



Biological activities of new salts of 2-carboxyphenyloxamoylamino acids

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ABSTRACT

New sodium salts of 2-carboxyphenyloxamoylamino acids were evaluated for their anti-inflammatory, analgesic, and ulcerogenic effects. All of the sodium salts showed high to moderate anti-inflammatory activities when compared to celecoxib and possess low ulcer index compared to indomethacin. The most active compounds also showed considerable analgesic activities. In addition, the hepatoprotective effect of a previously synthesized D-glucosamine salts of the 2-carboxyphenyloxamoylamino acids was also evaluated. Two of these salts showed considerable hepatoprotective activities when compared to silymarin. Thus, these findings imply potential pharmacological activities.

Keywords: 2-Carboxyphenyloxamoylamino acid salts, hepatoprotective, antiinflammatory, analgesic, ulcerogenic action.

INTRODUCTION

The oxanilic acids as well as their derivatives such as oxamoylamino acids were found to be associated with several pharmacological activities and excellent biological effects including anti-inflammatory, diuretic, and nootropic effects [1, 2, 3, 4, 5]. In addition, oxamoylamino acid derivatives are known to be useful synthetic intermediates that function as suitable building blocks in the synthesis of different salts. For instance, combination of N-substituted oxamoyl amino acids with biologically active base (athacidine, chloroquine, D-(+)-glucosamine) produced salts with significant antimicrobial, antileishmaniasis, antimalarial, anti-inflammatory and hepatoprotective activities [6, 7, 8, 9, 3].

Thus, the objectives of the present study were to: 1. prepare sodium salts of the previously synthesized 2-carboxyphenyloxamoylamino acids (1-5) and 2-carboxyphenyloxamic acid (A) [3]. 2. To study their anti-inflammatory, analgesic activities and ulcerogenic effects. 3. To study the hepatoprotective activity of their D-(+)-glucosamine salt (6-11).

MATERIALS AND METHODS

Chemistry: The UV-spectra were measured on a UV-160 IPC (SHIMADZU) spectrophotometer using samples (10^{-4} mol) dissolved in ethanol and the IR spectra result were obtained on a IR-FTIR-8300 (SHIMADZU) spectrophotometer in tablets of potassium bromide, at the range of 4000- 400 cm^{-1} . Elemental analyses (C,H,N) were performed on Perkin-Elmer 240 C analyzer (Perkin-Elmer). The melting temperatures were determined on melting point apparatus SMP3 (England). The purity of the targeted products was checked by TLC on Silufon 0.25 mm silica gel 60 F254 (Merk, Germany). All chemicals used for synthesis were purchased from (Sigma-Aldrich).

Pharmacology

Hepatoprotective activity: This study was performed to assess the hepatoprotective activity of the synthesized salts 2-D-(+)- glucosamium-2-carboxyphenyloxamoylamino acids (compounds 6-10,11) in rats against carbon tetrachloride (CCl_4) as hepatotoxin using silymarin as standard hepatoprotective.

Animals: Fifty four male albino rats weighing 175-225g acclimatized and maintained under standard husbandry conditions (Temp. $23 \pm 2^\circ\text{C}$. relative humidity $55 \pm 10\%$ and 12 h light dark cycle) were used for all studies. Animals were allowed to take standard laboratory feed and water. Ethical Committee on Animal Care and Experimentation has approved all animal handling procedures.

Evaluation of hepatoprotective activity: Fifty four male albino rats were divided into nine equal groups; control, silymarin, carbon tetrachloride (CCl_4) and test groups. Group (1) The rats of control group received three doses of 5% acacia mucilage each (1 ml/Kg, per oral) at 12 h intervals (0 h, 12 h and 24 h). Group (2) The rats of silymarin group received three doses of silymarin (25 mg/kg) at 0 h, 12 h and 24 h. CCl_4 (1.25 ml/kg i.p) was administered 30 min. after the first dose of silymarin. Group (3) The rats of CCl_4 group received three doses of vehicle at 12 h intervals and a single dose of CCl_4 (1.25 ml/kg i.p) diluted in liquid paraffin (1:1) 30 min after the administration of the first dose of vehicle. Group (4, 5, 6, 7, 8 and 9) were given the first dose of the tested compounds at a dose of (25 mg/kg p.o) in acacia mucilage at 0 h which was followed by a dose of CCl_4 (1.25 ml/kg p.o) after 30 min., while at 12 h and 24 h the second and third dose of the test compounds (25 mg/kg p.o) were administered. After 36 h of administration of CCl_4 blood was collected and serum was separated and used for determination of biochemical tests.

Assessment of liver function: Assessment of liver function and the results for compounds (6-11) were reported previously [3].

Histopathological examination: Histopathological examination of liver sections for compounds (6-11) was done according to Bancroft and Stevens [10]. Briefly, the liver was dissected out and fixed in 10% buffered neutral formalin solution, dehydrated in gradual ethanol (70-100%), cleared in xylene, and embedded in paraffin. Five-micron thick paraffin sections were prepared and then stained with hematoxylin and eosin dyes (HE), Oil red O for fats and Van Gieson's stain for collagen [10].

Antiinflammatory activity: The selected new salts (12-17) were tested for their anti-inflammatory activity. In addition, the ulcerogenic activity for these compounds was determined against celecoxib and indomethacin as references. The rat hind paw oedema method was applied to determine the anti-inflammatory activity of the test compounds using celecoxib as a standard [11]. Mature albino rats of both sexes weighting 200-250 g were used. The animals were divided into eight equal groups (each

of six). The first group was left as control, while the second group was injected (i.p.) with celecoxib at a dose of 18 mg/kg. The test compounds were injected (i.p.) to the remaining groups at a dose of 18 mg/kg. One hour later, oedema in the right hind paw was induced by injection of 0.1 cm³ of 10% carrageenan. The thickness of the paw was measured using skin caliper 1,2,3 and 4 hours after carrageenan injection to determine the antiinflammatory activity of the test compounds (Table 1).

