Plasma lipid profile and sex hormone levels in rabbits under paracetamol-induced oxidative stress

G. V. Vikulina, V. I. Koshevoy, S. V. Naumenko, M. L. Radzikhovskyi

Abstract
Changes in the redox balance at different levels, which lead to the accumulation of toxic radicals against the background of a decrease in the content of redox-active compounds, i.e., a state of oxidative stress (OS), are considered the leading pathogenic factor in pathological conditions. Processes of peroxide oxidation of biological substrates, especially cell membrane lipids and lipophilic compounds have both regulatory and pathological effects in the body of animals and humans. For now, it remains relevant to establish the regularities of interaction of metabolism during OS development. Among the large number of existing OS models, attention is drawn to those that can be reproduced in clinical practice, as a complication of treatment measures or an error in the medical strategy, due to a constant influence on peroxidation processes in the body. Important among such means is paracetamol (acetaminophen, APAP) – one of the most common antipyretic and analgesic drugs. In addition to the known hepatotoxic, nephro- and neurotoxic effects of its long-term and/or excessive use, there are data on reproductive toxicity and disorders of lipid metabolism. Therefore, the aim of our work was to determine the lipid profile and the level of sex hormones in male rabbits during paracetamol-induced OS. The study was performed on male rabbits of the Khila breed. According to the principle of analogs, a control group (n = 12) was formed, which were kept on a standard diet, and an experimental group (n = 12), whose animals were simulated the state of OS by oral injection of a solution of paracetamol with food at a dose of 300 mg/kg of weight body once during 21 days. The following results were obtained by determining the dynamics of changes in biochemical indicators in the rabbit body during chronic injection of paracetamol: in the rabbits of the experimental group, after the injection of paracetamol, the level of diene conjugates was 63.0 % higher, and the content of thiobarbiturate-active products was 1.26 times higher (P ˂ 0.05). At the same time, the level of steroid hormones also changed during the study – the level of total testosterone gradually decreased (on the 21st day – by 22.8 %, and at the end of the experiment – by 30.9 % lower than the values of the control group (P ˂ 0.05). At the same time, the level of estradiol had an upward trend and on the 21st day was higher than the control indicators by 10.7 % (P ˂ 0.05). Significant changes in lipid metabolism were found in male rabbits – the total cholesterol content on the 21st day was 35.4 % lower than the values of the control group (P ˂ 0.05). At the same time, the level of diene conjugates was 63.0 % higher, and the content of thiobarbiturate-active products was 1.26 times higher (P ˂ 0.05). At the same time, the level of steroid hormones also changed during the study – the level of total testosterone gradually decreased (on the 21st day – by 22.8 %, and at the end of the experiment – by 30.9 %, P ˂ 0.05). Significant changes in lipid metabolism were found in male rabbits – the total cholesterol content on the 21st day of the experiment exceeded the data of control animals by 25.3 %, the level of triacylglycerols – by 42.3 %, and the content of low-density lipoprotein cholesterol also increased by 1.1 times (P ˂ 0.05), and the high-density lipoprotein cholesterol level, on the contrary, was reduced by 17.0 % (P ˂ 0.05). In general, the obtained results indicate the relationship between the development of OS with changes in lipid metabolism and the balance of sex hormones, which allows us to use the model of paracetamol-induced OS in rabbits for further research.

Keywords: rabbits; sex hormones; lipid metabolism; paracetamol; oxidative stress.

1. Introduction

Acetaminophen (paracetamol, N-acetyl-p-aminophenol; APAP) was first described in 1878 as an analgesic and antipyretic drug that was rarely used clinically until phenacetin was withdrawn from the market due to renal toxicity. At the time of writing, APAP is probably the most widely available and widely used drug worldwide and is a very important analgesic (Athersuch et al., 2018; Naz et al., 2023). APAP was considered a safer drug compared to non-steroidal anti-inflammatory drugs (NSAIDs), especially in terms of a lower risk of renal injury, gastrointestinal damage, and asthma/bronchospasm induction (Ishitsuka et al., 2020).

Hepatotoxicity is the biggest pitfall of APAP and the most common cause of acute liver failure from its overdose (Elshazly et al., 2014). High doses of APAP are hepatotoxic and nephrotoxic in both humans and animals (Yousef et al., 2010; Ahmad et al., 2021a). Its long-term injection contrib-
utes to the production of active forms of oxygen (AFO), depletes the antioxidant defence system (ADS), and also causes tissue damage and cell death (Kehrer & Klotz, 2015; Du et al., 2016).

