GEORGIAN MEDICAL NEWS

ISSN 1512-0112

NO 5 (338) Май 2023

ТБИЛИСИ - NEW YORK

ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

Monthly Georgia-US joint scientific journal published both in electronic and paper formats of the Agency of Medical Information of the Georgian Association of Business Press. Published since 1994. Distributed in NIS, EU and USA.

GMN: Georgian Medical News is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board since 1994. GMN carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

GMN is indexed in MEDLINE, SCOPUS, PubMed and VINITI Russian Academy of Sciences. The full text content is available through EBSCO databases.

GMN: Медицинские новости Грузии - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения. Журнал индексируется в MEDLINE, отражён в базе данных SCOPUS, PubMed и ВИНИТИ РАН. Полнотекстовые статьи журнала доступны через БД EBSCO.

GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

ჟურნალი ინდექსირებულია MEDLINE-ის საერთაშორისო სისტემაში, ასახულია SCOPUS-ის, PubMed-ის და ВИНИТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებიდან.

WEBSITE

www.geomednews.com

К СВЕДЕНИЮ АВТОРОВ!

При направлении статьи в редакцию необходимо соблюдать следующие правила:

1. Статья должна быть представлена в двух экземплярах, на русском или английском языках, напечатанная через **полтора интервала на одной стороне стандартного листа с шириной левого поля в три сантиметра**. Используемый компьютерный шрифт для текста на русском и английском языках - **Times New Roman** (**Кириллица**), для текста на грузинском языке следует использовать **AcadNusx.** Размер шрифта - **12**. К рукописи, напечатанной на компьютере, должен быть приложен CD со статьей.

2. Размер статьи должен быть не менее десяти и не более двадцати страниц машинописи, включая указатель литературы и резюме на английском, русском и грузинском языках.

3. В статье должны быть освещены актуальность данного материала, методы и результаты исследования и их обсуждение.

При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

5. Таблицы необходимо представлять в печатной форме. Фотокопии не принимаются. **Все цифровые, итоговые и процентные данные в таблицах должны соответствовать таковым в тексте статьи**. Таблицы и графики должны быть озаглавлены.

6. Фотографии должны быть контрастными, фотокопии с рентгенограмм - в позитивном изображении. Рисунки, чертежи и диаграммы следует озаглавить, пронумеровать и вставить в соответствующее место текста **в tiff формате**.

В подписях к микрофотографиям следует указывать степень увеличения через окуляр или объектив и метод окраски или импрегнации срезов.

7. Фамилии отечественных авторов приводятся в оригинальной транскрипции.

8. При оформлении и направлении статей в журнал МНГ просим авторов соблюдать правила, изложенные в «Единых требованиях к рукописям, представляемым в биомедицинские журналы», принятых Международным комитетом редакторов медицинских журналов - http://www.spinesurgery.ru/files/publish.pdf и http://www.nlm.nih.gov/bsd/uniform_requirements.html
В конце каждой оригинальной статьи приводится библиографический список. В список литературы включаются все материалы, на которые имеются ссылки в тексте. Список составляется в алфавитном порядке и нумеруется. Литературный источник приводится на языке оригинала. В списке литературы сначала приводятся работы, написанные знаками грузинского алфавита, затем кириллицей и латиницей. Ссылки на цитируемые работы в тексте статьи даются в квадратных скобках в виде номера, соответствующего номеру данной работы в списке литературы. Большинство цитированных источников должны быть за последние 5-7 лет.

9. Для получения права на публикацию статья должна иметь от руководителя работы или учреждения визу и сопроводительное отношение, написанные или напечатанные на бланке и заверенные подписью и печатью.

10. В конце статьи должны быть подписи всех авторов, полностью приведены их фамилии, имена и отчества, указаны служебный и домашний номера телефонов и адреса или иные координаты. Количество авторов (соавторов) не должно превышать пяти человек.

11. Редакция оставляет за собой право сокращать и исправлять статьи. Корректура авторам не высылается, вся работа и сверка проводится по авторскому оригиналу.

12. Недопустимо направление в редакцию работ, представленных к печати в иных издательствах или опубликованных в других изданиях.

При нарушении указанных правил статьи не рассматриваются.