Analgesic activity evaluation: To evaluate the analgesic activity of the new salts (12-17), the hot plate method of Jacob and Bosovski was used [12]. Mature albino mice of both sexes weighting 20-25gm were classified into eight groups (each of six). The first group was left as control and injected (i.p.) with the solvent (DMSO), whereas the second group was injected (i.p.) with celecoxib at a dose of 1.7 mg/kg. Each of the remaining group was injected (i.p.) with a test compounds (12-17) at a dose of 1.7 mg/kg. Ten minutes later, each mouse was placed in a two litter beaker immersed in a water bath thermostatically controlled at 56°C . The time elapsed till the mouse licks its paw or jumps was considered as the reaction time (defensive reflex time in second) and was taken as a measure of the analgesic effect. Readings were taken at 10,20,30,60,90 and 120 minutes post treatment (Table 2).

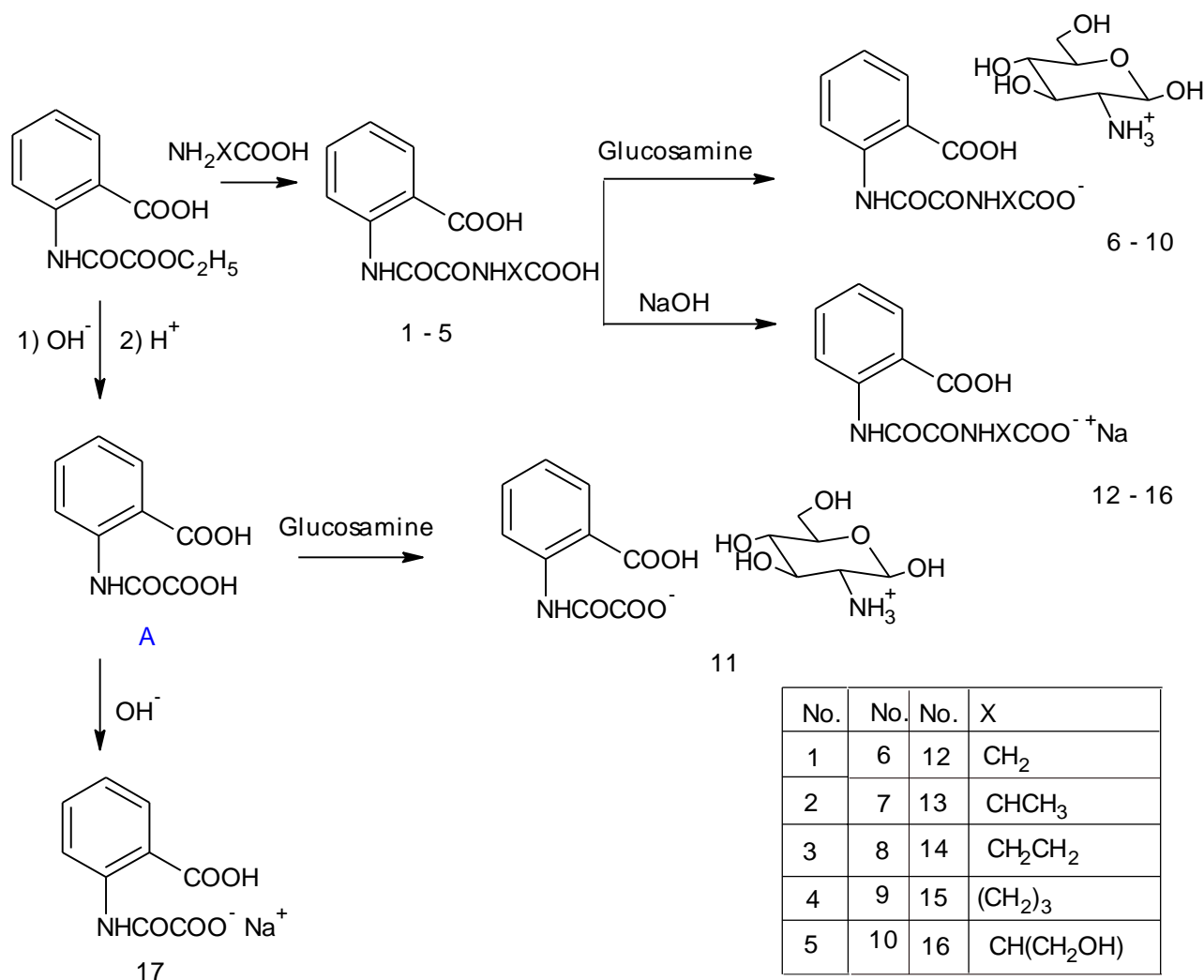
Ulcerogenicity: The ulcerative effect of test compounds (12-17) and celecoxib were investigated relative to the known ulcerogenic drug, indomethacin. Male albino rats weighing 150-200g were fasted for 12 hrs prior to drug administration. Water was given *ad libitum*. The animals were divided into 8 equal groups (each six).

The first group received 1% gum acacia (suspending vehicle) orally once a day and was left as control; whereas, the second group received indomethacin at a dose of 18mg/kg/day orally. The third group received celecoxib at a dose of 18 mg/kg/day orally. The remaining groups received the test compounds at a dose of 18 mg/kg/day orally. The test compounds were administered once a day for three successive days. The animals were killed by an over dosage of ether 6 hrs after the last dose. The stomach was removed, opened along the greater curvature, and examined for ulceration. The number and severity of discrete areas of damage in the glandular mucosa were scored (Table 3). The ulcer score was calculated according to the 1 to 5 scoring system [13]. Stomach ulceration was expressed in term of ulcer index (U.I) [14].

