# Oral Acute Toxicity of Polyenylphosphatidyl choline (PPC) in Rats

Sanja Krošnjar[1*], Maida Todić[2], Sanela Bakić[1], Begler Begović[3], Irfan Zulić[2], Midhat Vehabović[1]

1. "Bosnalijek", Pharmaceutical and Chemical Industry, Joint Stock Company, Jukićeva 53, 71000 Sarajevo, Bosnia and Herzegovina
2. Institute of Pharmacology and Toxicology "Pavel Stern", Faculty of Medicine, Čekaluša 90, 71000 Sarajevo, Bosnia and Herzegovina
3. Institute of Clinical Pharmacology3, Clinical Centre of University of Sarajevo, Koševska bb, 71000 Sarajevo, Bosnia and Herzegovina

* Corresponding author

## Abstract

Endogen phospholipids play a major role in determining the structure and nature of cell membranes. A deficiency of phospholipids in cellular membranes makes it almost impossible for the cell membrane to perform its function as a selective barrier between what passes in and out of the cell. Polyenylphosphatidylcholine chemical structure corresponds to that of endogen phospholipids, but it possesses functional superiority because of its content of unsaturated fatty acids. Polyenylphosphatidylcholine integrates in the cell membrane and organelle systems while becoming their constitutive elements. A healthy cell membrane leads to healthy cells and then healthy tissue and then to healthy organs or body systems and finally, healthy bodies and minds. For a long time, polyenylphosphatidylcholine in combination with vitamins has been used in the treatment of numerous health problems such as liver diseases, dyslipoproteinaemias and different intoxications with consequent liver failure. The main aim of toxicology studies is evaluation of the toxic potential and risks of human exposition to the substance (1). According to the Organization for Economic Cooperation and Development (OECD) acute oral toxicity refers to those adverse effects occurring following oral administration of a single dose of a substance or multiple doses given within 24 hours. LD50 (median lethal dose), oral, is a statistically derived single dose of a substance that can be expected to cause death in 50 per cent of animals when administered by the oral route (2). Our acute toxicity study was performed on albino Wistar rats. Animals were randomised in three experimental and one control group, each of 5 males and 5 females. Study was based on the administration of a single oral dose of the test substance (polyenylphosphatidylcholine) to each experimental animal. There were three dose-levels of the test substance: 300, 500 and 1000 mg/kg. Test substance administration day was the first day of the observation period that lasted 14 days. Control animals were given milk vehicle. At the end of the study, no statistically significant differences between experimental and control animals were observed concerning the recorded parameters: body weight, respiratory rate, tremor, faeces and phonation quality, indicating the absence of the test substance acute toxicity.

