Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2019, 11(1): 12-19

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Hepatoprotective Effect of Spissum Extract from Immature Walnut based on Model of Paracetamol-Induced Acute Liver Injury in Rats

Ye Zalyhina[1*], S Kaidash[1], N Khomiak[1], V Slesarchuk[1], N Lohvynenko[1] and I Selina[2]

[1]Department of General and Clinical Pharmacy, State Institution "Dnipropetrovsk Medical Academy of the Ministry of Health of Ukraine", Vernadskyi St., Dnipro, 49044, Ukraine

[2]Department of Health Protection of Dnepropetrovsk region, Department of Occupational Diseases and Clinical Immunology, Staff Physician of Regional Hematology Center, based on Dnepropetrovsk City Clinical Hospital No 4, Ukraine

---

ABSTRACT

According to the World Health Organization (WHO), more than 2 billion people in the world suffer from hepatopathy. Medicine-induced liver injury presents 12.8 to 14.0% of all adverse reactions to medicinal products.

Paracetamol (latin Paracetamolum) is one of the most commonly used analgesics, which is on the WHO Essential Medicines List. Paracetamol-induced liver toxicity is associated with accumulation of its toxic metabolite N-acetyl-p-benzoquinone imine (NAPQI) that is a free radical enhancing lipid peroxidation, disturbing the energy state and causing death of hepatocytes.

Nowadays, the problem of effective therapy of liver disease has yet to be resolved and it determines the relevance of searching for or creating new effective remedies. Speaking from this perspective, the medicinal plant walnut (Juglans regia L.) attracts attention because it is a promising object for creation of new gastroprotective remedies with hepatoprotective action.

The study of hepatoprotective action of SAAE 30 (spissum aqueous alcoholic extract) was conducted on the model of acute Paracetamol-induced liver injury (Paracetamol Darnitsa Tablets 500 mg No. 10). As a reference substance, the medicinal product Legalon 70 was used.

The results of the study revealed that application of SAAE 30 optimized a potential increase in the activity of antioxidant liver protection, as evidenced by a 3.3 times increase in content of reduced glutathione (RG) with regard to animals with hepatitis. Growth of antioxidant protection affected by SAAE 30 provided inhibition of lipid peroxidation intensity, thus the content of TBA-reactants decreased by 1.7 times ($p < 0.05$).

The investigated extract showed a potential anticytolytic activity: ALT activity decreased significantly by 1.6 and 1.8 times, respectively. Under the influence of SAAE 30, glycogen content returned to normal and reached the same level as in intact animals. Application of SAAE 30 contributed to a 1.4 times reduction of cholesterol content in bile, along with 1.2 times increase of bile acids level and 1.4 times decrease of AP activity.

As affected by SAAE 30, there was a 1.7 times increase of cholesterol-cholate ratio (CCR) with regard to the group of model pathology and a significant increase of bile secretion rate by 1.35.

Conclusion: The stated results show that in the presence of paracetamol-induced toxic hepatitis, SAAE 30 contributes to an increase in antioxidant liver protection, normalizes the glycogen-synthetic, chole-excretion and cholate-forming abilities of the organ and has a positive effect on the regulation of lipid metabolism.

Thus, under the conditions of oxidative stress of the hepatobiliary system, SAAE 30 shows a pronounced hepatoprotective effect, which is equated with the effect of silymarin-containing hepatoprotector Legalon 70.

Keywords: Paracetamol; Hepatitis; Hepatoprotective effect; Spissum extract; Walnut

---

INTRODUCTION

Hepatopathy holds a prominent place among digestive system diseases. According to the World Health Organization (WHO), more than 2 billion people in the world suffer from hepatopathy, that is 100 times higher than the prevalence of HIV-infection; from 500 thousand to 1 million patients are registered annually in the countries of the Commonwealth of Independent States. In Ukraine, the prevalence of hepatopathy increased by 20.1% over the past 10 years [1-5].