RESULT AND DISCUSSION

Chemistry: The compounds 1-5, 6-10, and 11 were synthesized previously and their structures were established using UV, IR, ¹HNMR and conformed by potentiometric titration (1-5) [3]. The salts 12-

16 were obtained by interaction of an ethanolic solution of 2-carboxyphenyloxamoylaminoacids 1-5 with an equivalent amount of an ethanolic solution of sodium hydroxide, as illustrated in the following scheme:



The obtained salts 12-16 looked white crystalline substances and soluble in water with the formation of neutral solution. The Addition of mineral acid to these salts solution led to precipitation of the initial compounds 2-carboxyphenyloxamoylamino acids. The purity and individuality of the synthesized compounds were checked by measuring the melting point and method of thin layer chromatography eluted with one system of solvents n-butanol-acetic acid-water (4:1:1). The UV spectra of the salts 12-16 were found to be identical to spectra of the previously synthesized compounds 1-5 [3]. The IR-spectrum of the salts 12-16 displayed stretching absorption bands of the all

functional groups corresponding to the proposed structures. These spectra were found to be similar to the IR-spectra of compounds 1-5 except the stretching absorption band of CO (COOH), which was found to be absence in the salt due to the resonance between CO- bonds [3]. Salt 17 was obtained by interaction of an ethanolic solution of 2-carboxyphenyloxamic acid A with equivalent amount of ethanolic solution of sodium hydroxide. The obtained salt 17 looked white crystalline substance and soluble in water with the formation of neutral solution. The addition of mineral acid to this salt solution led to precipitation of the initial compound 2-carboxyphenyloxamic acid.

Biology**Hepatoprotective activity****Group 1:** Control.

Neither gross nor microscopic abnormalities were detected in the livers of these examined groups, which showed the normal lobular architecture, central vein and radiating hepatic cords with well-defined nuclei and cytoplasm of constituent hepatic cells (Fig 1).

Groups (3) only received CCl₄ and **Group 8** received CCl₄ and compound 9.

Microscopic examination showed enlarged liver and soft exaggerated markings in the lobules. Grayish white, yellow or hemorrhagic streaks were also noticed throughout the hepatic surface. Nevertheless, it showed severely congested and over distended gallbladder with dark greenish viscid bile. Microscopic examination, showed toxic hepatitis with distorted architecture and central veins. Multifocal areas of coagulative necrosis and hemorrhage were visualized along the hepatic parenchyma (Fig 2). Severe diffuse hepatic vacuolations and ballooning degeneration were seen (Fig 3).

Group (2): received carbontetrachloride (CCl₄) and Silymarin. The liver was slightly congested with normal size and consistency. The lesions of CCl₄ in the liver were completely disappeared except for congestion of some portal blood vessels and slight vacuolations and ballooning degeneration in some hepatocytes (Fig 4). Few round cells were rarely seen in the portal areas. Almost hepatic lobules showed restored architecture with no evidence of hepatic necrosis.

Groups (4) and (5) both received CCl₄ and compound 6, compound 7, respectively. Lesions similar to that described with Silymarin group were seen and focal vacuolations and apoptosis were scattered through the hepatic tissue (Fig 5). The portal areas showed congestion of the portal veins and lymphocytic infiltration and mild proliferation of the biliary epithelia (Fig 6). Small periportal area of coagulative necrosis was noticed in the liver of one the rat which received compound 7 (Fig 7).

Groups (6), (7) and (9) all received CCl₄ and compound 8, compound 10 and compound 11, respectively. The lesions in the livers of these groups were moderate depending on how compound 8 was received and were severe depending on how compound 11 was how received. Histopathology of liver sections revealed that the normal liver architecture was disturbed by hepatotoxin in carbontetrachloride group; whereas, in the intoxicated liver sections of the rats treated with the D-(+)-glucosamonium 2-carboxy

phenyloxamoylglycinate and D-(+)-glucosamonium 2-carboxyphenyloxamoyl- α -alaninate (compounds 6,7) the normal cellular architecture was retained. Comparison of these results to that obtained using standard Silymarin (group 2), confirmed the significance of hepatoprotective effect of these compounds consistent with previously reported biochemical tests [3]. Compound 8 showed low hepatoprotective activity; whereas, compounds 9, 10 and 11 were found to be inactive. Thus, combination of D(+) glucosamine with 2-carboxyphenyloxamoylamino acid in one molecule is important for hepatoprotective activity and that the amino acid moieties play a crucial rule in determination of hepatoprotective activity among these compounds (6-11). This further implies specifically that the compounds with glycine and α -alanine moieties (compounds 6,7) were the most active hepatoprotective agents.

Anti-inflammatory activity: As shown in table 1, the test compounds (12-16) showed some decrease in paw thickness after one hour and reached its maximum effect after four hours The decreasing order of activity after 4 hr was as followed: Celecoxib (48%) > compound 12 (46%) > compound 14 (37%) > compound 17 (34%) > compound 13 (32%) > compound 16 (8.7 %) > compound 15 (2.2%). These findings are consistent with that of Khaldoon et al., in that N-substituted oxamoylamino acid and their salts exhibit anti-inflammatory activity [3,9]. This showed that the sodium salts of 2-carboxyphenyloxamoylamino acid are promising anti-inflammatory agents especially sodium 2-carboxy phenyloxamoylglycinate, Sodium 2-carboxy phenyloxamoyl- β -alaninate, Sodium 2-carboxy phenyloxamoyloxamininate, Sodium 2-carboxy phenyloxamoyl- α -alaninate (compounds 12,14, 17, 13). It can be further deduced that the type of amino acid moieties determined the effect on anti-inflammatory activities, where compound with glycine moiety was the most active one, followed by compound with β -alanine moiety.

Analgesic activity: Compounds (12-16) which are derivatives of anthranilic acid showed analgesic activities consistent with findings of Joshi JK et al. [15]. Table 2 showed that the compounds 12, 14 exhibited analgesic activity, increasing the time of the defensive reflex to 30.00 sec (130%) for compound 12 (2-carboxyphenyloxamoylglycinate) and to 20.00 sec (54%) for compound 14 (2-carboxyphenyloxamoyl- β -alaninate) vs. 13.00 sec (100%) for the control at peak activity compared with the initial data. The analgesic activity of the most active compound 12 was less than that of

Celecoxib which showed defensive reflex of 38.40 sec (195%) vs. control.