**KEY WORDS**:polyenylphosphatidylcholine, acute oral toxicity.

## INTRODUCTION

Endogen phospholipids are found in every cell membrane in all living matter. Endogen phospholipids play a major role in determining the structure and nature of cell membranes. They are made up of a hydrophilic head and a hydrophobic tail. The head group has a 'special' region that changes between various phospholipids (Figure 1). head group differs between cell membranes. The fatty acid tails can also differ, but there is always one saturated and one unsaturated "leg" of the tail. A deficiency of phospholipids in cellular membranes makes it almost impossible for the cell membrane to perform its function as a selective barrier between what passes in and out of the cell. Endogen phospholipids facilitate the regulation of important biochemical processes in different biological systems. Polyenylphosphatidylcholine (PPC), an antioxidant phosphatidylcholine mixture extracted from soybeans, 50% of which consists of the highly bioavailable dilinoleoylphosphatidylcholine, restores phospholipids of the damaged membranes and reactivates their enzymes, including phosphatidylethanolamine methyltransferase, needed for phospholipid regeneration. Polyenylphosphatidylcholine (PPC) chemical structure corresponds to that of endogen phospholipids, but it possesses functional superiority because of its content of unsaturated fatty acids. Polyenylphosphatidylcholine integrates in the cell membrane and organelle systems while becoming their constitutive element. A healthy cell membrane leads to healthy cells and then healthy tissue and then to healthy organs or body systems and finally, healthy bodies and minds. Demirbilek and co-workers concluded that polyenylphosphatidylcholine therapy might be a useful adjuvant therapy in controlling of the excessive production of the inflammatory cytokines in patients with severe sepsis (3). Since polyenylphosphatidylcholine appears to promote the breakdown of collagen, it might affect not only the progression of the liver disease, but it may also reverse pre-existing fibrosis in rats (4). Polyenylphosphatidylcholine was found to prevent alcohol induced steatosis and hyperlipemia in rats (5) and to exert potent antioxidant effect of possible relevance to fibrosis in baboons (6). It has been shown that polyenylphosphatidylcholine reduced levels of transaminases in patients with hepatitis C (7). PPC was beneficial in patients with alcoholic hepatitis, and it opposed fibrosis in heavy drinkers and decreased aminotransferases in patients with hepatitis C. Finally, replacing long-chain with medium-chain triglycerides opposed the fatty liver experimentally and clinically (8). Authors Lee SH. et al., while performing a study in rats, found that polyenylphosphatidylcholine expressed cytoprotective effects on pancreatic beta-cells after the diabetic induction by streptozotocin. Their results strongly suggest that polyenylphosphatidylcholine plays important roles not only in protecting beta-cells against cytotoxicity, but also in maintaining their insulin syn-