REQUIREMENTS

Please note, materials submitted to the Editorial Office Staff are supposed to meet the following requirements:

1. Articles must be provided with a double copy, in English or Russian languages and typed or compu-ter-printed on a single side of standard typing paper, with the left margin of **3** centimeters width, and **1.5** spacing between the lines, typeface - **Times New Roman** (**Cyrillic**), print size - **12** (referring to Georgian and Russian materials). With computer-printed texts please enclose a CD carrying the same file titled with Latin symbols.

2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

8. Please follow guidance offered to authors by The International Committee of Medical Journal Editors guidance in its Uniform Requirements for Manuscripts Submitted to Biomedical Journals publication available online at: http://www.nlm.nih.gov/bsd/uniform_requirements.html
http://www.icmje.org/urm_full.pdf
In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

9. To obtain the rights of publication articles must be accompanied by a visa from the project instructor or the establishment, where the work has been performed, and a reference letter, both written or typed on a special signed form, certified by a stamp or a seal.

10. Articles must be signed by all of the authors at the end, and they must be provided with a list of full names, office and home phone numbers and addresses or other non-office locations where the authors could be reached. The number of the authors (co-authors) must not exceed the limit of 5 people.

11. Editorial Staff reserves the rights to cut down in size and correct the articles. Proof-sheets are not sent out to the authors. The entire editorial and collation work is performed according to the author's original text.

12. Sending in the works that have already been assigned to the press by other Editorial Staffs or have been printed by other publishers is not permissible.

Articles that Fail to Meet the Aforementioned Requirements are not Assigned to be Reviewed.

ავტორთა საყურადღებოდ!

რედაქციაში სტატიის წარმოდგენისას საჭიროა დავიცვათ შემდეგი წესები:

1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე,დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx.** შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

3. სტატიაში საჭიროა გაშუქდეს: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

4. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: მიზანი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფჩხილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

GEORGIAN MEDICAL NEWS
No 5 (338) 2023

Содержание:

GEORGIAN MEDICAL NEWS
No 5 (338) 2023

ACUTE TOXICITY OF COUMACINES: AN *IN VIVO* STUDY

Wejdan Al-Shakarchi[1], Yasir Saber[2], Marwan M. Merkhan[1,2]*, Yasser Fakri Mustafa[1].

[1]*Department of Pharmaceutical Chemistry, College of Pharmacy, University of Mosul, Mosul, Iraq.*

[2]*Department of Pharmaceutical Chemistry, College of Pharmacy, Ninevah University, Mosul, Iraq.*

Abstract.

The design and synthesis of new drugs are increasingly challenging in chemistry settings. The synthesis is itself lured by the properties of the product after synthesis, including solubility, hygroscopicity, intensive adverse effects, and biological inefficacy; hence, the creation of a new drug should be considered in light of the avoidance of these downside features, if any. The present study is designed to investigate the acute toxicity of newly discovered heterocyclic frameworks derived from the coumarin backbone, namely coumacine I and coumacine II. To do so, a mouse model of 25 mice was subclassified into 5 groups (5 mice control, 5 mice coumacine I 1000 mg/kg, 5 mice coumacine II 1000 mg/kg, 5 mice coumacine I 2000 mg/kg, and 5 mice coumacine II 2000 mg/kg), a single dose was given, and mice were sacrificed after 4 hours post-dose. The blood sample and tissue were collected for biochemical and histopathological studies. Serums were analyzed for the measurement of renal function and liver enzyme activity using classical biochemical methods. A high dose of either compound caused deleterious changes, as evidenced by a significant ($p<0.05$) increase in creatinine, urea, GOT, and GPT, as well as disrupting tissue quasi-equilibrium at the cellular level in both kidney and liver. To sum up, coumacine I and coumacine II are relatively safe unless otherwise used in high doses, knowing that either dose in the present study is remarkably higher than the therapeutic dose of coumarins currently in use in clinical settings.

Key words. Coumacine, coumarin, acute toxicity, renal function.

Introduction.

The coumarin backbone is a term for benzo-(e)-pyrone, and its structure is shown in Figure 1 [1-3]. Coumarin is actually found in many plants as a secondary metabolite [4]. There are natural and synthetic structures derived from coumarin that make up a diverse and significant class of oxygen-containing heterocycles. These coumarins can be grouped into different categories based on their structural frameworks. For example, there are simple coumarins [5], furanocoumarins [6], pyranocoumarins [7], biscoumarins [8], and phenylcoumarins [9]. Each of these groups has unique properties and uses. So, while the term "coumarin backbone" may sound intimidating, it's actually an important subject that has many practical applications. From medicine to agriculture, the study of coumarins is an important field that has the potential to make a big impact.

