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INFLUENCE OF SUPPLEMENTS OF *CURCUMA LONGA*, *ANGELICA KEISKEI*, AND ROYAL JELLY, IN ASPIRIN AND OMEPRAZOLE ADMINISTRATION, ON ENZYMATIC AND LIPID PROFILE: A MURIN MODEL

INFLUENȚA SUPLIMENTELOR DE *CURCUMA LONGA*, *ANGELICA KEISKEI* ȘI LĂPTIȘOR DE MATCĂ, ÎN ADMINISTRĂRILE DE ASPIRINĂ ȘI OMEPRAZOL, ASUPRA PROFILULUI ENZIMATIC ȘI LIPIDIC: UN MODEL MURIN

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ABSTRACT | REZUMAT

Aspirin, or acetylsalicylic acid, is included in the category of non-steroidal anti-inflammatory molecules (NSA IDs) due to its anti-inflammatory, anti-thrombocyte, analgesic, and antipyretic effects, but long-term use is associated with various adverse effects (hepatotoxicity, nephrotoxicocity, gastrointestinal ulcers, and even kidney cancer). Along with aspirin, omeprazole is one of the most prescribed classes of drugs being used in stomach ulcers, with or without infection, with Helicobacter pylori, in gastroesophageal reflux, Zollinger-Ellison disease, dyspepsia, esophagitis and gastritis. A problem faced by clinicians in these cases is hepatotoxicity and harmful effects on liver enzymes and lipid profile, knowing that NSAIDs can become hepatotoxic, with asymptomatic increases in serum transaminases and alkaline phosphatases, or hepatic / cholestatic or mixed cytolysis. Therefore, the parameters of serum transaminases are important to track, and their increase can be a certain sign of early warning and are considered relevant indicators of liver toxicity. Certain biochemical markers; alanine-aminotransferase (ALT), aspartate-aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin can help diagnose liver damage. An enzyme that is mainly present in the liver, but also in the kidneys, and that participates in the oxidative deamination of glutamate is glutamat dehydrogenase. (GLDH). The present study proposes testing the effect of the extracts of Angelica keiskei, *Curcuma longa* and Royal jelly, known with anti-inflammatory, antioxidant, chemoprotective, tissue protective, antibacterial, antifungal, antiviral, immunomodulatory, and antineoplastic properties in liver stress induced by aspirin, correlated with the parameters of transaminases and lipid profile, at the level of the liver. The present study, designed on a murine model on 48 mice from the BALB/c line, comes to add information related to the administration of certain biological preparations with potential hepatoprotective and hypolipidemic activity and that the short-term administration of aspirin, does not lead to chronic hepatotoxic effects.

Keywords: aspirin, transaminases, lipid profile, murine model, phytotherapy

Aspirina sau acidul acetilsalicilic este inclusă în categoria moleculelor antiinflamatoare nesteroidiene (AINS) datorită efectelor sale antiinflamatorii, antitrombocitare, analgezice și antipiretice, dar utilizarea pe termen lung este asociată cu diverse efecte adverse (hepatotoxicitate, nefrotoxicitate, ulcere gastrointestinale și chiar cancer de rinichi).

Alături de aspirină, omeprazolul este una dintre cele mai prescrise clase de medicamente fiind utilizat în ulcerele gastrice, cu sau fără infecție, cu Helicobacter pylori, în refluxul gastro-esofagian, boala Zollinger-Ellison, dispepsia, esofagita și gastrita.

O problemă cu care se confruntă clinicienii în aceste cazuri este hepatotoxicitatea și efectele nocive asupra enzimelor hepatice și a profilului lipidic, știind că AINS pot deveni hepatotoxice, cu creșteri asimptomatice ale transaminazelor serice și ale fosfatazelor alcaline, sau mixte hepatice/colestatice, citoliză, prin ur-are, parametrii transaminazelor serice sunt importanți de urmărit, iar creșterea lor poate fi un anumit semn de avertizare timpurie și sunt considerați indicatori relevanți ai toxicității hepatice.

Anumiți markeri biochimici, alanina-aminotransferaza (ALT), aspartat-aminotransferaza (AST), fosfataza alcalina (ALP) si bilirubina pot ajuta la diagnosticarea leziunilor hepatice. O enzimă care este prezentă în principal în ficat, dar și în rinichi și care participă la dezaminarea oxidativă a glutamatului este glutamat dehidrogenaza (GLDH).

Prezentul studiu propune testarea efectului extractelor de Angelica keiskei, Curcuma longa și Lăptișor de matcă, cunoscute cu proprietăți antiinflamatoare, antioxidante, chimioprotectoare, protectoare tisulare, antibacteriene, antifungice, antivirale, imunomodulatoare și antineoplazice în stresul hepatic indus de aspirină, corelat cu parametrii transaminazelor și a profilului lipidic, la nivelul ficatului.

Prezentul studiu, conceput pe model murin, pe 48 de șoareci din linia BALB/c, vine să adauge informații legate de administrarea anumitor preparate biologice cu potențială activitate hepatoprotectoare și hipolipidemiante și că administrarea pe termen scurt a aspirinei nu duce la efecte hepatotoxice cronice.

Cuvinte cheie: aspirină, transaminaze, profil lipidic, model murin, fitoterapie

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The liver is an important metabolic organ that performs numerous vital functions. The physiological and biochemical processes of the body are dependent on the disorders of the liver: hepatitis B and C, alcoholic liver disease,non-alcoholic fatty liver diseases (NAF LD), liver cirrhosis, liver failure, and hepatocellular carcinoma (46). The liver is the central organ that c ls lipid homeostasis through complex but well-controlled biochemical and cellular signalling pathways. The biochemical and metabolic functions of the liver are controlled by hepatocytes, which are the main parenchymal cells of the liver. Hepatocytes control, including triglyceride metabolism. Additional types of cells inside the liver include Kupffer cells, cholangiocytes, star cell, and endothelial cells with specialised functions in liver physiopathology. (46).

