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The Physicochemical Composition of Sea Buckthorn (*Hippophae rhamnoides* L) Oil and Its Treatment Characteristics

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ABSTRACT
Sea buckthorn (*Hippophae rhamnoides* L.) oil is a unique oil source, emphasizing its potential as a dietary and medicinal supplement. Many studies have shown that the whole berry oil of sea buckthorn is rich in saturated and unsaturated fatty acids and fat-soluble vitamins. The oil also had been used in digestive ulcers and cardiovascular disease. The present study has determined the whole berry oil of sea buckthorn physicochemical parameters, such as refractive index, acid value, iodine value and peroxide value, and fatty acid composition. The dominating fatty acids in whole berry oil were oleic, linoleic, and linolenic. Also, the therapeutic properties of whole berry oil and its acute and chronic toxicity were determined. In addition, we carried out the pharmacological effect of the whole berry oil of sea buckthorn on indomethacin-induced gastric ulcers in rats. It has been shown that sea buckthorn oil positively affects the regeneration of stomach tissues in experimental rats in case of stomach ulcers. However, the bacterial mass in the mucous membranes of the stomach in some rats was increased.

Keywords: *Sea buckthorn, Fatty acids, Acute and Chronic toxicity*

1. INTRODUCTION

Sea buckthorn is a thorny shrub in the family *Elaeagnaceae* with narrow, greyish leaves and small, green flowers. It produces orange-yellow berries in the autumn. The botanical definition of a *berry* is a particular type of small fleshy fruit. Sea buckthorn is well adapted to Mongolia's climate, soil, and geographical conditions is exceptionally resistant to drought and cold and is of great importance for medical and environmental protection. The Mongolian sea buckthorn subspecies (*Hippophae rhamnoides* L. ssp mongolica Rous.) is distributed in the Orkhon-Selenge delta, Zavkhan Tes, Uvs Tes, Bokhmoron, Torkhilog Buyant, Khovd, Zavkhan, Borkh, and Bulgan river basins. It is also endemic to the Altai, Tuva, Lake Baikal, Russia, and China's Xinjiang Uygur Autonomous Region.

Sea buckthorn is a natural product with many biologically active compounds and nutritional value. It contains phenolic compounds, secondary metabolites. The main products of sea buckthorn are oil and juice, which contain 2.8-8% of the soft tissue of the fruit and 12-13% of oil from the seeds. The composition of fruits and berries depends on their appearance, colour, taste and aroma, environmental factors, care, irrigation, stage of education and storage conditions. Scientists from the Institute of Chemistry and Chemical Technology (ICCT) of the Mongolian Academy of Sciences have been conducting detailed studies of the composition and biologically active substances of sea buckthorn juice in Mongolia since 1968, and ascorbic acid 70.3-159

mg%, betaine 293-450 mg%, choline 29.5-33.5 mg%, flavon 94.5-220 mg%, catechins 81-243 mg%, anthocyanins 4-20 mg%, and free amino acids 188-200 mg%, and 0.41-0.53% of minerals in fruit juice [1]. Sea buckthorn is used as a raw material for cosmetics due to its high vitamins A, E and carotenoids tocopherols. α-, β-, and γ-tocopherols make up a small percentage of the non-saponifying fraction of sea buckthorn oil.

In traditional Mongolian and Tibetan folk medicine and Western and Eastern folk medicine, the sea buckthorn oil is used for many diseases such as external wounds, burns, frostbite, internal anaemia, stomach ulcers, diabetes, high blood pressure, and various cancers. The seed oil and whole berry oil differ significantly in terms of their content of active ingredients [2]. However, both oils contain a wide range of essential unsaturated fatty acids (UFA), particularly unique palmitoleic acid (C16:1), which is highly valued in cosmetology. Both oils abound in tocopherols, tocotrienols and plant sterols [2]. Unlike seed oil, whole berry oil has a high content of carotenoids [3]. In Mongolia, Russia and China whole berry oil is used topically to treat skin burns [4-6]. Thanks to a high content of carotenoids and tocopherols [7], sea-buckthorn oil can be used to treat burns, frostbites, bedsores and skin damage, e.g. resulting from exposure to sun or X-rays [2, 8]. A previous study revealed the physico-chemical characteristics of pulp oil by enzymatic process [9].

In this study, we determined the physicochemical parameters such as sea buckthorn whole berry oil refractive index, acid value, iodine value and peroxide value, fatty acid composition and studied therapeutic properties.