Ulcerogenicity: Result presented in table 3 revealed that compounds 13,15,16,17 produced 60% incidence of gastric ulceration. Compound 12 (sodium 2-carboxyphenyloxamoylglycinate) produced 20% incidence of gastric ulceration, compound 14 (sodium 2-carboxyphenyloxamoyl- β -alaninate) produced 50% incidence of gastric ulceration when compared to indomethacin 100%. It was observed that the most active anti-inflammatory and analgesic compounds (12, 14) produced the less incidence of gastric ulceration, which is worthy of further investigation on their biological activities and toxicities

CONCLUSION

In this study, six new sodium salts of 2-carboxyphenyloxamoylamino acids were prepared and 12 compounds were screened for their hepatoprotective, anti-inflammatory, analgesic and ulcerogenic potential. The D-(+)-glucosamonium 2-carboxyphenyloxamoylglycinate (compounds 6) and D-(+)-glucosamonium 2-carboxyphenyloxamoyl- α -alaninate (compounds 7) exhibited considered hepatoprotective activity.

Sodium 2-carboxy phenyloxamoylglycinate (compounds 12) and sodium 2-carboxy phenyloxamoyl- β -alaninate (compound 14) showed strong anti-inflammatory and analgesic activities with low incidence of gastric ulceration. Among the sodium salt of 2-carboxy phenyloxamoylamino acids, it was established the relationships between the antiinflammatory, analgesic activities and the nature of amino acid moieties. The most active compounds those that contain glycine and β -alanine amino acids moieties. The same compounds also exhibited less incidence of gastric ulceration. Among D-(+)-glucosamonium 2-carboxyphenyloxamoylamino acids (compounds 6-10), it was established that salts containing in their structure glycine and α -alanine amino acids moieties exhibited excellent hepatoprotective activities

Experimental

Chemistry

Sodium 2-carboxyphenyloxamoylglycinate(12): An ethanolic solution of 2.66g (0.01 mol) of 2-carboxyphenyloxamoylglycine was added into 0.40g (0.01 mol) of NaOH solution. The reaction mixture was allowed standing to pH neutral. The formed precipitate (salt) was filtered, washed with anhydrous diethyl ether and dried. Other salts were obtained similarly (13-16).

Yield, 1.8g (72%); mp., 182-184; IR spectrum(γ , cm^{-1}): 3415,3377 γ NH; 2949-2650 γ OH(COOH);

1680 γ CO; 1517CONH; 1600 γ C=C. Anal. Calcd. for $\text{C}_{11}\text{H}_9\text{N}_2\text{O}_6\text{Na}$ (288.1): C, 45.82; H, 3.12; N, 9.72. Found C, 45.62; H, 3.35; N, 9.96.

Sodium 2-carboxyphenyloxamoyl- α -alaninate (13): Yield, 1.95g (68%), mp., 212-214; IR spectrum(γ , cm^{-1}): 3342,3400 γ NH; 2971-2771 γ OH (COOH);1670 γ CO; 1514CONH; 1585 γ C=C. Anal. Calcd. for $\text{C}_{12}\text{H}_{11}\text{N}_2\text{O}_6\text{Na}$ (302.2): C, 47.65; H, 3.63; N, 9.27. Found C, 47.35; H, 3.77; N, 9.65.

Sodium 2-carboxyphenyloxamoyl- β -alaninate (14): Yield, 1.98g (69%); mp., 192-194; IR spectrum(γ , cm^{-1}): 3300,3215 γ NH; 2959-2528 γ OH (COOH); 1678 γ CO; 1514 CONH; 1587 γ C=C. Anal. Calcd. for $\text{C}_{12}\text{H}_{11}\text{N}_2\text{O}_6\text{Na}$ (302.2): C, 47.65; H, 3.63; N, 9.27. Found C, 47.45; H, 3.50; N, 9.55.

Sodium 2-carboxyphenyloxamoyl- γ -aminobutyrate (15): Yield, 2g (73%); mp., 316-318; IR spectrum(γ , cm^{-1}): 3346,3323 γ NH; 2961-2532 γ OH (COOH); 1674 γ CO; 1516CONH; 1570 γ C=C. Anal. Calcd. for $\text{C}_{13}\text{H}_{13}\text{N}_2\text{O}_6\text{Na}$ (316.2): C, 49.30; H, 4.11; N, 8.85. Found C, 49.90; H, 4.33; N, 8.45.

Sodium 2-carboxyphenyloxamoylserinate (16): Yield, 2g (68%); m.p., 198-200; IR spectrum(γ , cm^{-1}): 3446 γ OHass. 3360,3280 γ NH; 2900-2638 γ OH(COOH); 1685 γ CO; 1517 CONH; 1590 γ C=C. Anal. Calcd. for $\text{C}_{12}\text{H}_{11}\text{N}_2\text{O}_7\text{Na}$ (318.2): C, 45.25; H, 3.45; N, 8.80. Found C, 45.60; H, 3.33; N, 8.69

Sodium 2-carboxylphenyloxamine (17): An ethanolic solution of 2.31g (0.01 mol) of 2-carboxyphenyloxamic acid was added into 0.40g (0.01 mol) of NaOH solution. The reaction mixture was allowed standing to pH neutral. The formed precipitate (salt) was filtered, washed with anhydrous diethyl ether and dried. Yield, 1.5g (83.8 %); m.p., 235 ° C decom.; IR spectrum (γ , cm^{-1}): 3307, 3365 γ NH; 2682-2987 γ OH (COOH); 1670 γ CO; 1530CONH; 1585 γ C=C. Anal. Calcd. for $\text{C}_9\text{H}_6\text{NO}_5\text{Na}$, (231): C, 46.75; H, 2.60; N, 6.06. Found C, 45.50; H, 2.31; N, 6.22

Statistical analysis: Statistical data were analyzed using the computer program SPSS. The differences in mean values were determined one-way ANOVA followed by Duncan's multiple rank tests (means with different letters significantly different).

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Table 1. Anti-inflammatory activity evaluation of the test compounds 12-17 and celecoxib (n=5).