One of the most common causes of liver failure, drug-related hepatotoxicity is one of the biggest problems facing clinicians. Hepatotoxicity is caused by several mechanisms, including disruption of cell membranes, initiation of an immune response, alteration of cellular pathways of drug metabolism, accumulation of reactive oxygen species (ROS), lipid peroxidation, and cell death (19). Aspirin is classified in the category of non-steroidal anti-inflammatory agents (NSAIDs), also known as acetylsalicylic acid (ASA). The molecule, cheap and affordable, remains among the most widely used non-steroidal anti-inflammatory (NSA ID). Aspirin is used for a variety of purposes, including anti-inflammatory (in joint conditions), anti-thrombocyte (in cardiovascular conditions), analgesic, and antipyretic (1,19).

Nephrotoxicity, hepatotoxics, gastrointestinal ulcers, kidney cancer, and other adverse effects on several organs have sometimes been linked to long-term use of aspirin in treatments. Dose-dependent inhibition of cytoprotection by higher doses of aspirin amplifies the risk of bleeding and perforation. Aspirin administration over a longer period of time has caused significant histopathological changes in the liver, by inhibiting cytoprotective factors in the stomach mucosa, which can lead to new lesions of the mucous membrane or worsen existing ones (19,27,30).

Several studies have confirmed the toxicity of aspirin to the liver. ASA is metabolised by conjugation mechanisms in the liver to form salicylic acid and several metabolites with hepatotoxic potential. ASA disperses throughout the body after ingestion, and the highest concentrations found are in: blood plasma, liver, renal cortex, heart, and lungs (1,2).

Proton pump inhibitors (IPPs), such as omeprazole, are among the most commonly prescribed classes of drugs. These pump inhibitors are indicated for the treatment of ulcers, with or without Helicobacter pylori infection, for treating gastro-oesophageal reflux, Zollinger-Ellison disease, dyspepsia, oesophagitis, and gastritis, and for the prevention of peptic ulcer in patients receiving non-steroidal anti-inflammatory agents (NSAIDs) as well as those with upper gastrointestinal bleeding (26,42).

Omeprazole's mechanism of action involves effectively suppressing the secretion of gastric juice through specific inhibitions of H+K+-ATP-gastric acid. This proton pump is located in the secretory membranes of the parietal cell of the gastric mucosa and constitutes the final stage of acid secretion; however, hepatotoxicity of omeprazole has been very rare (26).

The mechanism of liver pathology is an important tool for identifying and characterising liver lesions, whether or not clinical biochemical changes are identified. The main patterns of liver damage resulting from a phenomenon of hepatotoxicity may include: zonal necrosis, hepatitis, cholestasis, steatosis, granuloma, vascular lesions, neoplasm, and occlusive venous diseases. The most common hepatotoxic phenomenon is steatosis. This type of liver injury may manifest itself as an accumulation of triglyceride molecules (48) leading to either small drops (microvesicular build-up) or large droplets (macrovesiculary build-up) in the liver. Aspirin, ketoprofen, tetracycline, nucleoside reverse transcriptase inhibitors, and valproic acid plants of the *Scutellaria* species may lead to microvesicular steatosis, while acetaminophen and methotrexate can lead to macrovezicular steatosis. (8, 25, 26).

From another perspective, amiodarone, chlorpheniramine can cause phospholipidosis (7) if the accumulation of phosfolipids leads to a similar pattern, with diseases with hereditary defects of phospholipid metabolism (41). Liver lesions can be diagnosed by certain biochemical markers, such as alanine aminotransferase (ALT), aspartataminotransférase (AST), alkaline phosphatase (AlP), and bilirubin.

Increases in serum enzyme levels are taken as relevant indicators of liver toxicity, while increases in both total and conjugated bilirubin levels are measures of the overall liver function. An increase in transaminase levels in combination with an increase in bilirubin levels to more than twice the upper normal level is considered a relevant marker for hepatotoxicity (39). Other studies have demonstrated RJ's hepatoprotective action in vivo in non-alcoholic fatty liver diseases. Oxidative stress in liver cells plays a significant role in the pathophysiology of this condition. Other treatments performed on laboratory rats, administered intragastrically with multiple doses of RJ; 150, 300, and 450 mg/kg BW), showed that RJ was effective in relieving liver steatosis and improving the serum fat profile. All these positive effects have arisen due to the antioxidant effect of RJ and can be attributed to various biological mechanisms, among which the most important may be the ability of the RJ to increase the enzymatic activity of liver-specific antioxidant enzymes, thereby reducing levels of oxidative stress (6,7,11). As a general mechanism of action, non-steroidal anti-inflammatory agents act by inhibiting cyclooxygenase (COX), an enzyme that turns arachidonic acid into prostaglandins, being a pain mediator. (4). Two isoenzymes of cyclooxygenase (COX-1 and COX-2) are generally recognised; COX-1 is involved in the gastric protection from gastric acid and in the formation of tromboxane by platelets. COX-2 is

induced by inflammatory mediators in a wide variety of conditions and has been associated with inflammation, but can also contribute to processes related to renal physiology, reproductive function, bone resorption, and neurotransmitters (5,44).

Within the metabolism of triglycerides, the bonding of triglyceride molecules (TG) is the main way through which the liver stores and exports fatty acids. Under normal conditions, the liver stores little triglycerides (TG), but exports considerable amounts in the form of VLDL (very low-density lipids), which supply fatty acids to muscle tissue, depending on their nutritional status. Previously, it was believed that excess triglyceride molecules (TG) in the establishment of non-alcoholic fatty liver disease (NAFLD) contribute to lipotoxicity, but new concepts suggest that increased triglyceride storage (TX) and VLDL secretion are, instead, protective. (14,20).

Cholesterol (CHL) is a basic component of all cell membranes and a precursor of several hormones, vitamins, and bile acids, being an essential molecule for the body. Although cholesterol (CHL) plays an important role in the body, its accumulation at high plasma levels is a risk factor for the development of atherosclerosis (2,46). Cholesterol homeostasis is mediated by 3 main pathways: biosynthesis, absorption from the intestine, and elimination as bile acids. Excessive intake of cholesterol increases catabolism to bile acids. Liver cholesterol metabolism is associated with triglyceride metabolism (15,47).