Hepatobiliary system dysfunction, especially liver dysfunction, is the most often result of the effect of aggressive compounds. These include the effects of poisons, bacterial and viral damage, free radicals. In addition, the hepatobiliary system can suffer because of hormonal and metabolic disorders, poor nutrition, alcohol, stress, etc. [6].

To date, the most dominant problem is application of numerous medicines for successful treatment of many diseases, which along with high efficiency have a number of side effects, in particular hepatotoxicity. Medicine-induced liver injury presents 12.8 to 14.0% of all adverse reactions to medicinal products. In contrast to the lungs and kidneys, which also suffer from toxic effects of intravenous and oral medicines, hepatopathy in most cases is caused by enteral administration, due to the peculiarities of blood supply and metabolism of medicinal products in liver [7-12].

Paracetamol (latin Paracetamolum) is one of the most commonly used analgesics, which is on the WHO Essential Medicines List. It is widely used as a monotherapy and in combination with other groups of medicines. For example, it is known that when paracetamol is used in combination with weak opioid (such as codeine), its effectiveness rises in about 50% of people, but the number of side effects increases too [13]. Paracetamol-induced liver toxicity is associated with accumulation of its toxic metabolite N-acetyl-p-benzoquinone imine (NAPQI) that is a free radical enhancing lipid peroxidation, disturbing the energy state and causing death of hepatocytes [14-19]. Nowadays, Acetaminophen (APAP) is a major cause of acute liver failure in the western world [20-21].

Despite a considerable progress of modern hepatology and a rather wide range of hepatoprotective agents, there is an increase in various damage of the biliary tract and a tendency to chronicity and prolonged, lingering course, therefore the problem of effective therapy of liver diseases remains unresolved. It determines the relevance of searching for or creating new effective remedies. Speaking from this perspective, the medicinal plant walnut (Juglans regia L.) attracts attention, since it has long been used in folk medicine as an effective remedy with gastroprotective, antioxidant, sugar-lowering, antiproliferative, antimicrobial, anti-inflammatory, reparative, astringent, hemostatic, antiproliferative action [22-26]. Based on the above, walnut is a promising object for creation of new gastroprotective remedies with hepatoprotective action.

The purpose of the research is to study hepatoprotective activity of a spissum aqueous alcoholic extract (ethyl alcohol 30%) from immature walnut in paracetamol-induced experimental hepatitis in rats.

EXPERIMENTAL MATERIALS AND METHODS

The study of hepatoprotective action of SAAE 30 was conducted on the model of acute paracetamol-induced liver injury (Paracetamol Darnitsa Tablets 500 mg No. 10).

24 white non-pedigree male rats weighing 200-220 g were used, which were kept under standard vivarium conditions.

Acute toxic hepatitis was caused by a single oral administration of paracetamol at a dose of 1250 g/100 g of body weight of animals in the form of a suspension on 2% starch paste for 2 days [27]. The animals were divided into 4 groups of 8 animals: the first one - intact control; the second one was a group of model pathology, where only paracetamol suspension was administered to rats. The animals of the third group, against toxin administration, received SAAE 30 intragastrically in arbitrary effective dose of 25 mg/kg established in accordance with antiulcerogenic action. Animals of the fourth group received the reference substance - silymarin-containing antioxidative hepatoprotector Legalon 70 (MADAUS GmbH) at a dose of 30 mg/kg (ED30). SAAE 30 and the reference substance were administered daily in preventive mode for 5 days and on the days of paracetamol administration - 1 hour before and 2 hours after its administration. 20 hours after the last paracetamol administration in animals, the processes of bile secretion and biliation were studied in the animals, thereafter the animals were decapitated, blood was taken and liver homogenate was prepared for biochemical studies.

