine Skopje, University Ss. Cyril and Methodius Skopje, Macedonia

²Department of Pathology, School of Medicine Skopje, University Ss. Cyril and Methodius Skopje, Macedonia

Abstract

The aim of our study was to determine whether short-term administration of clofibrate can lead to morphological changes in the Wistar rat livers and whether these changes were accompanied by changes in the specific activity of the enzymes involved in oxidative stress.

Male Wistar rats divided in two groups: control and experimental were treated with saline solution and clofibrate respectively (250 mg/1000 g/day) for 12 days. Tissue liver samples were taken for morphological examination and liver homogenates and sub-cellular fractions were used for measurement of proteins, reactive oxygen species (ROS) producing and ROS scavenging enzyme activities.

Following clofibrate treatment, liver histopathology in the treated group has shown deranged liver architecture, focal necrosis and presence of atypical lymphocytes. Electron light mitochondrial matrix and increased number of peroxisomes and mitochondria were also observed. These changes were accompanied by increase of the specific activity of ROS producing enzymes (urate oxidase and palmitoyl CoA oxidase). The most prominent increase of the activity with the increase of relative sub-cellular distribution was observed in the peroxisomal fraction (LMF). Specific activity of catalase was increased, whereas superoxide dismutase and glutathione peroxidase have shown almost a double decrease.

Morphological changes in the liver accompanied by ROS production which has prevailed over their removal pointed to a conclusion that clofibrate can cause morphological changes accompanied by biochemical changes.

Key words: clofibrate, Wistar rats, morphological changes, reactive oxygen species producing enzymes and scavenging enzyme specific activity.

Introduction

Clofibrate belongs to a group of chemically diverse exogenous and endogenous chemicals so-called peroxisome proliferators (PPs), such as phthalate-ester plasticizers, insecticides and herbicides, as well as endogenous fatty acids and eicosanoids, which give an increased activity of enzymes involved in fatty acid oxidation in the liver [1, 2]. In addition, the effects associated with peroxisomal proliferation have been also reported with the huge differences in the sensitivity to peroxisome proliferators among species, with mice and rats being most sensitive, whereas fibrates only elicit hypolipidemic effects in humans, and there is no evidence of peroxisome proliferation [3, 4, 5]. On the other hand clofibrate treatment induces moderate peroxisomal proliferation but does not cause oxidative stress in the liver of pigs [6]. Beyond the hypolipidemic effect of short-term administration of clofibrate in rodents, it was soon hypothesized that its long-term administration could be the perpetrator of the observed hepatocarcinogenesis [5]. The different mechanisms for this carcinogenicity include oxidative stress, increased cell proliferation, inhibition of apoptosis and promotion of spontaneously formed preneoplastic lesions in the liver [7, 8, 9]. The state of oxidative stress results from the imbalance of pro-oxidant and antioxidant enzymes, which leads to the

production of excess reactive oxygen species (ROS), e.g. superoxide anion, hydrogen peroxide, hydroxyl radical, etc. [5]. The cellular defense mechanisms against the building-up of ROS include non-enzymatic substances and antioxidant vitamins, such as glutathione, vitamins C, A, E, and antioxidant enzymes, such as catalase, superoxide dismutase (SOD) and peroxidases. Peroxisomes play an important role in oxygen metabolism due to the high concentration of H₂O₂-generating oxidases, such as D-aminoacid oxidase, urate oxidase and palmytoil-CoA oxidase. Considering antioxidant enzymes within peroxisomes, superoxide dismutase (SOD) converts superoxide to hydrogen peroxide, whereas catalase, which is abundant in peroxisomes, converts hydrogen peroxide to water and oxygen [10, 11]. The main function of glutathione peroxidase is the reduction of lipid hydroperoxides to alcohols and free hydrogen peroxide to water. In addition to the biochemical changes in mouse and rat livers, there are effects associated with peroxisomal proliferation, such as a decrease in body weight; liver weight enlargement; morphological changes with an increased number and size of peroxisomes, increased number of mitochondria and increased amount of smooth endoplasmic reticulum.