Herbal extracts and supplements generally show a wide range of beneficial effects, including anti-inflammatory, antioxidant, chemoprotective, antibacterial, antifungal, antiviral, immunomodulatory, antineoplastic, anti-aging and antidepressant properties, as described in the literature and by other authors (3, 12). The literature, confirmed that *Angelica keiskei* provides good results in the studies of prevention of obesity, hepatoprotection, diabetes mellitus, and increased plasma antioxidants, in patients with metabolic syndrome (46). The predominant components in *Angelica keskei*, including 4-hydroxiderricine (4-HD), isobavachalcona, xanthokeismine A, and xantoangelol B, E, D, and F, have been to possess a wide range of pharmacological qualities, including antioxidant and anti-inflammatory potential. Metabolomic and lipidemic analyses of human plasma showed that five components of Ak, including 4-HD, are responsible for preventive effects against liver disease, type 2 diabetes, obesity, and atherosclerosis (8, 36).

Curcumin is the main active polyphenolic component in turmeric. Curcumin is found mainly in the roots and rhizomes of the turmeric plant (*Curcuma longa*). Curcumin can be administered in the form of turmeric, curcuma extracts, essentially pure (95%) curcuminoids, or curcumin alone (43). Curcumin, the main substance, also known as the "yellow pigment of turmeric" has anti-carcinogenic, anti-inflammatory, hepatoprotective, and antioxidant properties. The protective effects of curcumin on the liver are attributed mainly to its antioxidant properties. Studies have shown that curcumin consumption leads to a decrease in structural changes in the liver and total bilirubin in the activities of ALT, AST, and GLDH and an increase in serum proteins (42,48). Royal jelly contains about 185 organic compounds, with royalactin being the most important protein present in royal jelly (22,24,40). The hepato-renal protective effect of royal jelly, is another good alternative for the treatment of liver and kidney dysfunctions. Administration of Royal Jelly for seven days as a hepatoprotective agent improved severe liver damage induced by anti-inflammatory drugs in mice (3, 10, 23). Macroscopic observations, especially histopathological observations and investigation of clinical biochemistry parameters, allow confirmation of hepatotoxicity. Hepatotoxicity can be characterised in two main groups, each with a different mechanism of lesion: hepatocellular and cholestatic (17, 31).

The aim of this study is to observe the influence of acetylsalicylic acid (ASA), administered for 3 consecutive days, on the liver enzymes, alanin-aminotransferase (ALT), aspartate-aminotransferase, glutamate dehydrogenase (GLDH), and lipid profile, cholesterol and triglycerides, in a line of laboratory mice, BALB/c, treated for 7 and 14 days, respectively, with herbal products (*Angelica keiskei, Curcuma longa*), royal jelly, as well as with omeprazole.

MATERIALS AND METHODS

Authorisation of the Bioethics Commission

The study was approved by the Bioethics Committee of USAMVB Timișoara, Romania, under No. 140/02.11.2022, being in accordance with all domains' national and international relevant regulations, all methods being reported in accordance with ARRIVE guidelines. Rodents were placed for seven days before beginning to adapt to laboratory settings in agreement with the UE Directive 2010/63/EU, upon the treatment of animals used for research purposes (16, 32).

Animals and Experimental Design

In the study were included 48 clinically healthy male and female mice, randomly chosen, from the BALB/c line, all with uniform weight (33±2g), aged 3-3.5 months. Animals were kept in a controlled environment and housed in standard polycarbonate cages (n = 8) (l × w × h = 750 × 720 × 360 mm) and fed *ad libitum* with the usual diet destined for rodents (Diet, Biovetimix, code 140-501, Romania). The temperature in the bio-base was 22 ±2°C and the relative humidity was 55 ±10%. The animals were exposed to a 12/12 h light-dark cycle.

The animals were divided into 6 experimental groups, as described in Fig. 1.

The 48 individuals included in the study were divided into 6 experimental groups (n = 8), and in order to induce liver stress, they received for three days, by oral gavage, a dose of aspirin of 300 mg/kg.BW. After the three days, the groups were treated with biological preparations that are known to have anti-inflammatory and hepatoprotective properties.

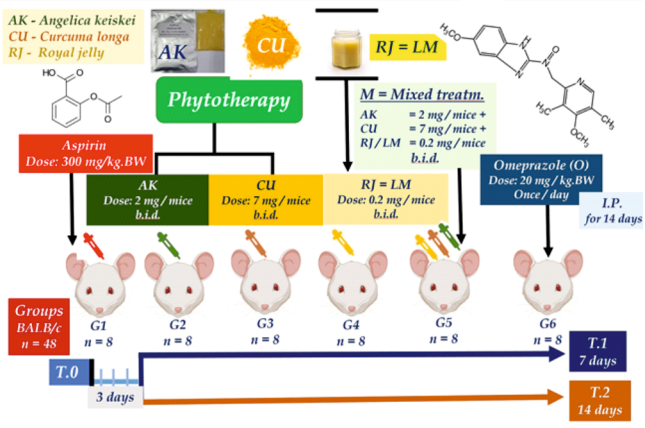

Fig. 1. Experimental design (T.0 = time of start of administration; T.1 = serum sampling 7 days; T.2 = 14-day sample harvesting). G1 (lot control) = received aspirin (ASA), dose = 300 mg/kg.BW., the first three days / once a day p.o.; G2 = received *Angelica keiskei* (AK), in the dose of = 2 mg/mouse, b.i.d. (*bis in diae* = twice a day) p.o.; G3 = received *Curcuma longa* (CU), dose = 7 mg/mouse, p.o., b.i.d.; G4 = received Royal Jelly (LM/RJ), 0.2 mg / mouse, p.o., b.i.d.; G5 = Mixed administration group (dose: 2 mg AK + 7 mg CU + 0.2 mg RJ/LM) / mouse; G6 = omeprazole (O), 20 mg/kg. BW, intra-peritoneal, once a day for 14 days

Aspirin is an essential drug molecule in human medicine, but less widely used in veterinary medicine. For this reason, Group 6 received omeprazole as treatment, because it is an established treatment for treating or preventing the occurrence of complications associated with the long-term administration of aspirin and is a classic treatment in the treatment of peptic ulcer.

Group 1 (Control group), aspirin only, for the first 3 days in a row, then the mice were administered only water *ad libitum*, until the end of the experiment.

Group 2 received *Angelica keiskei* extract for 14 days, dose = 2 mg/mouse. The administration was carried out twice a day, in gavage (*bis in diae* = b.i.d.)

Group 3 received 14 days of *Curcuma longa* powder, dose = 7 mg/mouse. The administration was carried out twice a day, in gavage, (b.i.d.).

Group 4 received royal jelly for 14 days, dose = 0.2 mg/mouse, in gavage, (b.i.d.).