2. EXPERIMENTAL

2.1. Sample Preparation

We used a mixture of four types of sea buckthorn cultivars, such as Chuiskaya, Zhivko, Elizabeth and Rubin, obtained from three areas in Mongolia included that the farmlands of "Uvs Khuns" LLC, Uvs province, the Food and Agricultural Research Institute in Ulaangom soum, Uvs province and "Kharkhorin Jims" LLC, Kharkhorin soum, Uvurkhangai province. The berries were frozen until use in the study. The juice was first extracted from berries on a blander press, and then wet pulp was dried at room temperature avoid to direct sunlight. The dried sample with a mixture of seeds and pulp-flakes were separated using a vibratory screen separator. The seed and peel were collected, and whole berry oil was used for further physico-chemical and therapeutic experiments. The oil was extracted by Soxhlet with hexane for 6 h, the most commonly used method to determine total oil content. Sea buckthorn whole berry oils proximate composition analyses were conducted in the Laboratory of Food Chemistry of ICCT. The study of treatment characteristics was conducted in the laboratory of the Research Center of the Institute of Traditional Medicine and Technology.

2.2. Determination of Physico-Chemical Parameters and Fatty Acid Composition

2.2.1. Refractive Index:

The refractive index of the liquid samples was determined by a refractometer. To determine solution concentrations, take a dropping sample using transfer pipettes, and place the drop on the cleaned prism top. Then move the dark and light border after taking the reading on the upper scale [10].

2.2.2. Acid Value

Acid value was determined by titrating an oil sample with an alkaline solution in the presence of a phenolphthalein indicator. Dissolve the oil in a mixture of alcohol, diethyl ether, or benzene. Dissolve the soap formed during the titration and add alcohol to dissolve the oil to prevent hydrolysis [10].

2.2.3. Iodine Value

The iodine content of sea buckthorn oil samples was determined by MNS ISO 3961: 99 "Determination of iodine content in animal and vegetable fats".

2.2.4. Peroxide Value

To determine peroxide value, a 1 g oil sample was weighed (accuracy of 0.0001 g) in weighing bottles, added a mixture of acetic acid and chloroform with a 1:1 ratio, and added 1 mL of 50% potassium iodate to make a homogeneous solution. Keep the mixture of solution for 20 min in a dark place and added 50 mL of distilled water. Subsequently, titrate the liberated iodine with 0.01 N thiosulfate solution to an equivalence point. Add 5-6 drops of starch solution as an indicator and titrate until colorless [10].

2.3. Determination of Fatty Acid Composition

2.3.1. Methylation of Fatty acids

There are several ways to saponify and esterify fatty acids, and we experimented with two methods: direct saponification using petroleum ether to extract and KOH alcohol solution, and the esterification method using Christian reagent [10]. Dissolve the methyl esters of the obtained fatty acids in hexane and determine their properties by thin-layer chromatography and infrared spectra.

2.3.2. Analytical Procedure

At the end of the saponification step, reaction broth might include triglycerides, diglycerides, monoglycerides, and fatty acid sodium salt. Thus, 0.33 mL HCl/CH_3OH of 9.22 M was added to convert soap into free fatty acids before hexane extraction. Gas Chromatography (GC, Thermo Scientific TRACE 1310, TSQ8000) analyzed hexane extraction with 5HT capillary column (15m×0.32mm i.d.×0.32μm film thickness, Zebron Corporation, USA). Conditions of the GC were 5 mL/min carrier gas (nitrogen), 320℃ injection temperature, 320℃ detector temperature, with the column temperature 100℃ initially, heated up to 155℃ (2 min hold) at 10℃/min followed by increasing to 175℃ at 5℃ /min and then increasing to 310℃ (2 min hold) at 30℃/min. Trioleate (Triglyceride, C18:1), Dioleate (Diglyceride, C18:1), Palmitic acid (Free fatty acid, C16:0), Oleic acid (Free fatty acid, C18:1) and FAME mix C16-C20 of GLC-20 from Sigma-Aldrich Corporation, USA were used as standard.