Compound	Initial thickness Zero time	Thickness of rat paw mm / % oedema inhibition relative to control			
		1 hr.	2 hr.	3 hr.	4 hr.
Control	0.32±0.012	0.65 ^a ±0.02	0.86 ^a ±0.02	0.99 ^{bc} ±0.03	1.38 ^a ±0.04
Celecoxib	0.32±0.012	0.44 ^d ±0.01	0.52 ^e ±0.01	0.66 ^e ±0.01	0.72 ^d ±0.01
		32%	39%	33%	48%
12	0.32±0.012	0.46 ^d ±0.01	0.54 ^e ±0.02	0.68 ^e ±0.01	0.74 ^d ±0.02
		29%	37%	31%	46%
13	0.324±0.014	0.61 ^{ab} ±0.01	0.76 ^b ±0.02	0.85 ^d ±0.02	0.93 ^c ±0.03
		6%	11%	14%	32%
14	0.32±0.012	0.58 ^{bc} ±0.01	0.68 ^c ±0.01	0.77 ^e ±0.02	0.86 ^c ±0.02
		11%	21%	22%	37.7%
15	0.32±0.010	0.67 ^a ±0.03	0.86 ^a ±0.02	1.01 ^a ±0.02	1.35 ^a ±0.03
		0	0	0.99%	2.2%
16	0.31±0.012	0.64 ^a ±0.01	0.82 ^a ±0.02	0.94 ^b ±0.02	1.26 ^b ±0.02
		1.5%	4.7%	5%	8.7%
17	0.324±0.011	0.53 ^c ±0.02	0.60 ^d ±0.02	0.70±0.02	0.91 ^c ±0.01
		18.5%	30%	29%	34%

Means within the same column having different alphabetical letters are significantly different ($P \leq 0.01$).

Table 2. Analgesic activity evaluation of test compounds 12-17 and celecoxib (n=5).

Compound	Time to defensive reflex, sec. after					
	10 min.	20 min.	30 min.	60 min.	90 min.	120 min.
Control	12.80 ^d ±0.37	13.40 ±0.51	13.40 ^d ±0.51	13.60 ±0.51	13.00 ^{de} ±0.32	13.00 ^d ±0.32
Celecoxib	24.00 ^a ±0.45	35.40 ^a ±1.44	38.40 ^a ±0.93	41.20 ^a ±1.39	41.60 ^a ±1.03	38.40 ^a ±0.93
12	24.60 ^a ±0.68	34.20 ^a ±1.83	34.80 ^b ±1.46	38.40 ^b ±0.93	38.60 ^b ±0.51	30.00 ^b ±0.32
13	12.60 ^d ±0.51	13.60 ^c ±0.24	13.60 ^d ±0.40	13.80 ^d ±1.33	13.60 ^{de} ±1.12	14.40 ^d ±0.24
14	19.60 ^b ±0.51	20.40 ^b ±0.51	23.40 ^c ±0.51	28.40 ^c ±0.37	22.60 ^c ±0.68	20.00 ^c ±1.58
15	14.40 ^c ±0.24	13.60 ^c ±0.40	14.20 ^d ±0.37	13.20 ^d ±0.49	15.40 ^{de} ±0.98	13.20 ^d ±0.37
16	12.80 ^d ±0.37	13.20 ^c ±0.01	14.00 ^d ±0.71	12.60 ^d ±0.40	14.20 ^{de} ±0.58	12.60 ^d ±0.40
17	12.80 ^d ±0.37	12.80 ^c ±0.58	13.60 ^d ±0.51	13.60 ^d ±0.51	14.00 ^{de} ±0.71	13.20 ^d ±0.58

Means within the same column having different alpha-betical letters are significantly different ($P \leq 0.01$).

Table 3. Ulcerogenic activity of the test compounds 12-17 and celecoxib (n=5)

Ulcerogenic Activity			
Compound	Incidence of gastric ulceration	Mean ulcer score	Ulcer index
Control	0.0	0.00	0.0
Celecoxib	0.0	0.00	0.0
Indomethacin	100%	4.60±0.24	460
12	20%	0.20±0.20	4
13	60%	1.00±0.45	60
14	50%	0.40±0.24	20
15	60%	0.80±0.37	48
16	60%	1.60±0.68	96
17	60%	1.00±0.45	60

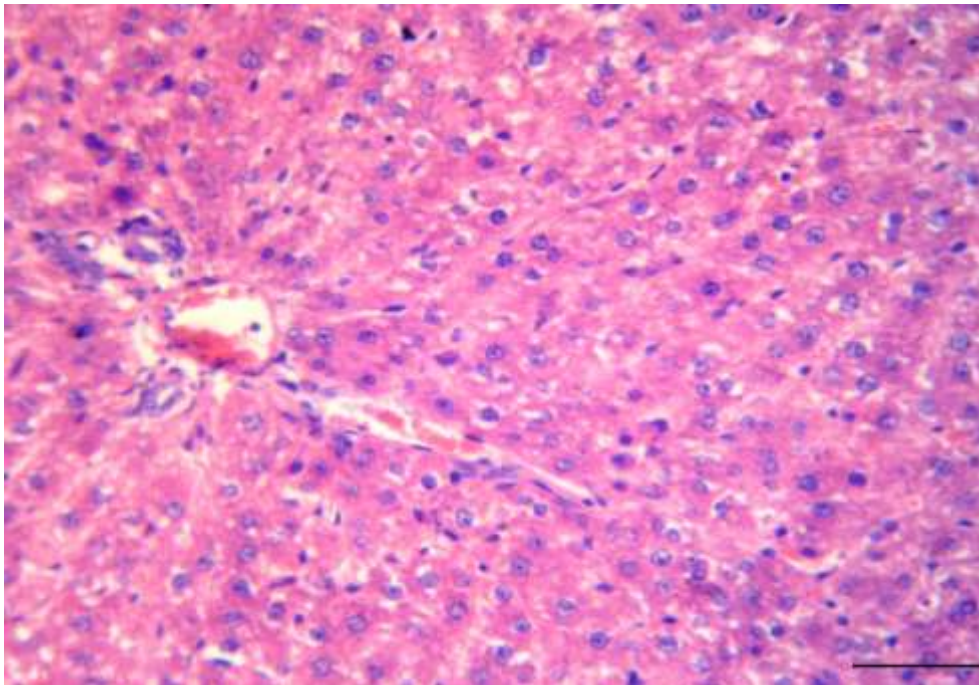


Figure 1: Rat liver of group 1 (control) showing normal hepatic architecture and sinusoids, H&E Stain (Bar =100 μ m).