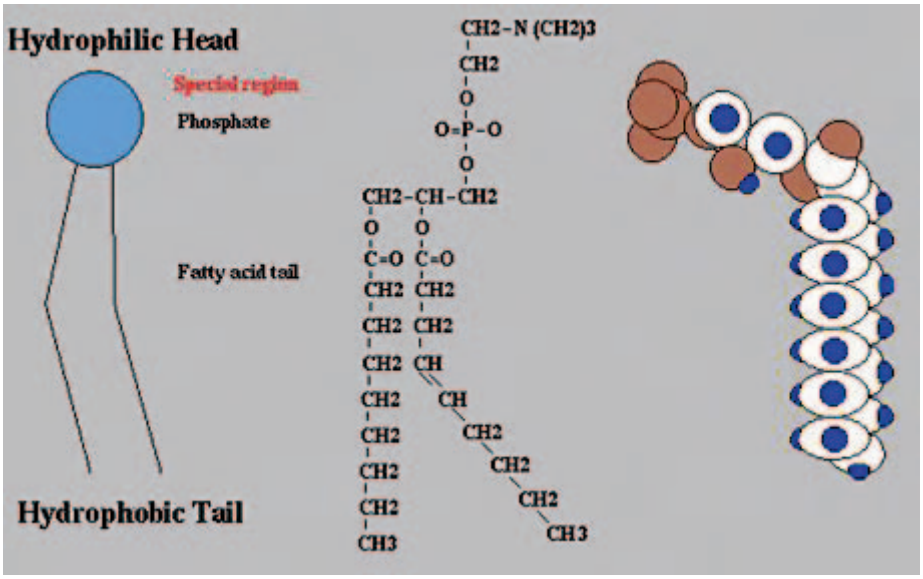



FIGURE 1.A model of phospholipid molecule

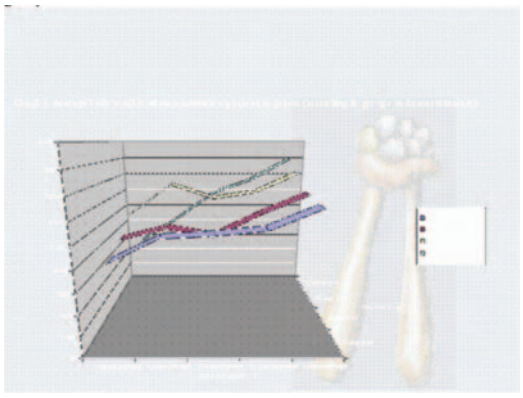
thesis and secretion for normal glucose homeostasis (9). Aleynik SI. and Lieber CS. found that polyenylphosphatidylcholine corrected the alcohol-induced hepatic oxidative stress in rats by restoring s-adenosylmethionine and replenishing hepatic glutathione (10). Research on nerve cells has shown that polyenylphosphatidylcholine can act as a substitute for the messenger substances acetylcholine. Dietary polyenylphosphatidylcholine decreased cholesterolemia in hypercholesterolemic rabbits. Phosphatidylcholine enriched diet developed significant higher cholesterol- and triacylglycerol-lowering effects by a two-step mechanism: 1) by reducing the beta-VLDLs, 2) by enhancing the secretion of bile cholesterol indicating promising effects of soybean phosphatidylcholine at the hepato-biliary level, in the treatment or prevention of hyperlipidemia and related atherosclerosis (11). For all of these reasons, polyenylphosphatidylcholine, alone or in combination with vitamins, has been used for a long time in the treatment of numerous health problems such as liver diseases, dyslipoproteinaemias and different intoxications with consequent liver failure. The major aim of our study was to determine oral acute toxicity of polyenylphosphatidylcholine in rats.

## Materials and Methods

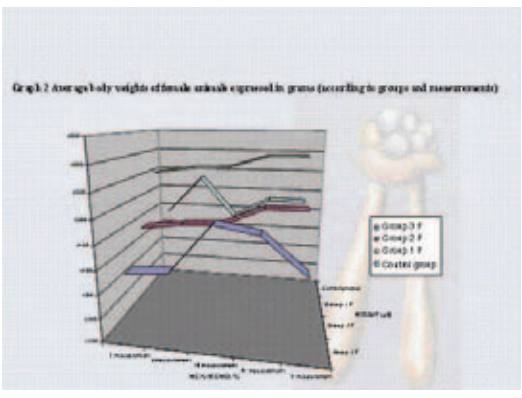
Our study was carried out on albino Wistar rats, during period from February 2nd until February 25th, 2003. Animals were put in 3 experimental and 1 control group, each of 5 males and 5 females (8 groups in total). Groups with male animals were signed with letter M. Groups with female animals were signed with letter F, while control groups of animals were signed with letter K. Animal groups signed with numbers 1, 2 or 3 were treated with following single doses of the test substance: 1 - 300 mg/kg; 2 - 500 mg/kg; 3 - 1000 mg/kg. Test samples were capsules, each containing polyenylphosphatidylcholine (PPC) 300 mg, nicotinamide 30 mg, pyridoxine hydrochloride 6 mg, riboflavin 6 mg, thiamine nitrate 6 mg, tocopherol acetate 6 mg. In order to prepare test substance the content of capsules was dissolved in milk heated in the water bath up to 60 °C. Prepared test substance was administered to test animals by oesophageal intubation. Control animals were given milk vehicle. Throughout the first day following the test substance application, 4 clinical examinations were performed in 2-hour intervals. Clinical examinations and mortality check-ups were carried out daily, in constant time intervals (every morning and evening). Each animal body weight determination was performed before the test substance administration, once a week and at the end of the study.

## Results

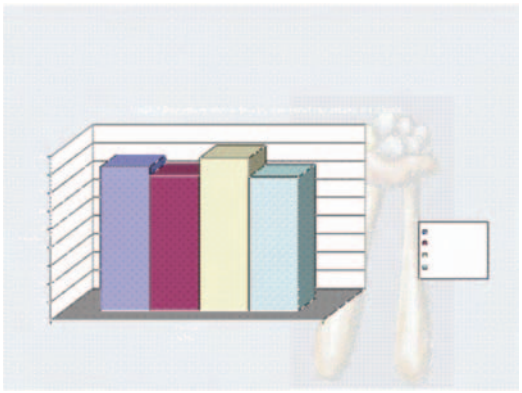
The first aim of our study was to determine the effect of the test substance administration on animal body weight. Experimental animals were randomised in such manner that the mean body weight did not significantly differ between groups (p=0,5). Variations inside experimental groups of animals were within two standard deviations. Statistical analysis of the body weight variations was performed separately for males and females (Graph 1, Graph 2). T-test for independent specimen failed to show any significant difference in body weights between groups of examined animals. The second study aim was to determine the effect of the test substance administra-