Palmitic acid (Free fatty acid, C16:0), oleic acid (Free fatty acid, C18:1), and FAME mix C16-C20 of GLC-20 and GLC-50 from Sigma-Aldrich was used as standard. As a result, samples after hexane extraction in this step were mainly FAME and possibly some unreacted FFA. Samples in the mixture were quantified in a GC-FID with a BPX-70 capillary column (30m×0.53mm i.d.×0.5μm film thickness, SGE analytical science, Australia) with 5 mL/min N_2 as the carrier gas, 250℃ injector temperature, 250℃ detector temperature and a column temperature increased from 85℃ to 150℃ (4 min hold) at 30℃/min followed by increasing to 170℃ (4 min hold) at 8℃/min.

2.4. Determination of Acute and Chronic Toxicity of Sea Buckthorn whole Berry Oil

The toxicity study was conducted with the approved methodology after having the approval decision of the meeting of the Ethics Review Committee of the Mongolian National University of Medical Sciences on March 18, 2018, under the guidelines of the "Biomedical Ethics for Animal Experiments".

2.5. Acute Toxicity Study

The whole berry oil was measured by the accelerated method of V.B.Prozorovsky [11]. The sample was injected into the abdomen of 16 laboratory white mice, each weighing 29-35 g, and the lethal dose of whole berry oil was determined within 72 h and evaluated by a method of K.K. Sidorov [12]. The classification of acute toxicity by Sidorov is shown in Table 1.

Table 1. Classification of toxicity assessment

Characteristic		LD_{50} mg/kg	
		Inject under the dermal	Inject into the abdomen
1	Extremely toxic	0.3	0.2
2	High toxic	0.4-15	0.3-10
3	Moderate toxic	16-150	11-100
4	Low toxic	151-1500	101-1000
5	Relatively harmless	>4500	>3000

2.6. Chronic Toxicity Study

Forty health Wistar rats, each weighing 180 to 220 grams, bred in the laboratory of the Research Center of the Institute of Traditional Medicinal and Technology (ITMT) used were in this study. All rats were randomly divided into four groups for 3 treatment groups (I, II, III) and one control group (IV). Rats in the treatment groups were given orally at 400, 800, and 1600 mg/kg of whole berry oil for 8 weeks. The control group received an equal volume of water for the same period. 8 weeks later, the experimental rats' body weight was measured, and collected blood samples were; the heart was injected with ketamine hydrochloride, and a sample was collected in a heparin-free and EDTA-containing tube. Blood samples are centrifuged at 3000 rpm for 15 min to separate the plasma for the blood test and biochemical analysis. Biochemical analysis was performed using the DIRUI DR-7000D semi-

automatic device. Blood tests were carried out using a fully automatic PE6800-vet machine.

2.7. Determination of the Effect of Sea Buckthorn Whole Berry Oil on Gastric Ulcers

The pathological model of gastric ulcer was performed by a method of Jung-Woo Kang *et al*., using indomethacin [13]. Fifty Wistar rats bred in the laboratory of the Research Center of the ITMT were used for the experiment to determine the effect of the whole berry oil on gastric ulcers. The rats were divided into 3 treatment groups with a dose of 400, 800, 1600 mg/kg whole berry oil, and a control group. A 10 fold magnification biological microscope evaluated the gastric ulcer and injury to calculate the ulcer index and protective activity using the following formula.

0.0 – normal stomach without pathological changes
0.5 – inflammation
1.0 – erosion
1.5 – chronic ulcer
2.0 – peptic ulcer >3 mm and <5 mm
3.0 – peptic ulcer >5 mm

The rat's stomach was immersed in 10% formalin solution for 24 h, and histopathological examination revealed gastric necrosis and necrosis. ELISA (Chromate 4300 microplate reader) measured plasma levels of inflammatory cytokines for TNF-α and IL-6 levels. Cytokine levels were determined after placing the rat blood at room temperature for 2 h and then rotating the serum at 2000 rpm for 20 min. For tissue microstructure analysis, rat tissue and organs samples were solidified in a 10% solution of buffered neutralized formalin for 24 h, washed under running water, soaked in ascending ethyl alcohol, xylene, and paraffin a while, and placed in a Yamato Konki sledge microtome. It was cut to a thickness of 2-5 μm, stained with hematoxylin-eosin, microscopically prepared, and analyzed under a Nikon Eclipse Ci microscope.

2.8. Statistical Analysis

All experiments were repeated three times, and analyses were carried out in duplicate. The biochemical experiments were based on the titration analysis, and deviation was calculated with the MS Excel program. The results were analyzed statistically using the SPSS-23, and in vivo, experimental results were performed using a one-way analysis of variance (ANOVA), paired T-test, and Tukey T-test.