The present study was designed to examine whether the short-term exposure to clofibrate could induce morphological changes in the livers of Wistar rats, and whether these changes were accompanied by the biochemical changes.

Material and methods

Chemicals

Clofibrate was purchased from Sigma Chemicals. In order to prepare the dose of 250 mg/1000g/24^h we have weighed 5 g of clofibrate and dissolved it in 80 ml of 0,2 mol/L NaOH, then neutralized with HCl to pH 7-8 and filled up with 100 ml sterile water. From this solution, appropriate volume was taken for i.p. application in order to give the dose of 259 mg/kg/day.

For the quantification of cholesterol and triglycerides in serum, commercial kits purchased from Randox Labs., Crumlin, North Ireland were used.

Glutaraldehyde, sodium cacodylate buffer Na (CH₃)₂AsO₂, osmium tetroxide, acetone, 5-(Hydroxymethyl)-uranyl acetate, lead citrate, durcupan embedding medium were purchased from Sigma chemicals and were used for electron microscopy.

For the quantitative determination of proteins, Bovine albumin and Coomassie Brilliant Blue G-250 were used. The chemicals used for measurement of the enzyme activities were either purchased from Sigma Aldrich Chemicals as follows: D-alanine; 2,4-Dinitrophenylhydrazine, Uric acid, Palmitoyl coenzyme A; β-NAD; KCN; sodium pyruvate; NADH; or from Randox Labs.; Crumlin, North Ireland.

Animals and treatment

The study was performed in male Wistar rats weighing 250-350 g, divided into two groups: a control and an experimental group consisting of 10 rats each. Animals were obtained from the Animal Facility of the Department of Preclinical and Clinical Pharmacology and Toxicology, Medical Faculty, Skopje. Animals were housed 5 in polycarbonate cages, with bedding of heat-treated pine-shavings at the animal facilities of the Pharmacology Institute, School of Medicine Skopje. The rats were allowed to acclimatize for at least one week before the start of the experiment including controlled light (12-hr light/dark) cycle, relative humidity of 40-60% and temperature conditions (22 ± 2^oC), with access to a normal rat chow and tap water *ad libitum* for at least one week before the start of the experiment. The clofibrate was applied in a dose of 250 mg/1000 g/ 24^h as a single dose by intra-peritoneal (i.p.) injection at the same time for 12 days. Animals from the control groups were injected i.p with sterile saline. The weight of the animals was monitored every day.

All the experiments performed on these animals were pre-approved by the Ethical Committee of School of Medicine, University Ss. Cyril and Methodius, Skopje. All of the procedures were performed according to accepted standards of Guide for the Care and Use of Laboratory Animals.

Collection of the blood and livers after clofibrate treatment

At the end of the experiment, each rat was bled under iso-flurane anesthesia and thereafter sacrificed by cervical dislocation. The blood samples for serum separation were collected in glass tubes. The livers were dissected out, weighed, and washed in cold PBS. Part of the liver was taken for electron microscopy preparation and the rest was used for preparation of the homogenate in 0,25 M sucrose pH 7,4 using three times up-and-down strokes of a Potter-Elvehjem homogenizer at 440 rpm. 20% whole liver homogenates were used for sub-cellular fractionation using high-speed centrifuge Janezki K 24. Nuclear, heavy mitochondrial (enriched in mitochondria); light mitochondrial (enriched in peroxisomes) and supernatant (mycosomal and cytosolic fraction) were obtained at the end of sub-cellular fractionation according to the deDuve [12]. All the preparations of the samples were performed on +4C⁰. Serum samples for lipid parameters were frozen at -20 C⁰ and homogenate and subcellular fraction samples for enzyme measurement were frozen at -70 C⁰.

Morphological changes

For morphological analysis, fresh liver tissue samples were fixed in 3% glutaraldehyde for 2-and- a-half hours. After overnight washing in phosphate buffer, the tissues were post-fixed in 1% osmium tetroxide for one hour. The dehydrated tissues with graduate concentrations of acetone were embedded in Durcupan resin. Semi-thin sections with a thickness of 0.5 nm were obtained on ultramicrotome Power Tome PC, Boeckeler RMC employing microtome glass knives. The sections for light microscopy were dyed with Jones stain- periodic acid silver methenamine.