In order to describe the effect of SAAE 30 on lipid peroxidation/antioxidant system, the intensity of the lipid peroxidation in the liver was measured by the level of TBA-reactants in the organ homogenate [28]. Determination of the RG level in the liver homogenate [29-30] allowed characterizing the functional state of the antioxidant system of the animal organism. The activity of cytolytic processes was determined by the level of ALT cytolysis marker in the blood serum [31]. In order to evaluate the activity of biligenic and biliary organ function, the rate of bile secretion, content of bile acids and cholesterol in bile were determined [32]. Severity of cholestasis was evaluated by AP activity, and the state of metabolic processes- by glycogen level in the liver, glucose level, total lipids, cholesterol and total protein in the blood serum [33]. The liver weight index (LWI) was also evaluated, which characterizes the intensity of the inflammatory process in the organ [34].

RESULTS AND DISCUSSION

According to the results of previous studies, the ability of SAAE 30 to suppress the intensity of lipid peroxidation and activate antioxidant protection of rats in acute alcohol-prednisolone ulcer associated with NSIAD-induced gastropathies was determined [35-37]. That is why it was practical to assess the effect of SAAE 30 on hepatibiliar system condition under the circumstances of acute oxidative stress of the liver caused by paracetamol administration, the pathologic behaviour whereof includes the decrease of antioxidant protection and escalation of lipid peroxidation processes against this background [38]. Paracetamol belongs to classical membranotropic toxins. As a result of its metabolism, a highly active N-para-benzoquinonine radical is formed which, firstly, binds to endogenous reduced glutathione and causes its depletion, and, secondly, induces free radical oxidation processes by interacting with the structural components of the membranes and leading to a damage to their integrity and function, and the organ as a whole [38].

Paracetamol administration led to the development of pathological disorders in the liver, as evidenced by strengthening of lipid peroxidation processes and decrease in antioxidant system activity of the body. The content of TBA-reactants in the liver homogenate in animals from the group of model pathology increased by 1.4 times (p

<0,05), and the level of reduced glutathione decreased by 3 times (p <0,05) as compared to indicators of intact animals (Table 1). It is against this background that activation of cytolytic processes was observed, as evidenced by 2,6 times increase (p<0,05) of ALT activity in the blood serum of animals from the group of model pathology. Damage to the structure of cell membranes and the activity of membrane-bound enzymes led to a decrease in the synthetic function of the organ, as evidenced by the tendency to reduction of the glycogen content by 1,2 times (Table 1).

A potential increase of LWI by 1.9 times in the group of model pathology indicates the development of alternative processes and oedema of the organ as a result of increased lipid peroxidation processes (Table 1).

Table 1. Effect of SAAE 30 on the liver condition indicators in the presence of acute paracetamol-induced hepatitis in rats, n=8

Test conditions	LWI, %	ALT, mmol/l	TBA-radicals of liver, mmol/g	RG in liver, µmol/g	Liver glycogen, mg/g
Intact control	2,88 ± 0,13	1,31 ± 0,15	76,28 ± 7,41	2,98 ± 0,16	25,40 ± 2,45
Model pathology	5,68 ± 0,11*	3,35 ± 0,21*	106,62 ± 9,78*	0,94 ± 0,20*	21,75 ± 2,39T*
SAAE 30, 25 mg/kg	4,50 ± 0,18*/**	2,06 ± 0,06*/**	62,82 ± 4,71**	3,12 ± 0,88**	24,15 ± 2,61**
Legalon 70, 30 mg/kg	4,29 ± 0,16*/**	1,89 ± 0,11*/**	55,61 ± 17,66*	4,00 ± 0,42**	24,21 ± 1,91**

Notes:

*Statistical deviations to intact control, p<0.05;

**Statistical deviations to control pathology, p<0.05;

*T–deviations tending to certainty 0.1<p<0.05;

n–Number of animals in the group

In connection with activation of lipid peroxidation processes, the most peculiar function of the liver - biligenic and biliary – were impaired in the group of model pathology (Table 2).