3. RESULTS AND DISCUSSION

3.1. The Results for Physico-Chemical Parameters and Fatty Acid Composition of Sea Buckthorn Whole Berry Oil

The physico-chemical analysis was conducted by using the standard analytical method, and fatty acid compositionswere analyzed chromatographic equipment. The results of the physico-chemical studies of whole berry oil of sea buckthorn are shown in Table 2.

As shown in Table 2, in general, the refractive index is a very stable parameter and can notice the oil's purity. The determined peroxide values were conducted at 1.31 meq H_2O_2/kg, which means the oil is fresh without oxidation. The peroxide value measures the oxidation present in animal and vegetable oils and can indicate flavor and fragrance materials. Peroxide values of fresh oils are less than 10 meq H_2O_2/kg, when the peroxide value is between 30* and 40 meq H_2O_2/kg, a rancid taste is noticeable. In addition, the result of the study showed that the iodine value was relatively similar to other studies [9, 14] with a value of 123.5 mg KOH/g indicating high content of unsaturated fatty acids.

Table 2. Physico-chemical properties of sea buckthorn whole berry oil

Parameters	**Whole berry oil**	***Hippophae salicifolia [14]***	***Hippophae rhamnoides [14]***	**Reported value [9]**
Refractive index	1.47	1.473	1.473	1.476
Acid value, mg KOH/g	16.2	4.66	4.01	6.4-15.0
Iodine value, mg KOH/g	123.5	154.95	150.35	124.0
Peroxide value, meq H_2O_2/kg	1.31	18.30	17.50	5.0

Table 3. The composition of fatty acids of whole berry oil

№	Fatty acids	Content, %
1	**Caprylic acid, C8:0**	0.47
2	**Capric acid, C10:0**	0.04
3	**Lauric acid, C12:0**	0.08
4	**Myristic acid, C14:0**	0.37
5	**Palmitic acid, C16:0**	14.84
6	**Palmitoleic acid, C16:1**	5.198
7	**Stearic acid, C18:0**	5.83
8	**Oleic acid, C18:1**	**26.87**
9	**Linoleic acid,C18:2**	**33.02**
10	**Linolenic acid, C18:3**	**11.89**
11	**Arachidic acid, C20:0**	0.89
12	**Behenic acid, C22:0**	0.16
13	**Erucic acid, C22:1**	0.23
14	**Lignoceric acid, C24:0**	0.11
	Monounsaturated fatty acid, %	44.91
	Polyunsaturated fatty acid, %	32.29
	Saturated fatty acid, %	22.8

According to the composition of the fatty acids, the unsaturated fatty acids such as oleic, linoleic, and linolenic acids were mainly contained in the whole berry oil of sea buckthorn. Therefore, the oil contained total 14 fatty acids, of which 22.8% were saturated fatty acids, and 44.91% were monounsaturated fatty acids. Saturated fatty acids were found to contain 14.84% palmitic acid and 5.83% stearic acid.

Researchers have found that sea buckthorn oil contains a certain amount of omega-3, 6, 7, and 9 fatty acids, essential for the human body. Linoleic acid (18:2, cis 9, 12), the shortest chain of the common omega-6 fatty acids, accounted for 33.02% of the total fat content, which has a significant effect on vitamin F in the human body and protects against UV rays and radiation. Oleic acid also accounts for 26.87%, and although it is synthesized in the human body, more critical for health when taken with food. α-linolenic acid (C18: 3, cis -9, 12, 15) is an essential omega-3 fatty acid that reduces blood cholesterol and triglycerides, provides vascular flexibility, reduces heart disease and stroke, and contain 11.89% of total fatty acids. The percentage of results is consistent with the results studied by Baoru Y *et al*. (6.2-18.1%) [15, 16].