However, the exact mechanism of APAP-induced liver damage remains unclear, and no effective treatment has been developed except for N-acetylcysteine and some herbal remedies (Zira et al., 2009; Alipour et al., 2013; Yan et al., 2018; Zhukova & Naumenko, 2022). In fact, APAP-induced hepatotoxicity remains the most common cause of acute liver failure (ALF) (Lee, 2013). In view of the concern caused by the hepatotoxicity of APAP, many efforts have been made to understand the mechanisms of its toxic action. Generally, APAP-induced oxidative stress and mitochondrial dysfunction play a central role in the pathogenesis of ALF (Jaeschke et al., 2012; Ahmad et al., 2021b). APAP overdose is a major cause leading to liver failure due to oxidative stress, mitochondrial and lysosomal dysfunction (Zubairi et al., 2014; Rostami et al., 2022). Treatment methods are also quite limited and are mainly represented by means with pronounced antioxidant and anti-inflammatory activity (Guo et al., 2019; Islam Shawon et al., 2024).

Lipid peroxidation (LPO) results from the attack of hydroxyl radicals on the fatty acyl chains of phospholipids and triacylglycerols and has attracted attention due to its effects on the cellular function of many organs. Although all organelles and compartments of the cell produce AFO, mitochondrial generation of hydrogen peroxide is considered the main source of oxidants (Sena & Chan, 2012). It is important to note that membrane phospholipids and triacylglycerols are primary targets for hydroxyl-mediated attack and formation of lipid radicals (Hauck & Bernlohr, 2016).

Therefore, the study of lipid metabolism under paracetamol-induced oxidative stress is an urgent scientific task. This is also explained by the fact that adipose tissue is the main depot of lipids in the body, and the increased mass of adipose tissue due to excessive accumulation of lipids is the morphological substrate of obesity (Olechnowicz et al., 2018). Obesity, in turn, is the main cause of chronic metabolic syndromes, such as dyslipidemia, diabetes, liver disease, and hyperuricemia (Wang et al., 2022).

Dyslipidemia is not only a common complication of chronic kidney disease, but also contributes to the occurrence of cardiovascular diseases (Ballew & Matsushita, 2018; Hu et al., 2019). The serum lipid profile, as well as various aspects of lipid metabolism, have been shown to be profoundly altered in proteinuria of nephrotic syndrome or chronic kidney disease (Bajaj et al., 2019). Effective means of reducing nephrotoxicity are being tested on the paracetamol-induced rabbit model (Makhdooom et al., 2022). Lipids are the main macromolecular components of the brain and important structural components of neuronal cell membranes. Lipids contribute to the processing of amyloid precursor proteins, and also affect synaptogenesis, myelin formation, inflammation, oxidative stress (Kao et al., 2020; Nie et al., 2024). Disorders of lipid metabolism lead to the accumulation of lipids in the liver, which affects various generators of reactive oxygen species (ROS), including mitochondria, endoplasmic reticulum and NADPH oxidase (Chen et al., 2020). Note that similar changes are observed with long-term use of paracetamol (Ghanem et al., 2016).

Studies in laboratory rodents demonstrate that exposure to near-therapeutic doses of paracetamol during the first days of life causes profound long-term neurological chang-

2. Materials and methods

Experimental studies were conducted on male rabbits of the Khila breed (n = 24) on the basis of the department of internal diseases and clinical diagnostics of animals and the department of veterinary surgery and reproductive technology of the State Biotechnology University. According to the principle of analogues, two groups of animals were formed – control (n = 12), intact animals kept on a standard diet (Table 1), and experimental (n = 12), which were injected with a paracetamol solution orally with food to simulate the state of oxidative stress in a dose of 300 mg/kg of body weight once for 21 days according to the method Ahmad et al. (2021a).

Table 1

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount of feed units (g/kg)</td>
<td>215.0</td>
</tr>
<tr>
<td>Root crops (g/kg)</td>
<td>190.0</td>
</tr>
<tr>
<td>Maize grain (g/kg)</td>
<td>70.0</td>
</tr>
<tr>
<td>Wheat bran (g/kg)</td>
<td>215.0</td>
</tr>
<tr>
<td>Bagasse (g/kg)</td>
<td>20.0</td>
</tr>
<tr>
<td>Meadow hay (g/kg)</td>
<td>70.0</td>
</tr>
<tr>
<td>Bean hay (g/kg)</td>
<td>60.0</td>
</tr>
<tr>
<td>Salt, (g/kg)</td>
<td>1.0</td>
</tr>
<tr>
<td>Phosphorus (g/kg)</td>
<td>1.0</td>
</tr>
<tr>
<td>Calcium (g/kg)</td>
<td>1.5</td>
</tr>
<tr>
<td>Carotene (mg/kg)</td>
<td>2.0</td>
</tr>
<tr>
<td>Digestible protein (per 100 g of feed in g)</td>
<td>14.0</td>
</tr>
<tr>
<td>Digestible energy (MJ/kg)</td>
<td>2.28</td>
</tr>
</tbody>
</table>