Ultrathin sections with a thickness of 50-70 nm were obtained on ultramicrotome Power Tome PC, Boeckeler RMC employing microtome diamond knives and were contrasted with 5-(Hydroxymethyl)-uranyl acetate and lead citrate for electron microscopy. Ultra structural analysis has been done TEM JEOL1400.

Biochemical changes

Total proteins were quantified according to the method of Bradford [13], where the absorption maximum of the Coomassie Brilliant Blue G-250 under acidic conditions results in color change from brown to blue in the presence of proteins.

Enzyme assays

The assay of *D-aminoacid oxidase (EC. 1.4.3.3)* was done according to Baudhin et al. [14]. The activity of urate oxidase (*EC. 1.7.3.3*) was measured using the method of Bergmeyer, where the decomposition of uric acid to allantoin by urate oxidase was monitored at the length weight of 292 nm [15].

Palmitoyl CoA oxidase (EC. 1.3.3.6) (cyanide insensitive) was assayed according to Hryb and Hogg method [16]. In brief, in the process of β -oxidation with Palmitoyl CoA oxidase, NAD⁺ is reduced to NADH that is recorded at 340 nm.

Catalase (*CAT; EC. 1. 11. 1. 6*) activity was assayed according to the method of Aebi [17] where the decomposition of substrate H₂O₂ was monitored spectrophotometrically at 240 nm for 3 minutes. Activity was expressed as k (rate constant of the first order reaction as defined by Aebi).

Cu, Zn SOD (EC.1.15.1.1) activity was measured with RANSOD kits (cat. No. SD 125; Randox Labs., Crumlin, North Ireland).

GPX (EC. 1.11. 1. 9) activity was determined with Ransel kits (Cat. No. RS 505; Randox Labs.; Crumlin, North Ireland), based on the method of Paglia and Valentine [18], with cumene hydroperoxide as a substrate, with the final concentration of the reagents in the assay according to the recommendations of the manufacturer.

Enzyme and protein assays of each sample were performed in duplicate and each result represents the results from the two independent experiments.

The specific activity of the enzymes was calculated from the measured enzyme activity in homogenate and sub-cellular fractions, and the concentration of total proteins in the very same samples. The spectrophotometric measurements were done by using a PU 8630 UV-VIS spectrophotometer.

Statistical analysis

Statistical analysis were performed using the commercial statistical package, Statistical for Windows Version 5.0. Results were expressed as means $\bar{x} \pm SD$. Comparisons were made using the Student "t" test. The level of significance was $p < 0.05$.

Results

General observations

Rats exposed to clofibrate in a dose of 250 mg/kg/day for 12 days, exhibited loss of body weight and hypertrophy of the liver (doubling liver: body ratio). There was an increase of the protein content, which was more prominent into mitochondrial fraction and the supernatant ($p < 0.001$) (Table 1). The increase of rat liver proteins in these fractions might be caused by a change in the size of these organelles during the treatment.

Table 1. Effect of clofibrate on body and liver weight and protein content.

	Control group	Experimental group
Body weight at the beginning of the experiment (g)	244.75	269.75
Body weight at the end of the experiment (g)	299.2	251.6
Liver weight (g)	5.942	10.775***
Liver somatic index (Liver g/body weight g)	2.207	4.279***
Protein content mg proteins/g liver in the:		
Homogenate	115.8 \pm 14.1	187.12 \pm 17.4***
Nuclear fraction	29.5 \pm 3.92	26.1 \pm 4.8
Heavy mitochondrial fraction	23.1 \pm 5.3	47.3 \pm 12.9***
Light mitochondrial fraction	13.9 \pm 1.7	17.5 \pm 3.9**
Supernatant	49.2 \pm 7.53	102.7 \pm 8.5***

Animals were treated with clofibrate as described in the Material and Methods. Each result represents the mean value \pm standard deviations for 10 animals (each group) from two independent experiments; ** $p < 0.01$; *** $p < 0.001$ compared to the control value.