Table 2. Effect of SAAE 30 on exocrine function of the liver in the presence of acute paracetamol-induced hepatitis in rats, n=6

Test conditions	Bile secretion rate, mg/min/100 g	Bile acid mg/100	Bile cholesterol, mg/100	CCR	AP U/l
Intact control	4,91±0,45	842,3±36,3	49,60±3,15	17,00	120,03±12,4
Model pathology	2,73±0,39*	538,2±11,3*	90,08±10,08*	6,00	190,85±14,2*
SAAE 30, 25 mg/kg	3,69±0,18**	670,1±18,3**	65,43±5,37**	10,24	140,39±8,9**
Legalon 70, 30 mg/kg	4,46±0,38**	744,3±28,2**	55,88±5,13**	13,32	143,67±9,8**

Notes:

*Statistical deviations to intact control, p<0,05;

**Statistical deviations to control pathology, p<0,05;

n– Number of animals in the group.

In response to a decrease of bile secretion rate by 1.8 times ($p < 0.05$), there was a 1.6 times reduction of the bile acid content ($p < 0.05$) and an increase of AP activity by 59% ($p < 0.05$) in animals with hepatitis with regard to intact control, the fact whereof points at the development of cholestasis syndrome. CCR also decreased, as the cholesterol content in the bile of animals from the group of model pathology increased by 1.8 times against a significant reduction of the bile acid concentration.

Acute paracetamol intoxication did not lead to significant impairment of metabolic processes. Glucose and total protein content was the same as in intact animals. There was a slight increase in the level of total lipids and cholesterol by 1.2 times. The foregoing indicates that under acute two-day intoxication with paracetamol, there is no disturbance of metabolic and, in particular, protein synthesis processes (Table 3).

Table 3. Effect of SAAE 30 on lipid and protein metabolism in the presence of acute paracetamol-induced hepatitis in rats, n=8

Test conditions	Glucose mmol/L	Total lipids, g/L	Cholesterol, mmol/ L	Total protein, g/L
Intact control	5,5 ± 0,21	3,15 ± 0,26	1,25 ± 0,28	80,5 ± 1,6
Control pathology	5,6 ± 0,13	3,69 ± 0,13[1*]	1,51 ± 0,17[1*]	78,3 ± 2,1[*]
Pathology + SAAE 30, 25 mg/kg	5,57 ± 0,21	3,14 ± 0,14[**]	1,28 ± 0,14[**]	79,8 ± 1,5[**]
Pathology + Legalon 70, 30 mg/kg	5,41 ± 0,18	3,38 ± 0,08	1,27 ± 0,16[**]	82,3 ± 1,2[**]

Notes:

[*]Statistical deviations to intact control, $p<0,05$;

[**]Statistical deviations to control pathology, $p<0,05$;

n– Number of animals in the group.

Application of SAAE 30 optimized a potential increase in the activity of antioxidant liver protection, as evidenced by a 3.3 times increase in content of reduced glutathione with regard to animals with hepatitis (Table 1). Growth of antioxidant protection affected by SAAE 30 provided inhibition of lipid peroxidation intensity, thus the content of TBA-reactants decreased by 1.7 times ($p < 0.05$). Administration of the reference substance silymarin-containing hepatoprotector Legalon 70 also contributed to an increase of RG content by 4,2 times ($p<0,05$) and reduction of TBA-reactants by 1,9 times ($p<0,05$) (Table 2).

Increase of the content of TBC-reactants and decrease of reduced glutathione level in the liver homogenate in animals from the group of model pathology points at a failure of antioxidant protection of the organ and, as a result of the toxic effect of paracetamol metabolites, there is an increase of lipid peroxidation, which leads to an increase in cytolytic processes. Glycogen-synthetic function of the body did not suffer significant changes in the presence of pathology.

The investigated extract and reference substance revealed a potentially anticytolytic activity: ALT activity statistically decreased by 1.6 and 1.8 times, respectively. Under the influence of SAAE 30 and Legallon 70, glycogen content returned to normal and reached the same level as in intact animals. Reduction of LWI also indicates normalization of the liver function under the influence of the investigated products: LWI rates were significantly lower than in the group of animals with hepatitis, but did not reach the level of the intact animals (Table 1).