During the research (on 1, 7, 14, 21 and 30 days), blood samples were taken from the ear vessels of rabbits for further biochemical studies. All blood samples were collected...
in centrifuge tubes with anticoagulant and centrifuged at 3000 rpm for 15 minutes at 4 °C to obtain plasma.

The content of OS markers in blood plasma was estimated using spectrophotometric methods in order to determine the concentrations of diene conjugates (DC) (based on the value of the molar extinction coefficient for conjugated dienes of polyunsaturated higher fatty acids wavelength \( \lambda = 233 \) nm) and thiobarbiturate acid-reactive compounds (TBA-RC) (based on the binding of malonaldehyde with thiobarbituric acid with the formation of a stable trimethine complex at a wavelength of \( \lambda = 532 \) nm).

Antioxidants were spectrophotometrically determined following Vlizlo (2012). Therefore, superoxide dismutase activity (SOD) was calculated by the degree of reaction inhibition by the enzyme to reduce nitroblue tetrazolium in the presence of nicotinamide adenine dinucleotide and phenazine methosulfate (at \( \lambda = 540 \) nm). Reduced glutathione (GSH) was assessed by the Butler method using Ellman’s reagent (at \( \lambda = 412 \) nm).

The total antioxidant activity (TAC) of plasma was assessed using FRAP analysis (the principle is to determine the antioxidant power of ferrum). A solution of ferric sulfate was used to create a standard curve, and the results were expressed in mmol of Fe(II) formed per liter of plasma. Plasma TAC was determined spectrophotometrically.

The level of sex hormones in the blood plasma – total testosterone and 17β-estradiol with the help of standard sets of reagents ELISA Kit (LifeSpan BioSciences, USA) according to the instructions on the immune enzyme analyzer Stat Fax 303 plus (Awareness Technology, USA).

The level of total cholesterol was determined by the enzymatic colorimetric method, the principle of which consists in the hydrolysis of cholesterol esters, which is oxidized by cholesterol oxidase into a ketone form with the release of hydrogen peroxide (in the presence of peroxidase, the dye quinonimine forms a red colour (at a wavelength of \( \lambda = 500 \) nm)). The intensity of the resulting colour is directly proportional to the concentration of triacylglycerols in the sample.

The principle of determining high-density lipoproteins (HDL) is that low-density lipoproteins (LDL) and very low-density lipoproteins (VLDL), which are in the blood plasma, form insoluble complexes with phosphorous-tungstic acid and magnesium ions, while in the blood only HDL remains, the level of which is determined using a reagent that functions similarly to the assay.

Instead, LDLs can be precipitated by polyvinylsulfonic acid, due to the fact that after centrifugation, LDLs remain in the supernatant. The concentration of LDL can be determined by the difference between total cholesterol and the supernatant.

All data were processed statistically by Microsoft EXCEL software. The obtained data from the rabbits of all groups were analyzed using one-way analysis of variance (ANOVA). The significant changes among indexes at probability were examined by Duncan’s Multiple Range Test. The records in the tables were presented as mean ± standard error means (SEM). The differences between groups were considered statistically significant at \( P < 0.05 \).

In organizing and conducting experiments, the authors of the article followed the provisions of the “European Convention on the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes” (Strasbourg, 1986), the 1st National Congress on Bioethics (Kyiv, 2001) and the Law of Ukraine “On the Protection of Animals from ill-treatment” (2006).