Reduction in the serum levels of triglycerides ($p < 0.001$) was observed by clofibrate treatment (triglyceride levels in the treated group were 0.66 ± 0.24 mmol/L compared to 1.53 ± 0.38 mmol/L in the control group).

Significant decrease ($p < 0.05$) in the serum cholesterol levels was also observed in the treated group (0.99 ± 0.11 mmol/L) compared to 1.58 ± 0.25 mmol/L in the control group.

Effect of clofibrate on liver morphology

In order to examine if there were morphological changes in the livers of Wistar rats after the short-term administration of clofibrate, the liver histopathology was done. The results in the treated group have shown deranged liver architecture, focal necrosis and presence of atypical lymphocytes in comparison to the control group (Figure 1a, 1b). In order to examine the size and number of mitochondria and peroxisomes, the electron microscopy of the selected sections was done.

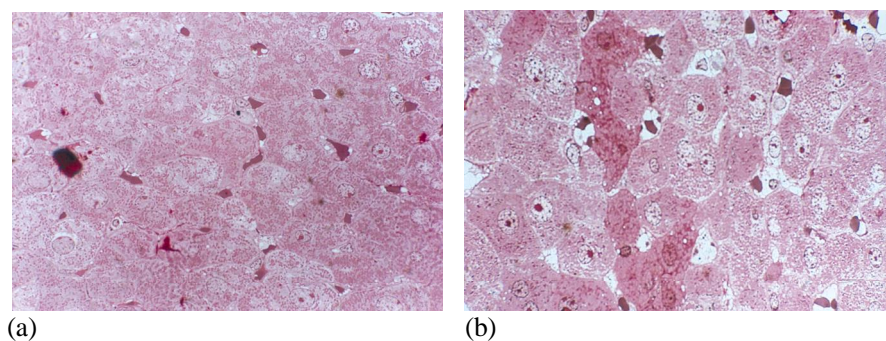


Figure 1. Liver histopathology after exposure to 250 mg/kg/day of clofibrate for 12 days (b) and in the control group (a). Sections of the liver were stained with periodic acid silver methenamine-Jones stain and visualized under the Leitz Orthoplan CM microscope. Magnification 1000.

Electron microscopic investigation of the liver sections has shown that there was a tendency of increased number and mitochondrial and peroxisomal volume density in rat livers after exposure to clofibrate (Figure 1 b), which suggested the presence of peroxisomal proliferation. These adverse changes were absent in the livers of control rats (Figure 1 a).

Electron light mitochondrial matrix and increased number of peroxisomes and mitochondria were observed after exposure to clofibrate (Figure 2b) in comparison to dispersed peroxisomes mitochondria with electron dense matrix in the control group (Figure 2 a).

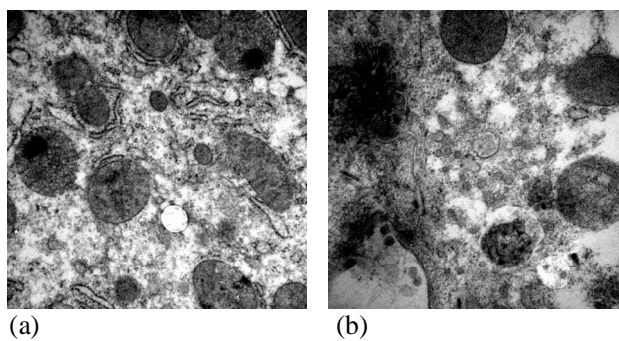


Figure 2. Electron microscopy of the liver sections in control group (a) and after exposure to clofibrate in a dose of 250 mg/kg/day for 12 days (b). Sections were stained with uracil acetate and lead citrate, and visualized under Electron Microscope JEOL JEM-1400 System. Magnification 30 000.

Effect of clofibrate on the enzyme activities

Table 2 show the effect of clofibrate treatment on the reactive oxygen species producing enzyme activities. Following the clofibrate treatment, the specific activity of these enzymes in the homogenate was increased for 120% and 2300% (urate oxidase and palmitoyl CoA oxidase respectively) in comparison to the control group ($p < 0.001$).