Group 5 received the combination of the three preparations (AK, CU, LM/RJ in gavage, in dose=2 mg AK + 7 mg CU + 0.2 mg LM/RJ/mouse), in gavage, (b.i.d.).

Group 6 was treated with omeprazole alone (20 mg/kg.BW), intra-peritoneally, once a day, for 14 days (Fig. 1).

The studied plants and molecules

Curcuma longa extract was purchased from Lipo Life Oradea, Romania, under the trade name of Liposomal Curcumin C3 complex (Drakes Lane Industrial Estate, Boreham, UK) (lot UELE 1707). In the concentration of 2% active substance (where 5 ml of extract contains 100 mg, 95% curcumin and curcuminoids, so 100 ml contains 2 g of active substances).

Angelica keiskei (Ashitaba Powder) powder was received as samples in standardised form of 99.99% pure powder, in 10 g sachets, directly from the manufacturer, under the name of ChalCurb-P8, (Japan Bio Science Lab. Co. Ltd. Osaka, Japan) (lot IACA 9910).

Royal jelly was purchased from the "Veceslav Harnas S.A." Beekeeping Complex, Bucharest, Romania. The 2 ml ampoules contain 0.2 g of pure freeze-dried royal jelly (lot 1/1515). The dose was calculated according to the manufacturer's instructions and after extrapolation of the dose.

In the case of *Curcuma longa*, *Angelica keiskei,* and royal jelly, the doses were calculated per animal by extrapolating the doses after body surface's area, using the Löwe's rule doses calculated for an individual were: 2 mg for Angelica keiskei, 7 mg for *Curcuma longa*, and 0.2 mg for Royal Jelly (13).

The drug molecules administered were:

Acetylsalicylic acid, chosen for our study, was the most soluble variant under the trade name of Alka-Seltzer (A.P.P. Bayer SRL, Bucharest, Romania) (lot BT 14001) and had a composition of 324 mg/tablet. In the case of aspirin, the calculated dose was 300 mg/ kg. BW, doses taken using data from the literature (37).

Omeprazole was purchased under the trade name Omez (Dr. Reddy's Laboratories, Bucharest, Romania) (lot 01733), each vial of powder for infusion contained 40 mg of omeprazole and excipients. The dose of omeprazole was calculated at 20 mg/kg. BW and was administered intraperitoneally throughout the treatment (14 days), and it was chosen using data from the literature (42).

Biochemical investigations

After 7 days (T1) in which the preparations were administered, half of the individuals of each group had their blood collected, and were euthanized. The same was done after 14 days (T2) of treatment. The liver, kidney, spleen, and stomach were harvested to be used in the cytohistological study.

Before the blood was drawn, the mice were kept on a fast for 8-12 hours. Blood was collected from the heart after anaesthesia (dose: ketamine 50-100 mg/kg.BW., i.m., xylazine 2-8 mgc/kg.BW., i.m.). The 0.5 mL blood was collected and transferred to a 1.5 mL Eppendorf tube without anticoagulant. To obtain the serum, after 30 minutes, the blood sample was centrifuged at 2400 rpm and 27°C, for 30 minutes. The serum was stored in the freezer (-4 °C) until biochemical analysis.The concentrations of triglycerides (TG), cholesterol (CHOL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and glutamate dehydrogenase (GLDH) were measured with the Randox Daytona Plus automatic biochemistry analyzer (Randox, Crumlin, U.K.), using the appropriate commercial kits (Randox,Crumlin,U.K). Calibration was performed with Randox Calibration Serum Level 3 (Cat. No. CAL 2351), and QC charts for reference materials were created using two levels of control serum: Randox Assayed Multisera Level 2 (Cat. Not. HN 1530) and re-

spectively level 3 (Cat.No. HE 1532).

Statistical Analysis

The statistical software used was Graph Pad Prism 6.0 for Windows (Graph Pad Software, San Diego, USA). Values were expressed as mean ±SEM (Standard Error of Means). The estimation of the difference between groups was ascertained using two-way analysis of variance (ANOVA) with Tukey's multiple comparison tests. The statistical values were considered as follows: $^{*}0.01 \leq p<0.05$, significant; $^{**}0.001 \leq p< 0.01$, highly significant; $^{***}p<0.001$, very high significant; ns: not significant.

RESULTS AND DISCUSSIONS

Biochemical marker analysis

Table 1 shows that there were no high serum values of the analysed markers compared to the reference values for the BALB/c line. Alanine-transferase (ALT), in the group that received only aspirin for 3 days, the average values registered the value of 55.93 U/L, falling within the reference value. Comparing the average value of lots, there was a slight increase in values, but not more than 20%. On day 14, the values fall within the reference ones, with a slight increase only in the case of the group that received *A. keiskei*. Compared to the ALT values on day 7, they are slightly higher, not within normal parameters; however, it cannot be deduced with certainty that biologics and omeprazole led to their increase and that there would be liver damage. Aspartate-aminotransferase (AST) tends towards the upper limit compared to the reference limits. In the case of the group that received *Angelica keiskei* for 7 days, there was an increase in AST of almost 50% compared to the reference values, and even more, compared to the Control group, which received only aspirin. For 14-day values, serum values were lower in all groups except the group that received aspirin, but the differences were not significant. Given that, in addition to the liver, this enzyme is also found in other organs such as the heart, muscle, brain, and kidneys, damage to any of these tissues can cause an increase in blood levels (34). Glutamate dehydrogenase (GLDH), in the case of the group that received Royal Jelly, recorded mean serum levels double compared to the Control group. At 14 days, there is an increase in the groups treated with *A. keiskei, C. longa,* and mixed administration, and in this case, we can deduce that there could be hepatocellular damage in the scenario of long-term administration of these biological products. This result is in line with the data in the literature where it is known that GLDH activity increases with hepatocellular deterioration, and in rodents, increases in GLDH activity have been reported to be of a much higher magnitude and to have a greater persistence over time after administration of liver stressors (4, 9, 34).

Cholesterol in general showed values above the reference levels, but triglycerides were within the parameters for all groups. Although cholesterol (CHL) has an important role in the body, its accumulation at high levels in plasma is a risk factor for the development of atherosclerosis and other metabolic diseases, but in association with normal triglyceride values, it cannot be confirmed with certainty that higher cholesterol values were influenced by the administration of aspirin, omeprazole, or administered biological products (2, 47). In this case, cholesterol can also have a protective effect, with additional clinical parameters (LDL /HDL - low density lipids/high density lipids) that could show with certainty whether higher cholesterol levels have a harmful hepatocellular effect.