3. Results and discussion

The conducted studies established the presence of complex changes in biochemical indicators in the body of rabbits under paracetamol-induced oxidative stress. The development of this condition caused by chronic administration of paracetamol caused a significant increase in the content of lipoperoxidation markers in the blood of rabbits of the experimental group (Fig. 1). In particular, the content of DC was characterized by significant changes compared to the control data: on the 7th day of the study, it increased by 13.5 %, after which there was a pronounced increase in this indicator – on the 14th day by 54.5 %, on the 21st – by 63.0 % and at the end of the experiment (on the 30th day) – by 62.3 % (\( P < 0.05 \)).
Depletion of ADS was characterized by a decrease in TAC, GSH content, and SOD activity. On the 7th day of the study, a decrease in TAC by 15.2 % compared to the control group of animals was established, and further a steady decrease of this indicator was noted – on the 14th day by 30.8 %, on the 21st – by 34.1 %, on the 30th – by 33.3 % (P < 0.05).

The liver is considered highly sensitive to the action of toxic agents, so the glutathione system protects hepatocytes by combining with the reactive metabolite of paracetamol, thus preventing their covalent binding to liver proteins (Vermeulen et al., 1992). Paracetamol is oxidized by cytochrome P450 to form the toxic electrophilic N-acetyl-p-chrome P450 to form the toxic electrophilic N-acetyl-p- (Vermeulen et al., 1992). Paracetamol is oxidized by cytochrome P450 to form the toxic electrophilic N-acetyl-p-chrome P450 to form the toxic electrophilic N-acetyl-p-

Table 2

<table>
<thead>
<tr>
<th>Day of the research</th>
<th>T-AOC, mmol×Fe²⁺</th>
<th>GSH, μmol/l</th>
<th>SOD, U/mgHb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>experimental</td>
<td>control</td>
</tr>
<tr>
<td>the first</td>
<td>0.734 ± 0.02</td>
<td>0.747 ± 0.02</td>
<td>7.82 ± 0.24</td>
</tr>
<tr>
<td>the seventh</td>
<td>0.771 ± 0.02</td>
<td>0.654 ± 0.02*</td>
<td>7.74 ± 0.27</td>
</tr>
<tr>
<td>the fourteenth</td>
<td>0.762 ± 0.02</td>
<td>0.527 ± 0.02*</td>
<td>7.91 ± 0.31</td>
</tr>
<tr>
<td>the twenty first</td>
<td>0.754 ± 0.02</td>
<td>0.497 ± 0.02*</td>
<td>7.79 ± 0.26</td>
</tr>
<tr>
<td>the thirtieth</td>
<td>0.769 ± 0.02</td>
<td>0.513 ± 0.02*</td>
<td>7.84 ± 0.23</td>
</tr>
</tbody>
</table>

Notes: * P < 0.05 – statistically significant changes in relation to the control group

Table 3

<table>
<thead>
<tr>
<th>Day of the research</th>
<th>Total testosterone, nmol/L</th>
<th>17β-estradiol, nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>experimental</td>
</tr>
<tr>
<td>the first</td>
<td>4.11 ± 0.13</td>
<td>4.19 ± 0.14</td>
</tr>
<tr>
<td>the seventh</td>
<td>4.07 ± 0.12</td>
<td>3.97 ± 0.12</td>
</tr>
<tr>
<td>the fourteenth</td>
<td>4.23 ± 0.13</td>
<td>3.64 ± 0.11*</td>
</tr>
<tr>
<td>the twenty first</td>
<td>4.16 ± 0.13</td>
<td>3.21 ± 0.09*</td>
</tr>
<tr>
<td>the thirtieth</td>
<td>4.21 ± 0.14</td>
<td>2.91 ± 0.08*</td>
</tr>
</tbody>
</table>

Notes: * P < 0.05 – statistically significant changes in relation to the control group

Changes in the balance of steroid hormones, in particular total testosterone and 17β-estradiol, were noted with the increase in the intensity of peroxide oxidation processes in the body of rabbits of the research group.

On the one hand, on the 7th day of the experiment, a tendency was established to decrease the level of testosterone, which on the 14th and 21st days was significantly lower by 13.9% and 22.8%, respectively, and at the end of the study it decreased by 30.9 % (P < 0.05). The obtained changes were due to the negative effect of active forms of oxygen and toxic peroxidation products on the interstitial endocrinocytes of the testicles of experimental rabbits (Koshevoy et al., 2021).

On the other hand, a tendency to an increase in the level of 17β-estradiol was determined, which, in our opinion, was caused by changes in aromatase activity and leptin metabolism under the influence of oxidative stress – this was pointed out in the studies of De Luca et al. (2021). It is worth noting that the data in Table 3 show that the level of 17β-estradiol on the 21st and 30th days of the experiment was significantly higher than the data of the control group by 10.7 % and 14.6 %, respectively (P < 0.05).
Changes in lipid metabolism, in particular, the content of total cholesterol in the blood plasma of rabbits and the level of triacylglycerols are shown in Fig. 2.