Table 2. Specific activity of peroxisomal reactive oxygen species producing oxidases expressed in nmol/mg proteins.

Fraction	D-amino acid oxidase		Urate oxidase		Palmitoyl CoA oxidase	
	Control	Experimental	Control	Experimental	Control	Experimental
H	7.9±1.3	7.1±1.5**	11.1±3.9	13.4±4.6***	0.91±0.12	21.6±7.7***
N	6.5±0.4	8.7±1.9**	5.5±1.5	13.8±5.5***	0.56±0.37	25.4±8.8***
HMF	9.6±1.5	5.5±1.4***	14.7±6.0	16.8±10.3	1.28±0.28	23.6±13.2**
LMF	11.3±1.7	16±4***	31.1±19.9	50.8±26.6*	4.3±0.6	120±33***
S	7.5±1.0	4.9±0.6***	6.3±1.3	4.4±0.9***	0.31±0.1	2.8±0.7*

Animals were treated with clofibrate as described in the Material and Methods. Each result represents the mean value ± standard deviations for 10 animals (each group) from two independent experiments; *p<0.5; **p<0.01; ***p<0.001 as determined by Student t-test. H- homogenate; N-nuclear fraction; HMF-heavy mitochondrial fraction; LMF-light mitochondrial fraction; S-supernatant.

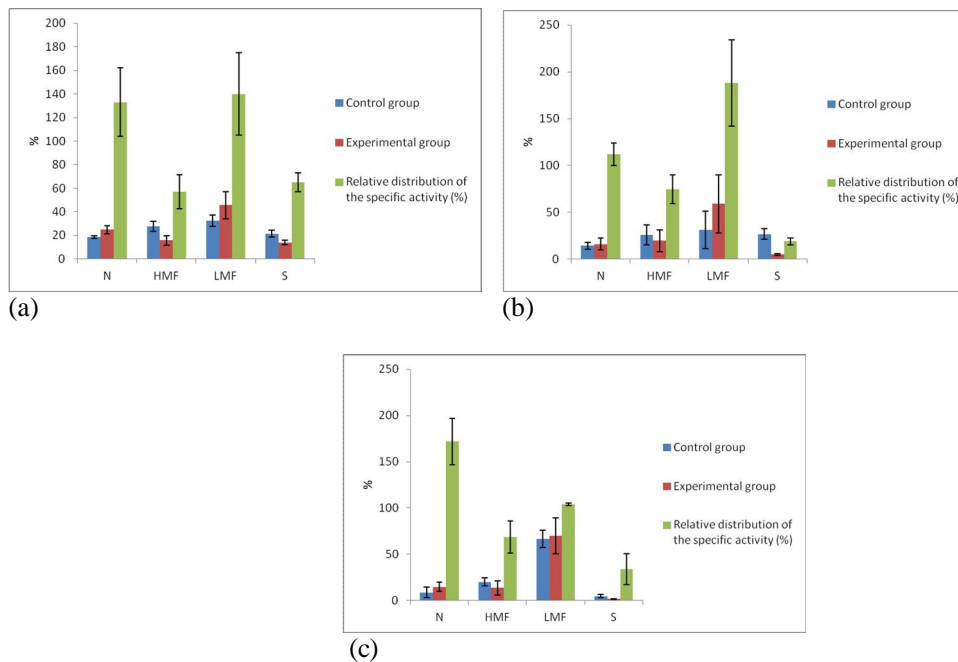


Figure 3. Subcellular distribution (%) of peroxisomal oxidases: D-amino acid oxidase (a); Urate oxidase (b) and Palmitoyl CoA oxidase (c) in the livers of Wistar rats in the control and experimental group treated with clofibrate, and relative distribution of the specific activity in the treated group expressed in % in comparison to the control group (100%). Each result represents the mean value ± standard deviations for 10 animals (each group) from two independent experiments. Corrections were not made for incomplete recovery in the subfractions. H- homogenate; N-nuclear fraction; HMF-heavy mitochondrial fraction; LMF-light mitochondrial fraction; S-supernatant.