Statistical data

In figures 2 and 3, the values of Cholesterol and Triglycerides are represented, observing that, in the Control group, both at T1 and T2, the values were higher than in the other groups that received *A. keiskei, C. longa*, mixed administration, and omeprazole. In

Table 1

Representation of mean serum values and standard error for ALT, AST, GLDH, Chol, and Try, at 7 and 14 days, respectively

Administration/ parameter/day	ALT U/L		AST U/L		GLDH mg/dl		CHOL mg/dl		TRY mg/dl	
	7	14	7	14	7	14	7	14	7	14
Angelica keiskei K. (AK)	**44.74** ±5.23	**82.32** ±27.21	**160.36** ±65.81	**73.56** ±14.71	**8.18** ±0.26	**24.00** ±8.66	**81.27** ±6.45	**95.00** ±16.01	**19.12** ±1.11	**27.97** ±9.07
Curcuma longa (CU)	**48.99** ±10.90	**91.71** ±17.55	**50.47** ±14.16	**78.57** ±14.81	**9.30** ±0.61	**27.00** ±6.24	**83.85** ±8.18	**82.69** ±6.18	**13.00** ±0.31	**25.31** ±8.20
Royal jelly (LM/RJ)	**46.51** ±4.52	**80.95** ±15.21	**121.43** ±23.11	**128.39** ±27.06	**22.35** ±7.88	**8.48** ±0.14	**112.00** ±1.55	**70.14** ±10.57	**23.83** ±1.25	**19.74** ±5.53
Omeprazole (O)	**44.49** ±4.39	**73.40** ±9.86	**79.04** ±11.11	**90.83** ±15.83	**9.38** ±0.89	**11.03** ±0.98	**80.50** ±11.92	**66.03** ±8.73	**21.06** ±3.63	**16.64** ±1.20
Control group (Aspirin)	**55.93** ±6.70	**47.53** ±4.92	**101.63** ±18.58	**118.50** ±23.66	**8.48** ±0.14	**8.18** ±0.26	**97.51** ±20.35	**102.76** ±12.39	**35.81** ±7.78	**20.42** ±8.91
Mixed administration (AK+CU+LM/RJ)	**48.58** ±12.18	**52.22** ±16.38	**114.06** ±73.98	**73.14** ±41.66	**8.10** ±0.30	**40.50** ±13.50	**67.00** ±32.24	**60.02** ±19.68	**21.60** ±11.09	**16.66** ±2.31
Reference values (11)	**29-81**		**69-111**		**-**		**20.53-53.66**		**14.76-33.67**	

the case of the G4 group, which received royal jelly, in T1, the cholesterol value was lower. Statistically significant changes ($^{**}p<0.01$) were observed for cholesterol in the groups that received omeprazole, the value at the T2 reference time being slightly lower.

In figure 3, it can be seen that the highest values in T1 are also found in the Control group. Statistically significant differences,compared to the Control group, were observed in all groups ($^{***}p<0.001$) except G5. The lowest average triglyceride values are found in T1 in the group that received *C. longa* for 7 days, confirming the data in the literature that it has a hepatoprotective and antioxidant effect. Lower mean values are also found in the group that received A*. keiskei,* royal jelly, or omeprazole, but in the G5 group, there was no significant decrease in tryglycerides.

In T2, the highest triglyceride values were found in the G2 group, being even higher compared to the Control group, demonstrating that, in certain cases, long-term administration of *A. keiskei* powder may have become potentially harmful. The lowest triglyceride values were in groups G5 and G6, and it can be found that long-term administration of a combination of herbs or administration of omeprazole, can lead to a hepatoprotective effect. The hepatotoxic effects of non-steroidal anti-inflammatory drugs, such as acetylsalicylic acid in the present case, range from asymptomatic increases in serum transaminases and alkaline phosphatases, he-patic/cholestatic or mixed cytolysis. Serum transaminases are useful parameters to monitor, their increase being a clear early warning sign in case of hepatocellular dysfunctions.

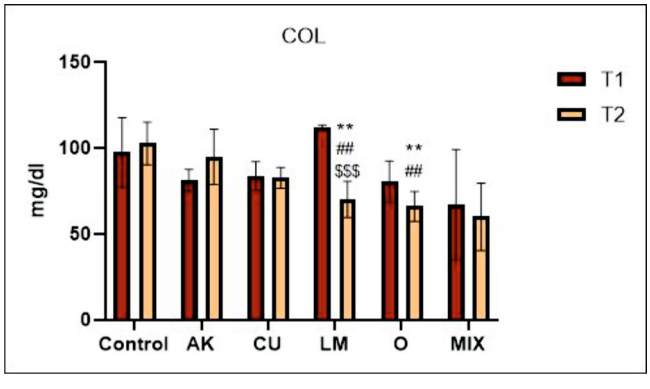

Fig. 2. Serum statistical values for Cholesterol. Comparative to C: $^{**}p<0.01$; AK: $^{\#\#}p<0.01$; T1 vs T2: $^{\$\$\$}p<0.001$
Where: $^{*}p<0.05$, significant; $^{**}0.001 \leq p<0.01$, highly significant; $^{***}p<0.001$, very high significant; ns: not significant.
Where: AK = *Angelica keiskei K.*, CU = *Curcuma longa*, LM/Rj= Royal Jelly, O = Omeprazol, Mix = AK+LM+CU

In the mechanism of NSAID-induced hepatotoxicity, two main mechanisms responsible are recognised: hypersensitivity and metabolic aberration (27). Hypersensitivity reactions contain significant anti-nuclear factor or antibody titers against smooth muscle, lymphadenopathy, and eosinophilia, and metabolic aberrations can occur as genetic polymorphisms and alter susceptibility to a wide range of drugs (28, 35, 45).