3.2. The Results for Acute and Chronic Toxicity of Sea Buckthorn Whole Berry Oil

3.2.1. Acute Toxicity Study

During the study, high doses of whole berry oil of sea buckthorn increased the experimental mice's heart rate, respiration, and mobility. The following table shows the conditions and results of the study on the acute toxicity of whole berry oil of sea buckthorn

Table 4. The results of acute toxicity of sea buckthorn whole berry oil

№	Weight of test mice, g	Sea buckthorn whole berry oil, 100%		Result
		Dose, mg/kg	Calculated by weight, g/kg	
1	35	3.5	100	Dead
2	35	3.5	100	Dead
3	34	3.0	88	Dead
4	34	3.0	88	Dead
5	33	2.8	84	Alive
6	33	2.8	84	Alive
7	32	2.5	78	Alive
8	32	2.5	78	Alive
9	32	2.2	68	Alive
10	30	2.2	73	Alive
11	29	2.0	68	Alive
12	29	2.0	68	Alive
13	29	1.8	62	Alive
14	29	1.8	62	Alive
15	28	1.6	57	Alive
16	28	1.6	57	Alive

According to K.K.Sidorov, The acute toxicity of whole berry oil of sea buckthorn is LD50=81.5 (71-84) g/kg, which belongs to the non-toxic category. The active dose was determined by I.P.Zapadnyuk [17] as ED=1630 (815-3260) mg/kg.

3.2.2. Chronic Toxicity Study

The experimental rats' general condition, weight, and mucous membranes did not differ significantly from the control group, and no deaths were observed during the experiments. The following table shows the body weights of the experimental rats.

Table 5. Effects of sea buckthorn whole berry oil on rat weight and body mass index

Group		Bodyweight		Solid-organ weight (100 g)				
		Before	After	Liver	Spleen	Lung	Kidney	Heart
Control		188±4.9	207±2.9	3.8±0.1	0.4±0.1	0.6±0.1	0.7±0.1	0.4±0.1
I	400 mg/kg	200±1.3	222±7.8	4.1±0.2	0.4±0.1	0.7±0.1	0.7±0.1	0.4±0.1
II	800 mg/kg	280±3.8	297±6.1	4.0±0.2	0.4±0.1	0.8±0.2	0.7±0.1	0.4±0.1
III	1600 mg/kg	218±2.4	241±7.7	4.3±0.3	0.4±0.1	0.9±0.1*	0.7±0.1	0.4±0.1

Compared to the control p<0.05* One-way RM-ANOVA

Table 6. Effect of sea buckthorn whole berry oil on parameters of general blood test in rats

Group		Erythrocytes 10^{12}/L	Leukocytes 10^9/L	Platelets 10^3/L	Haemoglobin g/dL
Control		8.4±0.1	6.5±0.2	588±18	14.5±0.7
I	400 mg/kg	8.7±0.5	7.3±0.5	536±22	14.6±0.6
II	800 mg/kg	9.1±0.2	9.6±0.8*	594±51	15.1±0.7
III	1600 mg/kg	8.2±0.8	9.1±0.5*	620±18	14.7±0.5

Compared to the control p<0.05* One-way RM-ANOVA

There were no statistically significant changes in the control group rats' body weight or solid-organs weight compared to the three experimental groups. However, the lung volume of animals treated with sea buckthorn oil at a 1600 mg/kg dose was statistically increased (*p=0.05).

Results of general blood and biochemical tests after oral treatment of whole berry oil of sea buckthorn to rats for 60 days are shown in Table 6.

Table 6 shows that long-term use of whole berry oil does not change the quantity and quality of some hematological parameters in the blood. The leukocytes count in the control group were 6.5±0.2 10^9/L, and the leukocytes counts in the experimental rats treated with the oil at 800, and 1600 mg/kg were 35-40% higher than in the control group (*p=0.05).

The results of the study show that each test group is compared with the control group, increased levels of AST (u/L) in the group treated with sea buckthorn whole oil at doses of 800 and 1600 mg/kg, while the total protein content decreased statistical probability in the group treated with a dose of 400 and 800 mg/kg groups (p=0.001).

Table 7. Effect of sea buckthorn whole berry oil on biochemical composition in rat blood

Parameters	Group			
	Control	Experiment		
		Sea buckthorn whole berry oil		
		400 mg/kg	800 mg/kg	1600 mg/kg
AST (u/L)	74.3±4.2	72.2±9.2	62.7±5.0*	62.5±5.4*
ALT (u/L)	125.8±5.2	131.2±27.5	122.7±4.4	122.7±5.1
ALP (u/L)	232.5±10.6	243.1±16.9	237.1±17.8	237.1±17.8
Albumin (g/L)	24.1±0.9	22.1±1.3	24.9±1.4	24.9±1.4
Creatinine (mmol/L)	45.6±1.4	47.4±4.2	43.7±2.3	42.1±3.5
Urea (mmol/L)	4.6±0.1	4.3±0.9	4.9±0.6	4.3±0.8
Triglycerides (mmol/L)	1.6±0.1	1.8±0.1	1.6±0.2	1.6±0.1
LDH (mmol/L)	0.1±0.1	0.2±0.03	NR	NR
Cholesterol (mmol/L)	1.3±0.1	1.0±0.05	1.1±0.04	1.2±0.02
Uric acid (mg/dL)	2.4±0.1	2.1±0.05	2.1±0.01	2.1±0.1
Total protein (g/L)	70.4±2.2	56.6±0.05**	51.8±0.6**	63.9±6.2