Although the activity of D-amino acid oxidase in the homogenate was decreased, there was a significant increase of the activity in the peroxisomal fraction (light mitochondrial fraction) ($p < 0.001$), as well as in the nuclear fraction ($p < 0.01$) (Table 2 and Figure 3a). Similar increase has been observed in the activity of urate oxidase (Table 2 and Figure 3 b). The most prominent increase of the activity with the increase of relative sub-cellular distribution was observed in the peroxisomal fraction (LMF) (Figure 3 b). The activity of palmitoyl CoA oxidase as a marker for hepatic β oxidation was increased in the homogenate and in all sub-cellular fractions (Table 2). Relative distribution of the specific activity was increased in nuclear and peroxisomal (LMF) fractions (Figure 3 c).

In general, following the clofibrate treatment, increased specific activity of reactive oxygen species producing enzymes in the LMF was observed.

The effect of clofibrate on the reactive oxygen species scavenging enzymes is presented in Table 3 and Figure 4 a, b and c. The specific activity of catalase was increased in the liver homogenate ($p < 0.001$) (Table 3). On the other hand, superoxide dismutase and glutathione peroxidase have shown almost double decrease of the activity ($p < 0.001$) following the short-treatment with clofibrate (Table 3). As presented in Table 3 and Figure 4a, catalase has shown the increase of the activity into the light mitochondrial fraction and cytosolic fraction for about 13%. Upon exposure of rats to clofibrate, the hepatic cytosolic catalase activity increased more than peroxisomal activity (Figure 4 a). The index of peroxisomal proliferation in the supernatant (cytosol/homogenate $\times 100$) was 8.8 (compared to control 2.2) and in the light mitochondrial fraction (light mitochondrial fraction/homogenate $\times 100$) was 10.3 (compared to 5.2 in the control group). These data suggested that there might be a presence of peroxisomal proliferation.

On the contrary, the superoxide dismutase activity was decreased in the homogenate and in the sub-cellular fractions (Table 3). The most prominent decrease was found into mitochondria, in the peroxisomes and cytosol (58% and 33% respectively) ($p < 0.001$) (Figure 4 b). Superoxide dismutase was present in the peroxisomes, but only at the level of 20% of the cytosolic activity. Compared to the activity of catalase in this compartment, the activity of superoxide dismutase was more than 600 times lower. The down regulated activity and decreased relative sub-cellular distribution in the mitochondria and peroxisomes (Figure 4 b), the two compartments accounted for the production of reactive oxygen species, suggested that, in the treated group, the removal of superoxide anion might not be so efficient in comparison to its production.

Table 3. Specific activity of the reactive oxygen species scavenging enzymes expressed in $\mu\text{mol}/\text{mg}$ proteins.

	Catalase		Superoxide dismutase		Glutathione peroxidase	
	Control	Experimental	Control	Experimental	Control	Experimental
H	910 \pm 130	1070 \pm 70***	19.6 \pm 1.7	11.6 \pm 1***	153.8 \pm 25.9	80 \pm 20***
N	270 \pm 70	360 \pm 100*	1.2 \pm 0.2	1.1 \pm 0.2	106.2 \pm 54.4	100 \pm 49
HMF	1130 \pm 350	960 \pm 100*	7.4 \pm 1.9	2.6 \pm 0.1***	121.5 \pm 27.5	59 \pm 20***
LMF	2090 \pm 350	2450 \pm 300*	13.3 \pm 0.99	3.8 \pm 2***	29.4 \pm 6.1	23 \pm 9*
S	880 \pm 98	1000 \pm 200	33.6 \pm 7.6	15.6 \pm 2***	216.4 \pm 42.7	96 \pm 20***

Each result represents the mean value \pm standard deviations for 10 animals (each group) from two independent experiments; * $p < 0.5$; ** $p < 0.01$; *** $p < 0.001$ as determined by Student t-test. H- homogenate; N-nuclear fraction; HMF-heavy mitochondrial fraction; LMF-light mitochondrial fraction; S-supernatant.