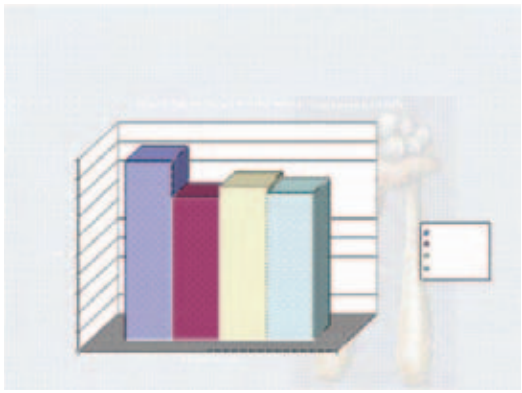
GRAPH 1.



GRAPH 2.



GRAPH 3.



GRAPH 4.

| GROUP | NUMBER OF RECORDINGS | MEAN | STD. DEV. | STD. ERROR | C.I. OF MEAN |
|---|---|---|---|---|---|
| 3M | 5 | 93,600 | 13,740 | 6,145 | 17,061 |
| 2M | 5 | 74,400 | 10,431 | 4,665 | 12,951 |
| 1M | 5 | 80,000 | 18,330 | 8,198 | 22,760 |
| KM | 5 | 76,800 | 12,458 | 5,571 | 15,469 |
| 3F | 5 | 79,200 | 23,900 | 10,688 | 29,676 |
| 2F | 5 | 74,400 | 15,126 | 6,765 | 18,782 |
| 1F | 5 | 84,000 | 17,205 | 7,694 | 21,362 |
| KF | 5 | 73,600 | 26,169 | 11,703 | 32,493 |

TABLE 1. Descriptive statistics for respiratory rate

tion on animal respiration rate. Respirator of female and male animals prior and post test substance application are presented in Graph 3 and Graph 4. Respiratory rates recorded in experimental and control groups of animals were within physiological limits for rats. Results did not significantly differ prior and post test substance administration (Table 1). The third aim of our study was to find out effect of the test substance administration on the appearance of clinical signs: tremor, animal phonation and faeces quality. Observed tremor was more frequent and more intensive in female animals than in male animals. Tremor was registered in 18 records from 160 records in total - 11,25%: 8 times in 3F group (in 4 animals), 6 times in 2F group (in 3 animals), and 4 times in 1F group (in 2 animals). Tremor was found to be mild in 13 cases (72,22%) or moderate in 5 cases (27,78%). In almost all cases (94,5%) tremor was registered during the first day of the test substance administration. Tremor was not observed in control group of animals. Sporadically, hoarse animal phonation was registered, predominantly in female animals. Hoarse phonation was noticed in control group of animals, as well. It was most frequently expressed during the 3$^{rd}$ and 4$^{th}$ day of the observation period. Such distribution of the changed animal phonation indicates that it was not a consequence of the test substance administration. Mildly to moderately lose faeces was observed sporadically and predominantly in male animals. Loose faeces was noticed in control animals, as well. Loose faeces were recorded 13 times in male animals (3,61%): 4 times in 3M group, 2 times in 2M group, 3 times in 1M group and 4 times in control group of animals. Loose faeces were found to be mild in 6 cases while moderate in 7 cases. Loose faeces were recorded 4 times in females (1,11%); 1 case per each group of animals. Loose faeces were found to be mild in 1 case while moderate in 3 cases (females). A statistical analysis performed on the end of our study revealed: **body weight** - the test substance administration did not induce statistically significant differences in body weights between experimental and control animals; **respiratory rate** - the test substance administration did not induce statistically significant differences in respiratory rates between experimental and control ani-

mals; **tremor** - the test substance administration did not induce statistically significant differences in observed tremor between experimental and control animals; **changed phonation** - the test substance administration did not induce statistically significant differences in phonation quality between experimental and control animals; **loose faeces** - the test substance administration did not induce statistically **significant** differences in faeces quality between experimental and control animals.