Compared to the control p <0.05*, p<0.01** One-way RM-ANOVA, NR: No reference value

3.3. The Result of the Effect of Sea Buckthorn Whole Berry Oil on Gastric Ulcers

Indomethacin inhibits the production of prostaglandins, which are responsible for maintaining the gastrointestinal mucosa by inhibiting the activity of the enzyme cyclooxygenase (COX-1 and COX-2). High doses can cause severe bleeding wounds or perforated wounds. 24 hours later, the animals in the study were slaughtered, their stomachs were removed, and the control group scored 2.93±0.6. According to the study results, 1.1±0.6 points were calculated for 400 mg/kg sea buckthorn oil, 1.7±0.3 points for the 800 mg/kg group, and 1.5±0.5 points for the 1600 mg/kg group (p=0.05). This indicator indicates the degree of injury and trauma that occurred during the pathological model. In other words, high, low, and medium doses of whole berry oil indicate a small amount of ulceration in the control group and may indicate gastric protection.

One indicator specific to the blood during burn wounds is the tumor necrosis factor (TNF-α), identified in the blood.

Table 8. Effects of sea buckthorn whole berry oil on some indicators of indomethacin-induced gastric ulcer inflammation

Group		IL-6 pg/mL	TNF-α pg/mL
Normal		23.6 ± 1.7	40.1 ± 0.8
Control		24.2 ± 2.4	46.5 ± 1.9
I	**400 mg/kg**	24.9 ± 0.8	39.7 ± 0.7*
II	**800 mg/kg**	22.7 ± 0.6	40.8 ± 0.8*
III	**1600 mg/kg**	23.6 ± 0.4	44.1 ± 0.5

Compared to the control p <0.05 * One-way RM-ANOVA

The results showed that IL-6 was not statistically probable in the control and experimental groups in indomethacin-induced gastric ulcers, while TNF-α levels increased by 6% in the control group. At the doses of 400 and 800 mg/kg, the results from the control group decreased by 12.2-14.6%. Figure 1 shows a microstructural analysis of the gastric mucosa of 3 rats selected from each group (100-fold magnification).

According to the results of the pathological examination, the gastric mucosa and lower mucosa of the 1st rat (R1) of the control group were loosened, did not regenerate, and ulcers were formed. In contrast, the mucous membranes of the 2nd (R2) and 3rd (R3) rats were loosened, and inflammatory cell infiltration, necrosis, ulceration, and large bacterial masses were observed between the lower mucosa and the musculature.

The results of the histopathology of the experimental rats showed that no recovery had taken place in the gastric ulcers of the control rats. There was a sizeable bacterial mass in the mucosa and necrosis of the inflammatory cells. Gastric ulcer treatment at 400 and 1600 mg/kg doses of sea buckthorn oil was good, but gastric ulcers at 800 mg/kg doses were not well performed.

All these results show that the use of whole berry oil in gastric ulcers is not suitable. Although the oil has been shown to positively affect the regeneration of gastric ulcers in some experimental rats, it allows bacteria to multiply in the gastric mucosa.

4. CONCLUSION

This study determined the physico-chemical properties and the fatty acid content of the whole berry oil of sea buckthorn. The whole berry oil of sea buckthorn mainly contains oleic acid and linoleic acids. The experimental rats' general condition, weight, and mucous membranes did not differ significantly from the control group, and no deaths were observed during the chronic toxicity experiments. In addition, long-term use of the oil does not change the quantity and quality of some hematological parameters in the blood. According to the results of histopathology examination of rats with gastric ulcer induced by indomethacin, although it has been shown to have a positive effect on the healing of gastric ulcers in some rats, it is unsuitable for use in chronic gastric ulcers because of the ability of bacteria to multiply in the gastric mucosa of some rats. However, according to K.K. Sidorov's classification, the result of accelerated studies of the toxicity of the whole berry oil, LD_{50}, is 81.5 g/kg or non-toxic, and the active dose is ED = 1630 (815-3260) mg/kg. Studies show that the whole berry oil is more suitable for external wounds.