The cytosolic activity of glutathione peroxidase was less than 10% of the total catalase activity (Table 3). The potential capacity of glutathione peroxidase to transform hydrogen peroxide to water

was 6 times greater than the capacity of catalase in the corresponding compartments (cytosol and peroxisomes) and over 100 times greater from the capacity of acyl CoA oxidase to produce reactive oxygen species (into peroxisomes) (calculated from Table 2 and Table 3). The activity of glutathione peroxidase into peroxisomes accounted for about 8%. There was increase of the relative sub-cellular distribution in this fraction in comparison to the control group (Figure 4c). This finding might suggest that this activity could play an important role in hydrogen peroxide metabolism under certain conditions.

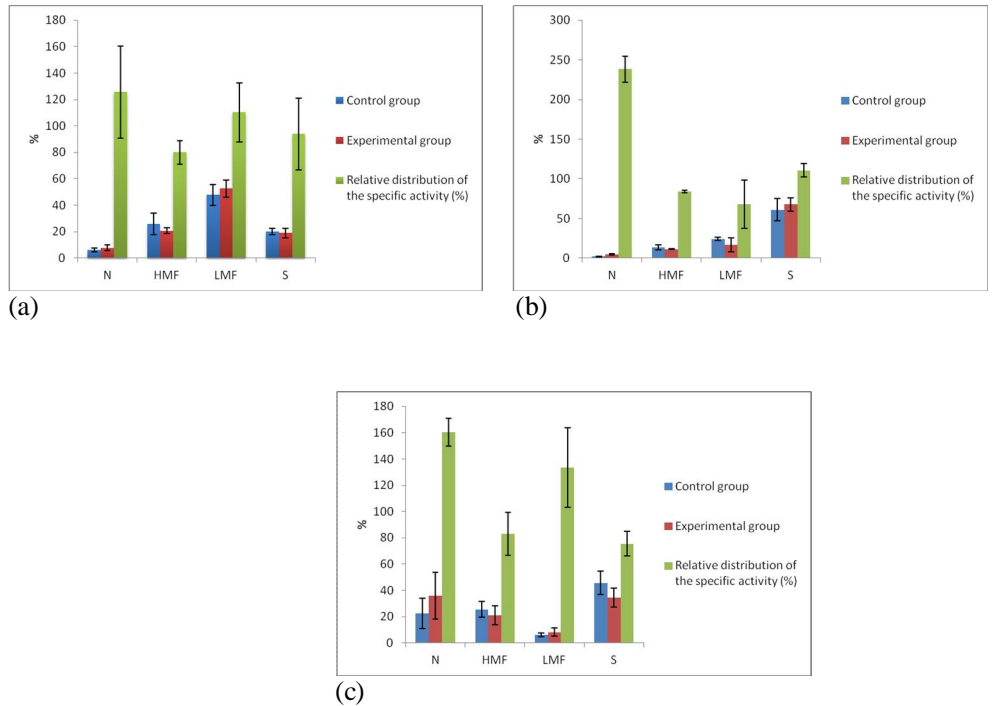


Figure 4. Subcellular distribution (%) of the reactive oxygen species scavenging enzymes: Catalase(a); Superoxide dismutase (SOD) (b) and Glutathione peroxidase (GpX) (c) in the livers of Wistar rats in the control and experimental group treated with clofibrate, and relative distribution of the specific activity in the treated group expressed in % in comparison to the control group (100%). Each result represents the mean value \pm standard deviations for 10 animals (each group) from two independent experiments. Corrections were not made for incomplete recovery in the subfractions. H-homogenate; N-nuclear fraction; HMF-heavy mitochondrial fraction; LMF-light mitochondrial fraction; S-supernatant.

Discussion

Clofibrate, a member of the fibrate family, has been used as a lipid lowering drug, which acts through the peroxisome proliferator-activating receptor alpha (α) - a major regulator of lipid homeostasis [19]. The short-term application of clofibrate, as well as other hypolipidemic agents cause peroxisomal proliferation in rodents and the long-term has been found to cause hepatocarcinogenesis [20, 21]. One of the proposed mechanisms underlying this effect is the hypothesis of oxidative stress. Hereby, we also hypothesised that short-term exposure to clofibrate can cause morphological changes in livers of Wistar rats accompanied by modulation of enzymes involved in the reactive oxygen species homeostasis.