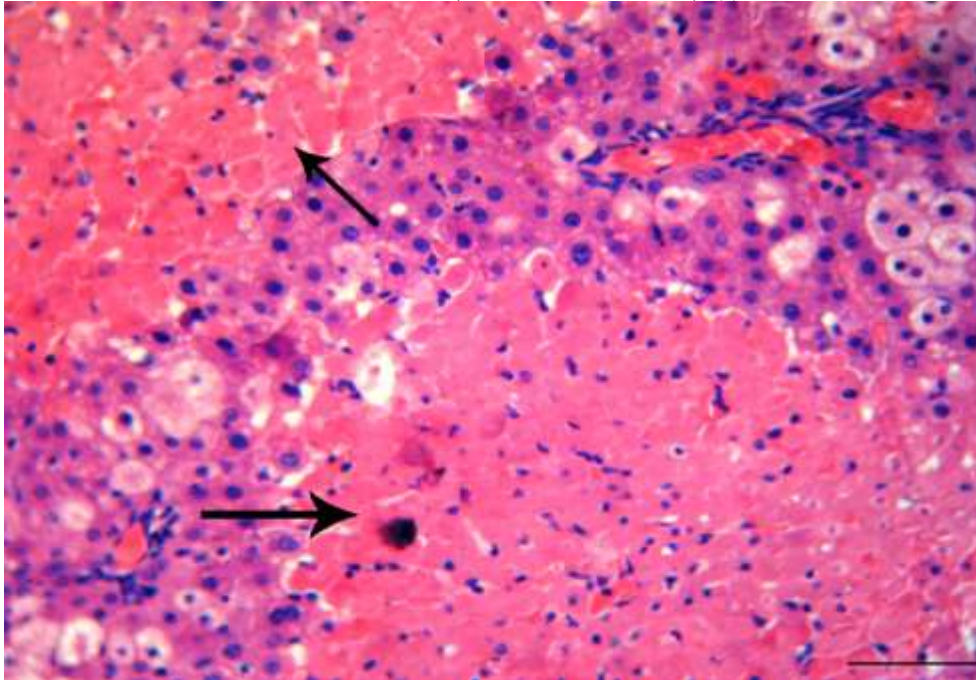


Figure 2: Rat liver of group 3 (Carbon tetrachloride) showing multifocal areas of coagulative necrosis (arrow), H&E Stain (Bar =100 μ m).

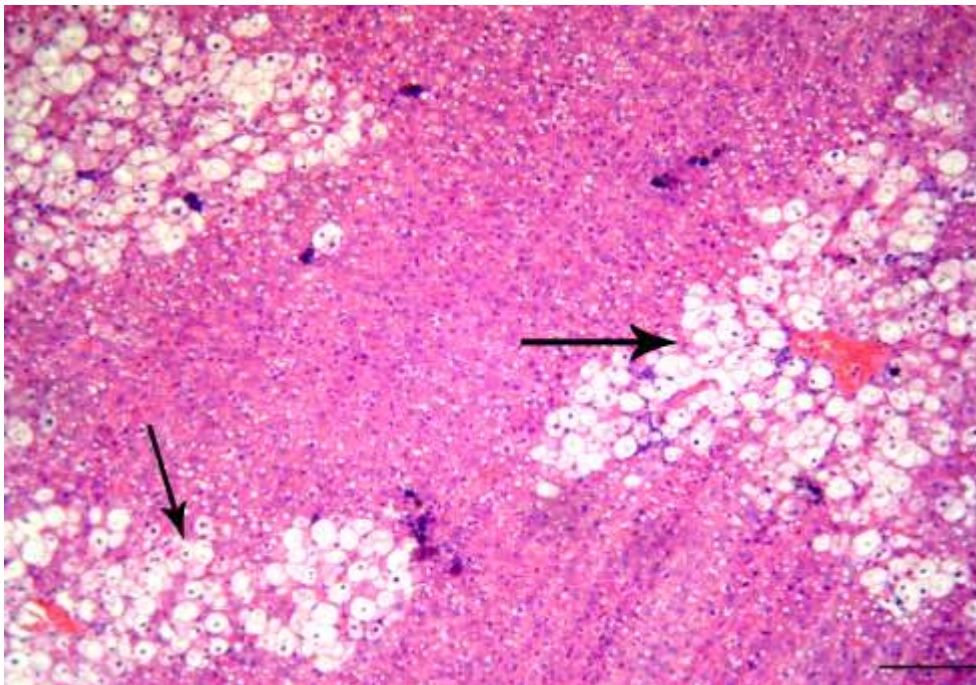


Figure 3: Rat liver of group 8 (Carbon tetrachloride + compound 9) showing vacuolations and ballooning degeneration (arrows), H&E Stain (Bar =100 μ m).