The constant state of oxidative stress, the formation of which we noted during the study, contributed to changes in lipid metabolism in the body of the rabbits of the experimental group. Thus, the content of total cholesterol and the level of triacylglycerols significantly increased by 18.5% each from the 7th day of the study (P < 0.05). Then, on the 14th day, an increase in the level of these indicators was noted – total cholesterol by 18.4%, and triacylglycerols – by 41.7% (P < 0.05). On the 21st and 30th days, the cholesterol content was higher than the control values by 25.3 % and 17.4 %, respectively. Similar changes were noted in the level of triacylglycerols – on the 21st day of the study, they increased by 42.3 %, and at the end of the experiment - by 16.7 % (P < 0.05). It should be noted that the hyperlipidemia noted for the injection of paracetamol after the ending of its injection was characterized by a compensatory decrease, which is consistent with the data of Almajwal & Elsadek (2015).

Quantitative data on the cholesterol content of lipoproteins of different fractions are shown in Fig. 3.

Changes in the content of cholesterol fractions of lipoproteins were noted in the males of the research group. Thus, the number of HDL-cholesterol at the beginning of the experiment tended to decrease, and starting from the 14th day, a significant decrease of this indicator was established by 21.2 %, on the 21st day – by 17.0 %, and at the end of the study – by 26.0 % (P < 0.05). On the contrary, the number of LDL-cholesterol increased significantly during the experiment, in particular by 47.6 % on the 7th day of the study, by 38.5 % on the 14th, by 1.1 times on the 21st day, and after the end of the administration of paracetamol remained higher than the data of the control group by 73.9 % (P < 0.05).

Similar changes were found in paracetamol-induced rats, with serum triacylglycerols, LDL-cholesterol and VLDL-cholesterol levels showing significant increases, while HDL-cholesterol levels showed a significant decrease compared to control rats (Almajwal & Elsadek, 2015), also for APAP-induced hepatotoxicity in mice – in addition, isorhamnetin treatment significantly modulated lipid profiles (triacylglycerols, LDL-cholesterol, and HDL-cholesterol levels) that were altered in response to APAP administration (P < 0.05) (Gungor et al., 2023). It is possible to correct changes in lipid metabolism and redox status in the body of rabbits by introducing N-acetylcysteine, which was shown by us in a previous study (Koshevoy et al., 2022).

Prospects for further research. Taking into account the obtained data and the analysis of literary sources, the authors of the article consider it urgent to search for new means of reducing the toxic effect of paracetamol on the body of animals and humans, especially among nanostructured materials with distinct redox properties in order to evaluate their antioxidant and hypolipidemic activity (Koshevoy et al., 2022; Naumenko et al., 2023).
4. Conclusions

Biochemical changes in the body of rabbits after chronic injection of paracetamol are indicating the development of oxidative stress, which affects the level of sex hormones and lipid metabolism:

1. An increase in the intensity of peroxidation processes in the rabbits of the research group was experimentally proven: after the injection of paracetamol in the animals, the level of DC was higher by 63.0 %, and the content of TBA-RC was 1.26 times higher (P < 0.05). At the same time, a decrease in the activity of ADS was noted – the amount of GSH decreased by 35.4 %, and the activity of SOD – by 25.6 % (P < 0.05). The obtained changes were consistent with the comprehensive indicator of total antioxidant activity, which on the 21st day of the experiment was 34.1 % lower than the values of the control group (P < 0.05).

2. The levels of steroid hormones also changed during the study – the level of total testosterone gradually decreased (on the 21st day – by 22.8 %, and at the end of the experiment – by 30.9 %, P < 0.05), while the level of 17β-estradiol tended to before growth and on the 21st day was higher than the control by 10.7 % (P < 0.05).

3. Under the influence of paracetamol, significant changes in lipid metabolism were established in male rabbits – the content of total cholesterol on the 21st day of the experiment exceeded the data of control animals by 25.3 %, the level of triacylglycerols – by 42.3 %, the content of LDL-cholesterol also exceeded the data of control animals by 25.3 %, the level of total cholesterol on the 21st day of the experiment exceeded the data of control animals by 25.3 %, the level of triacylglycerols – by 42.3 %, the content of LDL-cholesterol also increased by 1.1 times (P < 0.05), and the level of HDL-cholesterol, on the contrary, was reduced by 17.0 % (P < 0.05).

Conflict of interest

The authors declare no conflict of interest.

References