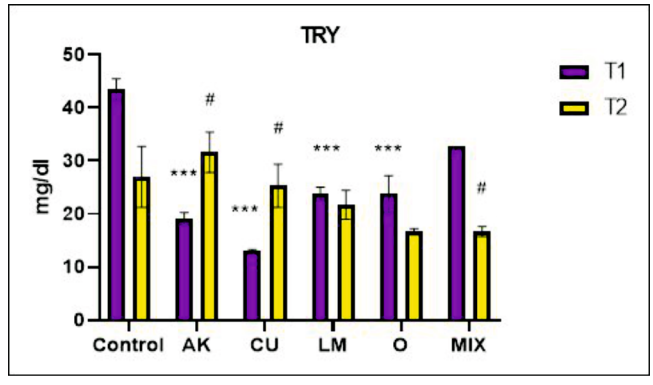

Fig. 3. Statistical serum values for Triglycerides. Comparative to C: $^{***}p<0.001$; Comparative T1 vs T2: $^{\#}p<0.05$

A standard clinical marker of hepatotoxicity is AST, a liver enzyme that plays an important role in amino acid metabolism and gluconeogenesis. It catalyses the transfer of an amino acid group from alanine to α-ketoglutarate to produce glutamate and pyruvate. Normal levels are accepted in the range of 29-81 U/L, with elevated levels of this enzyme being associated with liver damage. Since it is mainly found in the liver, the estimation of this enzyme is a test with great specificity for detecting liver abnormalities (3,17,18).

Figures 4 and 5 present the statistical analysis for ALT and AST. In the case of alanine aminotransferase (ALT) (Fig. 4), in the Control group, in T2, we find lower values compared to the ALT values from the other groups in the study. Significant and very statistically significant values can be found in T2 in the case of Lots G2, G3, G4, and G6, and in Lot G5, finding slightly higher values than in the case of the Control group but not statistically significant, neither in T1, or in T2.

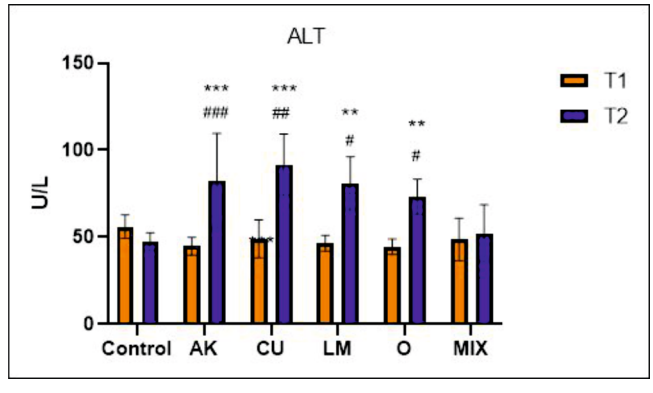

Fig. 4. Serum Alanine aminotransferase (ALT) values. Comparative to C: $^{**}p<0.01$; $^{***}p<0.001$; Comparative T1 vs T2: $^{\#}p<0.05$; $^{\#\#}p<0.01$; $^{\#\#\#}p<0.001$

In the case of aspartate-aminotransferase (AST) (Fig.5), in T1 and T2, in the G3 and G5 groups, we observe that the values were lower than in the case of the Control group, this aspect suggesting, in the case of these groups, that the administered preparations have

a potential protective effect at the hepatic level. In group G4, the values in T1 and T2 were slightly higher, and in group G6, in the case of T1, the AST level was slightly higher than in the case of the Control group. The highest value of AST recorded was found in T2, in group G2, the value being above the reference ones. Within the same group, however, it is observed that in T2, there was a significant reduction in AST with significant differences compared to Control. Aspartate aminotransferase is another liver enzyme that helps produce protein. It catalyses the reductive transfer of amino acids from aspartate to α-ketoglutarate to produce oxalo-acetate and glutamate. In addition to the liver, it is also found in other organs (heart, muscles, brain). Normal accepted levels are in the range of 69-111 U/L. Although considered a less specific enzyme for liver damage, AST helps detect hepatocellular necrosis, but may also indicate an abnormality of the heart, muscles, brain, or kidneys (17, 35, 38). Consequently, the AST/ALT ratio can be used to differentiate liver damage from other organ damage (4, 31).

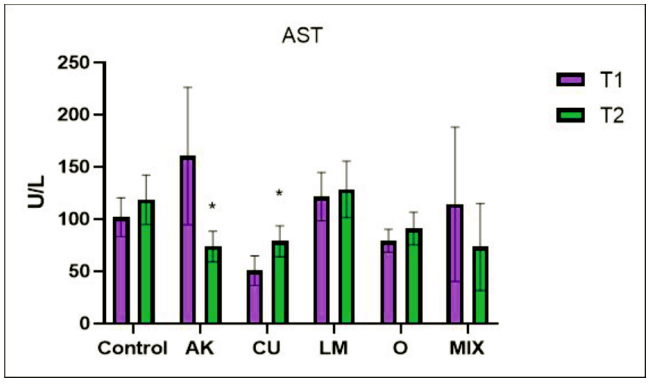

Fig. 5. Serum values
Aspartate aminotransferase (AST)
Comparative to C: $^{*}p<0.05$

Figure 6 shows the statistical analysis of the evolution of the GLDH. In the case of the Control group, we find normal references, both in T1 and T2. We also observe that in all groups, in T1, there were relatively low values, with no statistically significant differences between lots, except for lot G4, where the differences were very significant. In T2, in the case of lots G2, G3, and G6, the values were much higher, compared to the Control group. Comparing the GLDH values with the ALT and AST values in T1 with T2, higher values were observed in T2, in the case of groups G2, G3, and G6.

In T2, in the case of the G6 group, the GLDH value was even 5 times higher than in the case of the Control group, this aspect may indicate that in the medium term (over 7 days), the administration of the associated biological supplements can have a stressful potential at the level of liver metabolism. Also, correlating the statistical values, we can say that in T1 there are no major increases in the case of the administered molecules, the only exception being found in the case of the group G4, the values being even twice as high.

GLDH activity is more specific to the liver than transaminases and is not substantially affected by skeletal muscle damage (36). So GLDH is an enzyme involved in the oxidative deamination of glutamate, present mainly in the liver and to a small extent in the kidneys. The normal range of GLDH activity in humans is reported to be 7-11 U/L for mice of the BALB/c lineage, and there are no reliable data specifying reference values for the mouse species. What is known, however, is that GLDH activity increases with hepatocellular damage. In rodents, increases in GLDH activity were reported to be of a much greater magnitude and persisted much longer after administration of liver stressors (4,9,32).