Application of SAAE 30 contributed to a 1.4 times reduction of cholesterol content in bile, along with 1.2 time's increase of bile acids level and 1.4 times decrease of AP activity, indicating improvement of cholate synthetic function of the liver and reduction of cholestasis against extract administration. 1.7 times increase of CCR with regard to the group of model pathology shows a reduction of lithogenic properties of bile, which also reflects the restoration of the process of cholate formation from cholesterol. The reference substance worked at the level of the studied SAAE 30 (Table 2).

As affected by SAAE 30, there was a statistical increase of bile secretion rate by 1.35 and 1.6 times, respectively, indicating an improvement in bile-excretion function of the organ under investigated medicines. The reference substance increased this index 1.2 times more pointedly than SAAE 30 did, but these differences were not statistical (Table 2).

The analysis of the indicators describing the condition of metabolic processes in the animal organism, caused by the functional state of the liver, showed that under the influence of SAAE 30 there was a pronounced statistical normalization of the content of total lipids and cholesterol in the blood serum of animals. SAAE 30 did not affect the content of glucose and total protein (Table 3). Legalon 70 showed an effect similar to that of SAAE 30 in regulating metabolic processes in the liver.

CONCLUSION

The stated results show that in the presence of paracetamol-induced toxic hepatitis, SAAE 30 contributes to an increase in antioxidant liver protection, normalizes the glycogen-synthetic, chole-excretion and cholate-forming abilities of the organ and has a positive effect on the regulation of lipid metabolism.

Thus, under the conditions of oxidative stress of the hepatobiliary system, SAAE 30 shows a pronounced hepatoprotective effect, which is equated with the effect of silymarin-containing hepatoprotector Legalon 70.

Obtained results prove a promising outlook of application of SAAE 30 as a hepatoprotector, as well as its effectiveness in combined pathologies of gastrointestinal tract: stomach ulcer and hepatitis.

REFERENCES

[1] IG Bakulin; Sandler SOUTH. *Consilium medicum.* **2010,** 2, 22–25.

[2] GA Pogotova. *Visnyk Prob Biolog Med*. **2015,** 1 (117), 16–27.

[3] VT Ivashkin. *Moscow: Publ. M-Vesti House.* **2005,** 536.

[4] S Yuskeev. *Bulletin Med Internet conferences.* **2013,** 3 (3), 805-806.

[5] TME Davis; KE Peters; DG Bruce; WA Davis. *J Clin Endocrinol Metabol*. **2012,** 97 (5), 1581–1588.

[6] TD Zvyagintsev; SV Glushchenko. *Health of Ukraine*. **2010,** 1, 46-47.

[7] KO Kalko; O Mishchenko; LV Derymedvid; M Zolotaikina; TM Gontova; VV Mashtaler; SA Kutsenko. *Res J Pharm Technol*. **2018,** 11, 4393-4396.

[8] KO Kal'ko; SM Drogovoz; A Pozdnyakova; NV Zakharko. *Eksperimental'naya i Klinicheskaya Farmakologiya*. **2016,** 79 (7), 25-28.

[9] ND Bunyatyan; EA Kal'ko; SM Drogovoz ; AV Kononenko. *Bulletin Exp Biol Med*. **2018,** 165 (6), 754-757.

[10] M Robles; RJ Andrade. *Rev Esp Quimioter.* **2008,** 21 (4), 224-233.

[11] OI Naboka; SZ Khuari; OY Koshova. *Pharmacol That Likarska Toxicol*. **2015,** 2, 72-77.

[12] LS Bondarev; AA Zaplotnaya. *Bulletin of emergency restorative Med*. **2008,** 9 (2), 226-229.

[13] T Laurence; D Sheena; M Andrew ; JQ Henry. *Cochrane Database Syst Reviews*. **2009,** 1, CD001547.

[14] M Ghonghadze; N Antelava; K Liluashvili; M Okujava; K Pachkoria. *Georgian Med News.* **2017,** 263, 99-105.