Our results have shown that upon clofibrate treatment, in addition to the modulation of pro-oxidant and anti-oxidant marker enzymes for oxidative stress in the rats' livers, there was a presence

of hepatomegaly, focal necrosis and increased number and volume density of peroxisomes and mitochondria which indicated a presence of peroxisomal proliferation (Figure 1b and Figure 2b).

On the other hand, recent studies have shown that clofibrate has an *in vitro* hepatoprotective effect against oxidative stress and was found to be associated with hepatoprotection [22, 23]. Stimulation of PPAR α with clofibrate was suggested to increase the antioxidant defense, leading to improved cardiac function in Wistar rats [24]. The rat liver peroxisome accounts for about 35% of total H_2O_2 production, mainly through the activity of peroxisomal oxidases that is decomposed by ROS scavenging enzymes in the cell. Thus, following short-term exposure to clofibrate, here, we have detected a high up-regulation of the specific activity of palmitoyl CoA oxidase and of urate oxidase as hydrogen peroxide producing enzymes in the peroxisomes (Table 2). The ROS generating enzymes have shown the increase in the light mitochondrial fraction (peroxisomal fraction) (Fig. 3 a, b and c). On the other hand, significant down-regulation of the specific activity of superoxide dismutase and glutathione peroxidase with the moderate up-regulation of catalase activity (the ROS scavenging enzymes) was observed (Table 3 and Figure 4 a, b and c). These findings suggested that clofibrate treatment may lead to both increased ROS production and their prolonged presence in the liver. The weak side of our work was that we were not in a possibility to strength these findings with the measurement of hydrogen peroxide (H_2O_2) and lipid peroxidation products, as well as to study the gene expression of oxidative stress related genes. Different peroxisomal proliferators can cause different gene regulation, where the induction of the genes of lipid β -oxidation (particularly of acyl CoA oxidase) are induced very strongly in comparison to the moderate induction of catalase. Indeed, in our present work, the specific activity of palmitoyl CoA oxidase was up-regulated more than 2000 times. On the other hand, the specific activity of catalase was fairly up-regulated and the activity of superoxide dismutase and glutathione peroxidase (two other reactive species scavenging enzymes) was down-regulated. This disproportionate increase of H_2O_2 -generating oxidases, particularly of palmitoyl CoA oxidase, in comparison to H_2O_2 scavenging enzymes was suggested to be responsible for oxidative stress leading to the development of hepatic tumours in rodents [21, 25, 26, 27, 28]. Other factors, such as suppression of apoptosis perturbation of cellular proliferation have also been suggested to play roles in the pathogenesis of tumors associated with peroxisome proliferators [21]. On the contrary, experiments performed on mice and *in vitro* suggested that clofibrate could also be an effective agent in ovarian cancer treatment [29] and fibrates were found to induce apoptosis and suppress tumor growth in Manle cell lines [30] and to have a suppressive effect on lung metastasis development in animals [31].

It would be beneficial if anticancer effects of clofibrate could be tested and evaluated in comparison to its peroxisomal proliferation effect in rodents.

Conclusion

In this study we have shown that short-term treatment of male Wistar rats with clofibrate resulted with morphological changes in the liver such as focal necrosis and increased number and volume density of peroxisomes and mitochondria which indicated a presence of peroxisomal proliferation. These findings were accompanied by remarkable up-regulation of reactive oxygen species producing liver enzymes (especially a marked increase of palmitoyl CoA oxidase) and down-regulation of reactive oxygen species scavenging liver enzymes superoxide dismutase and glutathione peroxidase with a moderate up-regulation of catalase. These results pointed to a conclusion that short-term administration of clofibrate induces hypertrophy, hepatic peroxisomal proliferation, and oxidative stress caused by alteration in the balance between pro-oxidant and antioxidant liver enzymes.

Conflict of interests

The authors declare that there is no conflict of interests.

Acknowledgments

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