## Discussion

The main aim of toxicology studies is evaluation of the toxic potential and risks of human exposition to the substance (12). According to the *Organization for Economic Cooperation and Development* (OECD) acute oral toxicity refers to those adverse effects occurring following oral administration of a single dose of a substance or multiple doses given within 24 hours. LD50 (median lethal dose), oral, is a statistically derived single dose of a substance that can be expected to cause death in 50 per cent of animals when administered by the oral route. LD50 for acute oral toxicity means that dose of the material administered to both male and female young adult albino rats which causes death within 14 days in half the animals tested. The number of animals tested must be sufficient to give statistically valid results and be in conformity with good pharmacological practices. The result is expressed in mg/kg body mass. Acute oral toxicity is the adverse effects occurring within a short time of oral administration of a single dose of a substance or multiple doses given within 24 hours. LD50 (median lethal dose), oral, is a statistically derived single dose of a substance that can be expected to cause death in 50 per cent of animals when administered by the oral route. The LD50 value is expressed in terms of weight of test substance per unit weight of test animal (mg/kg) by United States Environmental Protection Agency. When the chemical substance produce toxicity only in certain circumstances, e.g. in extremely high doses, it is not necessary to evaluate the effects of irrationally high doses to demonstrate the toxicity. Information that substance in rationally high doses does not induce any lethality can be sufficient. Dose of 2 g/kg is accepted as a limit dose by the FAO/WHO Expert Committee on Food Additives, while US Environmental Protection Agency (EPA, 1994) accepts the limit of 5 g/kg. Applied doses in our study are approximations of the proposed future human single (therapeutic) doses. If one capsule contains therapeutic human dose, the applied doses in our study correspond to following: If each test sample capsule contains a single therapeutic dose of polyenylphosphatidylcholine-PPC, doses administered in our study were:

- 300 mg/kg is corresponding to a 50 times higher dose than human dose, (precisely 52,6 times higher dose);
- 500 mg/kg is corresponding to a 85 times higher dose than human dose, (precisely 87,7 times higher dose);
- 1000 mg/kg is corresponding to a 175 times higher dose than human dose, (precisely 175,4 times higher dose).

Dose of 2 g/kg is accepted as a limit dose by the FAO/WHO Expert Committee on Food Additives (WHO, 1996), while US Environmental Protection Agency (EPA, 1994) accepts the limit of 5 g/kg. A substance with LD50 between 0,5 and 5 g/kg is classified as moderately toxic (11). We did not register any lethality after the 1 g/kg-dose application, so the test substance may be classified as substance with moderate toxicity, low toxicity or practically with no toxicity. The maximal tested dose in our study was 1g/kg. Taking in consideration a fact that the overdose with a dose 200 times higher than the single therapeutic dose is hardly possible we did not find a testing of the higher test substance doses rational. Authors Gad & Changelis consider that testing of the doses 100-300 times higher than the proposed human doses is sufficient (13).

## Conclusion

Polyenylphosphatidylcholine did not express acute toxicity, when administered orally in rats in single doses of 300 mg/kg, 500 mg/kg or 1000 mg/kg. After the administration of a dose of even 1 g polyenylphosphatidylcholine per kg in rats that is corresponding to 175 times higher dose than human dose, no lethality was observed. According to a definition, by which a substance whose LD50 is between 0,5 and 5 g/kg is classified as moderately toxic substance (11), our test substance, polyenylphosphatidylcholine, might be classified as a substance of low toxicity or practically non-toxic substance.

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