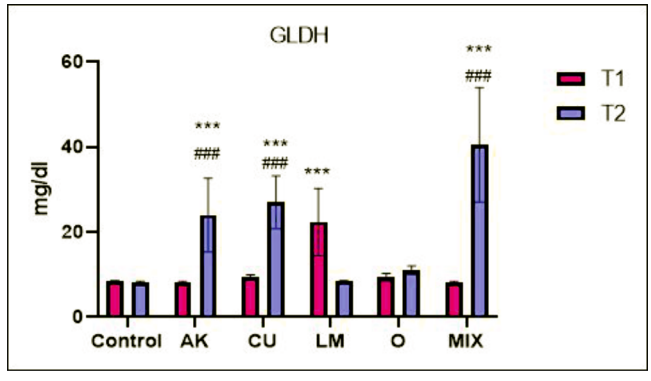

Fig. 6. Serum Glutamate Dehydrogenase (GLDH) values
Comparative to C: $^{***}p<0.001$;
Comparative T1 vs T2: $^{###}p<0.001$

The mechanism of hepatotoxicity due to the administration of NSAIDs, or responsible for hepatic adverse effects, is mainly due to idiosyncratic liver damage and alteration of liver metabolism, which could be caused by enterohepatic recirculation of NSAIDs. NSAID toxicity increases due to the concomitant use of other hepatotoxic drugs, but also in the case of combination with certain medicinal plants (5, 14, 17, 21, 44).

CONCLUSIONS

Phytotherapy with *Angelica keiskei K*., *Curcuma longa,* and royal jelly has been shown to have good hepatoprotective and hypolipidemic activity, suggesting that the administration of the preparations has the potential to reduce liver stress. In this sense, the additional histological investigation of the liver, correlated with the values of liver transaminases, is defining for a complex picture. Next, the investigation of liver transaminases remains the gold standard for determining early signs of microsomal injury. In the present study, it was shown that at 7 and 14 days, the administration of aspirin and omeprazole did not induce liver stress or increased liver enzymes. In general, the ALT and AST levels were within the reference values; in the case of all groups, both in T1 and T2, there was, however, a significant increase in AST, in the case of the group that received *Angelica keiskei* for 7 days (T1). It cannot be confirmed with certainty that higher choles-

terol levels were influenced by the administration of aspirin,omeprazole or biological products. In this case, cholesterol can have a hepatoprotective effect.

REFERENCES

1. *Adnan S., Ahmad M., Janabi A.l.*, (2005), Pharmacological effects of low dose of aspirin on corpus luteum functions in mature cycling female mice. Institute of the Embryo Research and Infertility Treatment, Middle East Fertility Society Journal, 10 (2):150-162
2. *Alberti S., Angelin B., Björkhem I., Diczfalusy U., Feltkamp D., Gustafsson J.A., Pettersson S., Parini P., Schuster G., Rudling M.*, (2001), Hepatic cholesterol metabolism and resistance to dietary cholesterol in LXRbeta-deficient mice. J Clin Invest, 107(5):565-573
3. *Amacher D.E.*, (2002), A toxicologist's guide to biomarkers of hepatic response. Hum Exp Toxicol, 21:253-262
4. *Singh A., Om P., Sharma Tej., Bhat K.,* (2011), Clinical biochemistry of hepatotoxicity. Journal of Clinical Toxicology, S4-001
5. *Björnsson E.*, (2010), Review aricle: drug-induced liver injury in clinical pracice. Aliment Pharmacol Ther, 32:3-13
6. *Botezan S., Baci G.M., Bagameri L., Dezmirean D.S., Pașca C.*, (2023), Current status of the bioactive properties of royal jelly: a comprehensive review with a focus on its anticancer, anti-inflammatory, and antioxidant effects. Molecules, 28(3): 1510
7. *Brind A.M.*, (2007), Drugs that damage the liver. Medicine, 35:26-30
8. *Chang C.Y., Schiano T.D.*, (2007), Review article: drug hepatotoxicity. Aliment Pharmacol Ther, 25 (10):1135-1151
9. *Church R.J., Aubrecht J., Boucher G.G., Eaddy J.S., Kreeger J.M., Schomaker S.J., Watkins P.B.*, (2020), Glutamate dehydrogenase as a biomarker for mitotoxicity; insights from furosemide hepatotoxicity in the mouse. PLoS One, 15(10):e0240562
10. *Collazo N., Carpena M., Nuñez-Estevez B., Otero P., Prieto M.A, Simal-Gandara J.*, (2021), Health promoting properties of bee royal jelly: food of the queens. Nutrients, 13:543
11. *Coman C., Dinu A., Vlase E.*, (2018), HematologicaL and plasma biochemical values in four specific pathogen-free mice strains produced in the animal facility at Cantacuzino Institute, Bucharest. Scientific Works. Series C. Veterinary Medicine, LXIV (1):115-122
12. *Cristina R.T.,Mohamed E.A.,Tulcan C., Dumitrescu E., Muselin F., Orășan S.A., Mateoc-Sirb T., Vlad D.,* (2024), The oxidative stress markers' protective influence of sea buckthorn and grape extracts in atorvastatin-treated hyperlipidemic rats. Nutrients, 16:1954
13. *Cristina R.T., Teusdea V.*, (2008), Ghid de farmacie și terapeutică veterinară, (Ed.) Brumar, Timisoara, Romania
14. *Davies N.M., Saleh J.Y., Skjodt N.M.*, (2000), Detection and prevention of NSAID-induced enteropathy. J Pharm Pharm Sci, 3:137-155
15. *Day C.P., James O.F.,* (1998), Steatohepatitis: A tale of two "hits"? Gastroenterology, 114: 842-845
16. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the Protection of Animals Used for Scientific Purposes. Available at: http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:en:PDF (Accessed July 20, 2024)
17. Drug Safety Update (2009). Volume 2, Issue 7.
18. *Dufour D.R., Gretch D.R., Koff R.S., Lott J.A., Nolte F.S.,* (2000), Diagnosis and monitoring of hepatic injury: I. Performance characteristics of laboratory tests. Clin Chem, 46:2027-2049, 2050-2068
19. *Gilman E.A., Cheng K.K., Langman M.J.,* (2000), Effect of anti-inflammatory drugs on overall risk of common cancer: case-control study in general practice research database. BMJ, 320:1642-1646
20. *Idehen I.C., Aigbiremolen E., Airhomwanbor K., Bankole J.K.,Dic-Ijiewere E., Ehimara R., Okparaku S., Osarobo E.*, (2018). Histopathological effect of varying dose of acetylsalicylic acid (aspirin) on liver of adult wistar rats. Journal of Biotechnology and Biomedicine, 1(2018):028-033
21. *Itoh S., Itabashi M., Marutani K., Matsuo S., Nishijima T.*, (1995), Liver injuries induced by herbal medicine, syo-saiko-to (xiao-chai-hu-tang). Dig Dis Sci, 40(8):1845-1848
22. *Kamakura M.*, (2011), Royalactin induces queen differentiation in honeybees. Nature, 473(7348): 478-483
23. *Kanbur M., Atasever A., Beyaz L., Eraslan G., Liman B.C., Silici S.,* (2009), The effects of royal jelly on liver damage induced by paracetamol in mice. Exp Toxicol Pathol, 61:123-132
24. *Kunugi H., Mohammed A.*, (2019), A. Royal jelly and its components promote healthy aging and longevity: from animal models to humans. Int J Mol Sci, 20(19):4662
25. *Langman G., Hall P.M., Todd G.*, (2001), Role of non-alcoholic steatohepatitis in methotrexate-induced liver injury. J Gastroenterol Hepatol, 16: 1395-1401
26. *Lars O., Carlsson E., Lindberg P.*, (2003), A Proton-pump Inhibitor Expedition: The Case Histories of Omeprazole and Esomeprazole. Nat Rev Drug Discov, 2(2):132-139
27. *Luigi D.L., Laura G., Francesco R.*, (2001), Aspirin, exercise and pituitary hormones. Official Journal of the Am Col Sports Med, 33:2029-2035
28. *Manov I., Frumin I., Iancu T.C., Motanis H.*, (2006), Hepatotoxicity of antiinflammatory and analgesic drugs: Ultrastructural aspects. Acta Pharmacol Sin, 27:259-272
29. *Alves-Bezerra M., Cohen D.E.*, (2017), Triglyceride metabolism in the liver. Compr Physiol, 8(1):1-8
30. *Mitchell C., Garcia-Rodriguez L.A., Landolfi R.*, (1973), Low-dose aspirin for the prevention of atherothrombosis. N Engl J Med, 3:2373-2383