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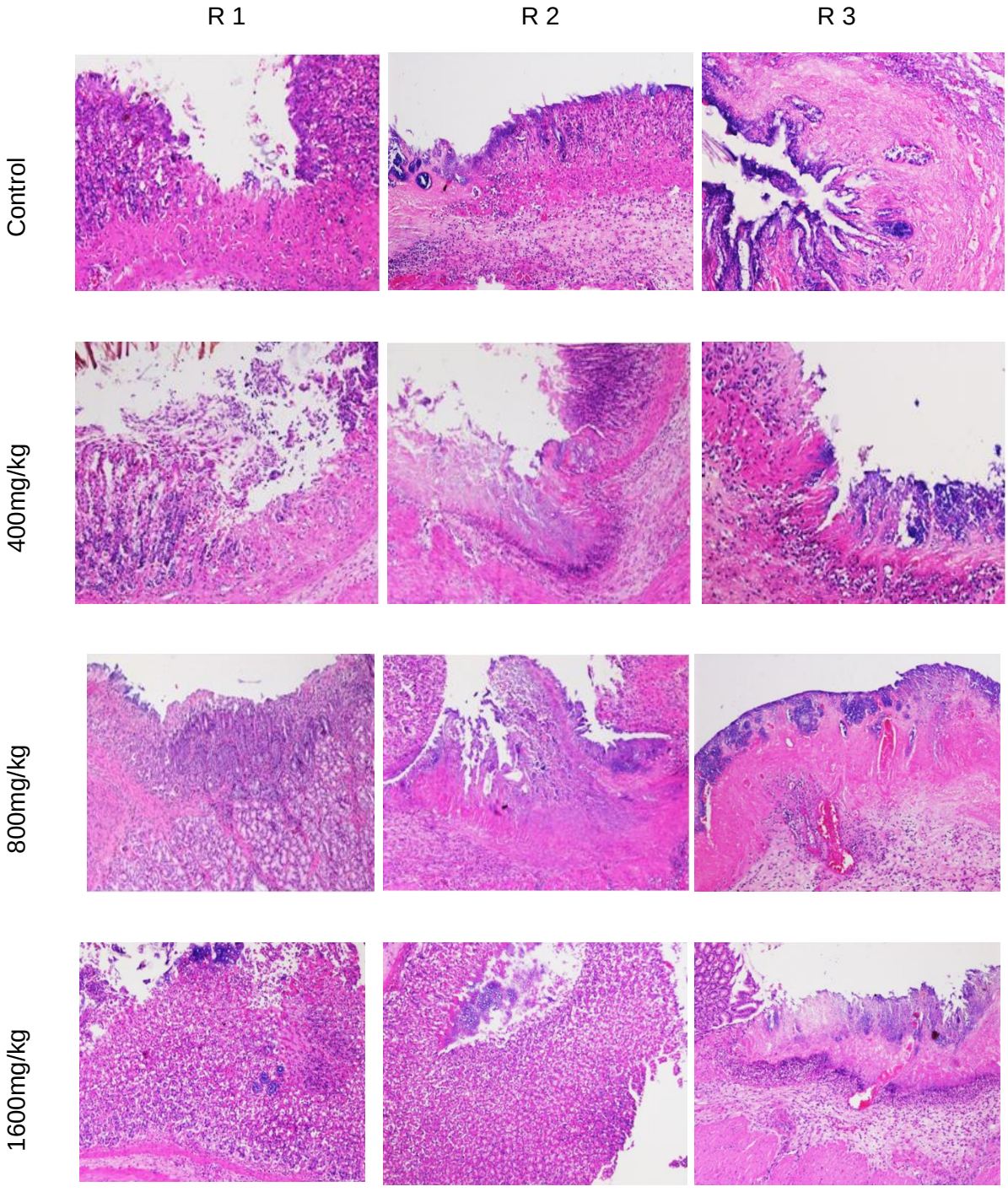

Figure 1. Histopathology (H&E stain100x) of gastric mucosal of rats (one group consisted of 3 rats; R1, R2, R3) in a control group and treated groups with the seabuckthorn oil at a dose of 400 mg/kg, 800 mg/kg, and 1600 mg/kg

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