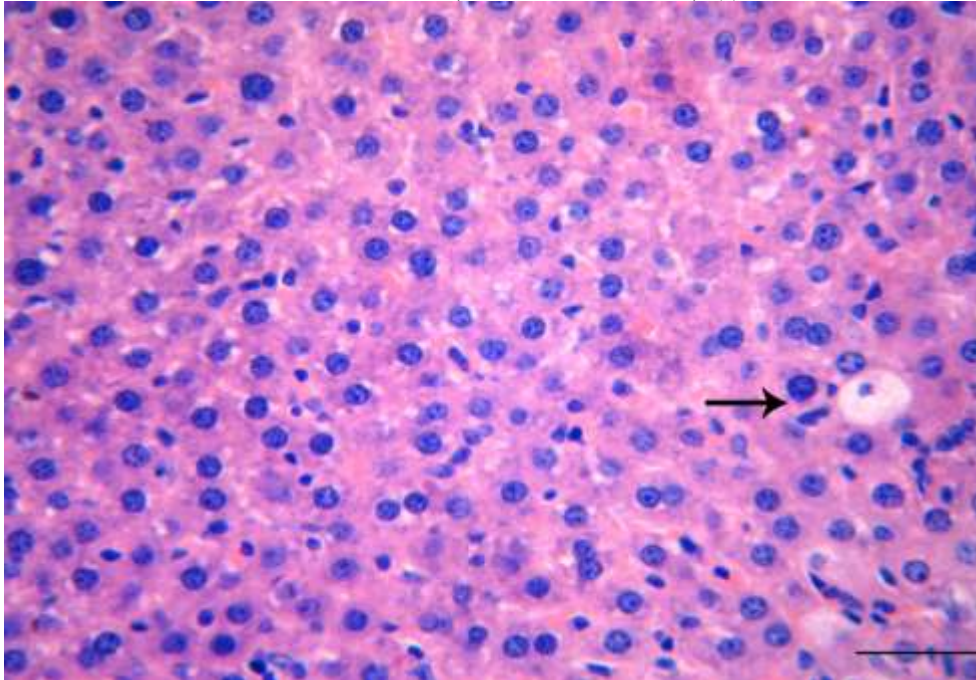


Figure 4: Rat liver of group 2 (Carbon tetrachloride + Silymarine)) showing slight vacuolations and ballooning degeneration in some hepatocytes (arrow), H&E Stain (Bar =100 μ m).

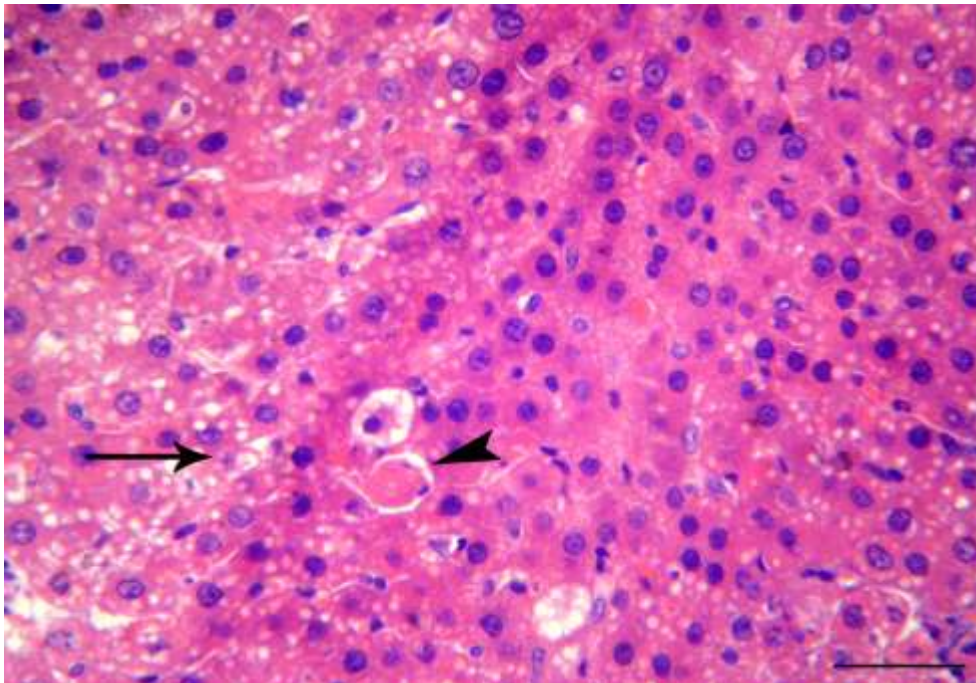


Figure 5: Rat Liver of group 4 (Carbon tetrachloride + compound 6) showing vacuolations (arrow) and apoptosis (arrowhead), H&E Stain (Bar =100 μ m).

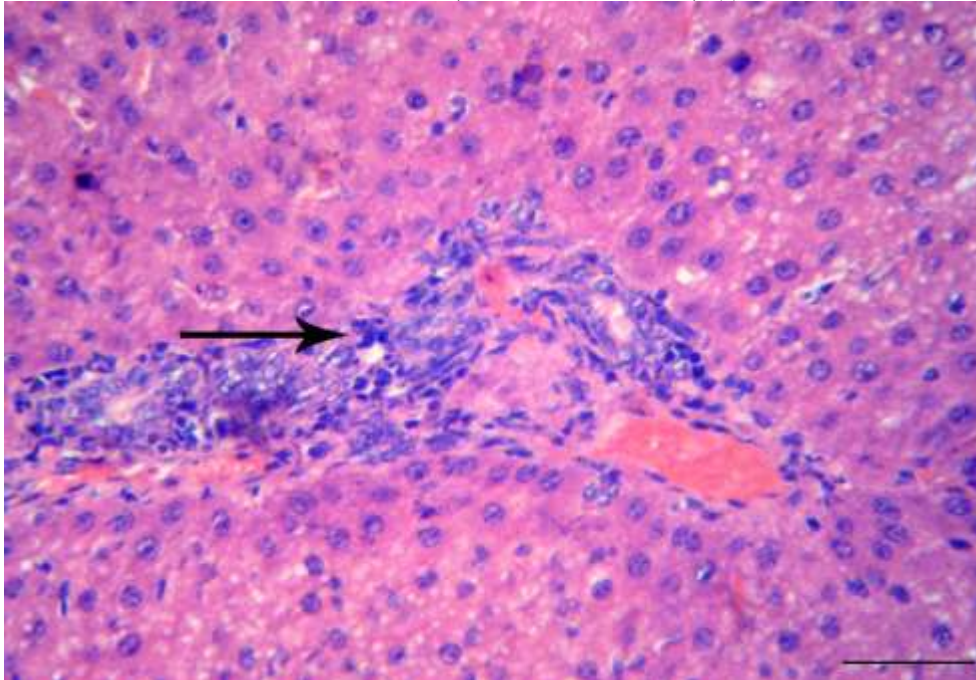


Figure 6: Rat liver of group 4 (Carbon tetrachloride + compound 6) showing lymphocytic infiltration and mild proliferation of the biliary epithelia in the portal area (arrow), H&E Stain (Bar =100 μ m).