31. *Nathwani R.A., Kaplowitz N., Pais S., Reynolds T.B.*, (2005), Serum alanine aminotransferase in skeletal muscle diseases. Hepatology, 41:380-382
32. *National Research Council (NRC),* (2011), Guide for the Care and Use of Laboratory Animals, 8th ed., (Ed.) The National Academies Press, Washington, D.C., USA
33. *Navarro V.J., Senior J.R.*, (2006), Drug-related hepatotoxicity. N Engl J Med, 354:731-739
34. *O'Brien P.J., Kramer K., Polley S.R. Slaughter M.R.*, (2002), Advantages of glutamate dehydrogenase as a blood biomarker of acute hepatic injury in rats. Lab Anim, 36:313-321
35. *O'Connor N., Dargan P.I., Jones A.L.*, (2003), Hepatocellular damage from non-steroidal anti-inflammatory drugs. QJM, 96:787-791
36. *Oh H.A., Lee H., Lim Y., Kwon O., Jung B.H., Kim D., Kim J.Y., Park S.Y,.* (2019), Analysis of plasma metabolic profiling and evaluation of the effect of the intake of *Angelica keiskei* using metabolomics and lipidomics. J Ethnopharmacol, 28(243): 112058
37. *Oncel S., Basson M.D., Gupta R., Wang Q.*, (2021), ZINC40099027 promotes gastric mucosal repair in ongoing aspirin-associated gastric injury by activating focal adhesion kinase. Cells, 10(4):908
38. *Ozer J., Bailey W., Ratner M., Schomaker S., Shaw M.*, (2008), The current state of serum biomarkers of hepatotoxicity. Toxicology, 245:194-205
39. *Piroth L.*, (2005), Liver steatosis in HIV-infected patients. AIDS Rev, 7:197-209
40. *Reuben A.*, (2004),Hy's law.Hepatol, 39(2):574-578
41. *Stohs S.J., Harry G.P., Luke R.B., Jin J., Oliver C., Sidhartha D.R.*, (2020), Highly bioavailable forms of curcumin and promising avenues for curcumin-based research and application: a review. Molecules, 25(6):1397
42. *Sri R.H., Balakrishnan S.J., Joseph J.H.M., Kandasamy M., Selvaraj D.B., Vergil-Andrews J.F.*, (2022), Omeprazole treatment manifests anxiolytic effects in a cysteamine hydrochloride induced mouse model of gastrointestinal disorder. Heliyon, 8(6):e09787
43. *Sunil N., Devashree N., Mridul A., Pradeep K.S., Sparsh N., Sohael K.*, (2015), Hepatotoxicity and changes in liver enzymes due to use of non-steroidal ani inlammatory drugs (NSAIDs) in non-traumatic musculoskeletal painful disorders. Molecular Enzymology and Drug Targets, 1(2):3
44. *Tanaka S., Ikeda S., Ishiguro M., Kagawa T., Kozai M., Nakahashi O., Masuda M., Takeda E., Taketani Y., Yamamoto H.,* (2013), Dietary phosphate restriction induces hepatic lipid accumulation through dysregulation of cholesterol metabolism in mice. Nutr Res, 33(7):586-593
45. *Vasconcellos R., Alvarenga E.C., Lima S.S., Parreira R.C., Resende R.R.*, (2016), Exploring the cell signalling in hepatocyte differentiation. Cell Signal, 28:1773-1788
46. *Wahyuni I., Aulifa D.L., Levita J., Rosdianto A.M.*, (2024), The pharmacology activities of Angelica Keiskei Koidzumi and its efficacy and safety in humans. Heliyon. 13;10(2):e24119
47. Wylly R.G.N., Pedraza-Chaverrí J., (2014), Protective effect of curcumin against heavy metals-induced liver damage. Food Chem Toxicol, 69:182-201
48. *Yu A.S., Keeffe E.B.*, (2002), Nonalcoholic fatty liver disease. Rev Gastroenterol Disord, 2(1):11-19.