[15] NB Voloshina; MF Osipenko; NV Litvinova. *Eksp Klin Gastroenterol.* **2016,** 9, 103-106.

[16] T Nabi; S Nabi; N Rafiq; Shah A. *Saudi J Gastroenterol.* **2017,** 23 (3), 169-175.

[17] P Sarges; JM Steinberg; JH Lewis. *Drug Saf.* **2016,** 39 (9), 801-21.

[18] D Katarey; S Verma. *Clin Med.* **2016,** 16 (Suppl 6), s104-s109.

[19] MD Leise; JJ Poterucha; JA Talwalkar. *Mayo Clin Proc*. **2014,** 89 (1), 95-106.

[20] FF Daly; JS Fountain; L Murray; A Graudins; NA Buckley. *Med J Aust.* **2008,** 188 (5), 296-301.

[21] M Khashab; AJ Tector; PY Kwo. *Curr Gastroenterol Rep.* **2007,** 9 (1), 66-73.

[22] F Toshiyuki ; A Cesarettin; S Fereidoon, editors. Tree Nuts. (Walnut polyphenols: structures and functions). Tree Nuts: Composition, Phytochemicals, and Health effects. Nutraceutical Scence and Technology. Ed. by Fereidoon Shahidi. Boca Raton London New York: CRC Press Taylor & Francis Grup. **2009,** 307.

[23] D Nasiry; AR Khalatbary; H Ahmadvand. *Iran J Basic Med Sci*. **2017,** 20 (11), 1275-1281.

[24] AS Negi; S Luqman; S Srivastava. *Pharm Biol*. **2011,** 49 (6), 669-673.

[25] J Iran; S Rahimzadeh M; S Jahanshahi; S Moein; MR Moein. *Basic Med Sci.* **2014,** 17 (6), 465-469.

[26] S Hosseini; L Jamshidi; S Meharzadi. *Italics: J Ethnopharmacol.* **2014,** 152 (3), 451-456.

[27] OB Stefanov. Doklinichni doslidzhennya l_karskih zobob_v: method. rec. Kyiv: Avitsenna; **2001,** 528 s.

[28] ID Steel, TG Garishvili. *Moscow: Medicine*. **1977,** 46 .

[29] SS Chernadchuk. Guidelines for the implementation of experimental studies on a large special workshop "Methods for assessing the state of oxidative and antioxidant systems of biological objects, Odessa. **2010,** 52.

[30] LM Voronina. A student to a laboratory and a family to occupy a biological logbook. Kharkiv: Basis; **1996,** 432.

[31] S Reed. Handbook of clinical and biochemical studies and laboratory diagnostics. 3rd ed. Moscow: MEDpress Inform; **2009,** 896.

[32] MP The mount. Nevdkladna dopomog with Gostokh otruєnnyakh: navch. posib - 2nd type, special stages. Ternopil: TDMU, Ukrmedkniga. **2005,** 243.

[33] VS Kamyshnikova. Methods of clinical laboratory research. 8th ed. Moscow: MEDpress-inform; **2016,** 736.

[34] TM Potapova; ЄB Zaligina; KV Sokolova; V Slєsarchuk; OA Podpletnya. Gastrocytoprotective power of the Livarsky Roslin in pharmacotherapy of acid-base acidosis ShKT: materi mizhnar. Science.-Pract. conf. "Actual problems of the clinical, theoretical, professional medicine, dentistry and pharmaceuticals"; 2016 app. 8-9; Odes. Odes. **2016,** 149-152.

[35] EV Zalygina; OA Podpletnya. *Pharmacol That Likarska Toxicol*. **2017,** 3 (54), 42-49.

[36] EV Zalygina; EA Podpletnya. *Actual Nutrition of Pharma Med Sci Practice*. **2017,** 10.3 (25), 324-328.

[37] E Yoon. *J Clin Transl Hepatol*. **2016,** 4 (2), 131-142.

[38] https://www.ed.ac.uk/news/2017/paracetamol-study-sheds-light-on-liver-damage