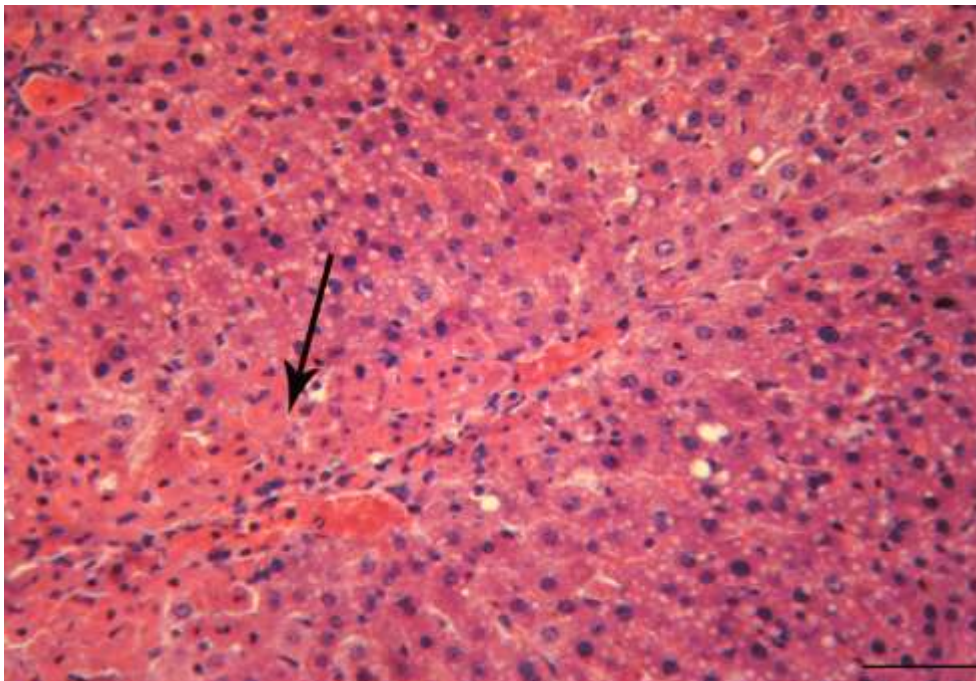


Figure 7: Rat liver of group 5 (Carbon tetrachloride + compound 7) showing small periportal area of coagulative necrosis (arrows), H&E Stain (Bar =100 μ m).

REFERENCES

1. Cheney BV et al. Structure-activity correlations for a series of antiallergy agents, oxanilic, quinaldic, and benzopyran-2-carboxylic acids. *J. Med.Chem.* 1978; 21(9): 936-40.
2. Vereitinova VP et al. Synthesis and biological activity of 2 oxoindolindine -3- hydrazides of oxanilic acids. *Pharm Chem J.* 1986; 20(12): 844-46.
3. Khaldoon MA et al. Synthesis and Biological Activities of Carboxyphenyloxamoylamino Acids, their Salts with 2-Ethoxy-6,9-Diaminoacridine and D-glucosamine *Adv. Pharmacoepidem Drug Safety* 2013; 2(2): 1-6.
4. Khaldoon MA et al. [Synthesis and biological activity of arensulphonyloxamoyl- and arensulphohydrazidooxalyamino acids and their salts with 2-ethoxy-6,9-diaminoacridine. *Farm Zh* 2008; 5: 62-6 [in Ukrainian].
5. Petyunin GP et al. Synthesis and nootropic activity of oxamoylamino acids. *Pharm Chem J.* 1988; 22(11): 838-40
6. Khaldoon MA. [Synthesis and antimicrobial activity of 3,4-dimethyloxamoylamino acid's salts with aminoacridines base. *Farm Zh* 2008; 2: 73-6 [in Ukrainian].
7. Khaled MN et al. The effect of new compound (Rivanol salts) and yemeni honey on cutaneous leishmaniasis. *Assuit Univ. J. of Zoology* 2014; 43(1): 1-20.
8. Khaldoon MA. et. al. Synthesis, antimalarial and antimicrobial activities of arensulphohydrazidooxalyl and arensulphonyloxamoylamino acids salts with 2-ethoxy-6,9-diaminoacridine and chloroquine. *J. of actual problem of pharm & med sci.* 2010; (23)4: 4-7.
9. Khaldoon MA. et al. Synthesis and biological activity of 3,4-dimethylphenyloxamoylamino acids and their salts with D(+)-glucosamine. *j. of org. & pharm. Chem.* 2008; 3(23): 53-8.
10. Bancroft JD, Stevens A, Dawswon MP. *Theory and Practice of Histological Technique*, 4TH ed.; Churchill Livingstone: New York, London, San Francisco, Tokyo, 1996; pp.273-92.
11. Winter CA et al. Carrageenin-induced edema in hind paw of the rat an assay for anti-inflammatory drugs. *Proc. Soc. Exp. Biol. Med.* 1962; 111: 544-47.
12. Jacob J, Bosovski M. *Screening methods in pharmacology.* Academic Press INC: New York and London, 1961; pp104-57.
13. Wilhelmi, G et al. Gastric Mucosal Damage Induced by Non-steroid Anti-Inflammatory Agents in Rats of Different ages. *Pharmacology* 1972; 8: 321-28.
14. Pauls, F. et al. An assay method for anti-ulcer substances. *Gastroenterology.* 1947; 8(6): 774-82.
15. Joshi JK et al. Synthesis and anti-inflammatory activity of N-anthranilic acid derivatives. *Indian Journal of Pharmaceutical Science.*2007; 69(5): 697-99.