Coumarin-based compounds that can be isolated from natural sources or synthesized by various synthetic schemes were suggested for use in clinical medicine because of their privileged biochemical characteristics [10]. For the therapy of numerous medical diseases, coumarin-based compounds ought to be assessed, perhaps for the treatment of high protein oedema [11], chronic infections [1], cancer [12], oxidative-overload-related illnesses [13], inflammation-based diseases [14], and thrombotic disorders [15].

The structural characteristics of the coumarin chemical backbone enable the production of various derivatives with various pharmacological properties. Coumarin itself and some of its derived products have been used in the past as a rodenticide, or a substance used to kill rats and mice [16]. These chemical compounds work by inhibiting the synthesis of the vitamin K-dependent clotting factors in the liver, which leads to internal bleeding and death [17]. The present study aimed at investigating the renal and liver toxicity of newly synthesized coumacines that were delivered from a coumarin backbone by our laboratory of pharmaceutical chemistry, namely coumacine I (CM I) and coumacine II (CM II), with structural formulas shown in Figure 1.

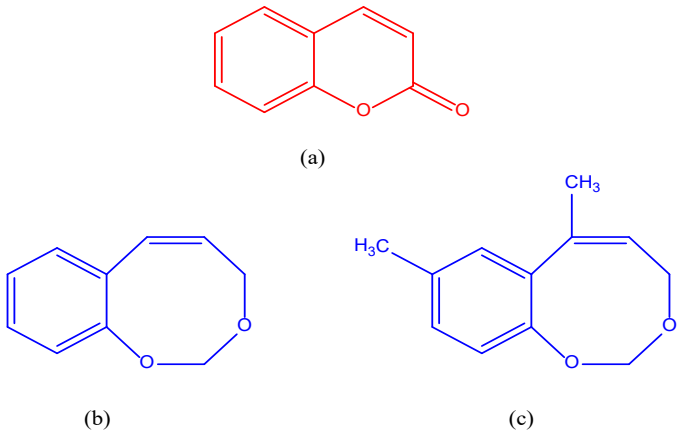

Figure 1. *Structural formulas of coumarin backbone (a), CM I (b), and CM II (c).*

The chemical compounds being studied are no ordinary substances. They have the power to stop the synthesis of vitamin K that is responsible for clotting factors in the liver. The result is internal bleeding and death [16]. This research delves deeper into the newly synthesized coumacines, CM I and CM II, which have a coumarin backbone [17]. Our laboratory of pharmaceutical chemistry is the leading laboratory behind these compounds, and we're eager to investigate their potential toxicity on the liver and kidneys. Check out Figure 1 for a sneak peek at their unique structural formulas.

Materials and methods.

Chemicals: The guidelines for drug design and development have been extensively used to introduce new chemical entities as

potential candidates for clinical use, especially as antimicrobials. In this regard, Mustafa YF has developed and characterized new dioxoheterocyclic compounds named CM I and II as potential killers of common aerobic and anaerobic pathogenic bacterial strains [1]. The synthesized coumacines were readily soluble in a 10% solution of hydroxypropyl beta cyclodextrin (HPBCD) for application in the present study.

***In vivo* study:** In this study, we enlisted the 25 male Albino mice to investigate the effects of CM I and II at different doses. The mice were divided into three groups - the first group was given high doses of CM I and II, the second group was given low doses of CM I and II, while the third group served as a control and was treated only with vehicle. We closely monitored the mice to see how they responded to the different doses, and the results were quite intriguing.

The present study was conducted on a mouse model using 25 male Albino mice to be exposed to high and low doses of CM I and II compared to the control group treated only by vehicle. Before starting the study, the mice were acclimated to the laboratory environment (temperatures of 23–25ºC and humidity of 50–55%) a week before experimentation. They had free access to food and water, and a light-dark cycle was applied. The 25 mice in total means 5 mice in each group (5 mice control, 5 mice CM I 1000 mg/kg, 5 mice CM II 1000 mg/kg, 5 mice CM I 2000 mg/kg, and 5 mice CM II 2000 mg/kg) received a single dose and were sacrificed after 4 hours post-dose. Blood samples were collected, and serum was separated for biochemical analysis.

Measurement of liver enzyme activities: The principle of measurement of AST/GOT (Kit Cat No. REF4191, Giesse Diagnostics, Italy) is based on the enzymatic removal of an amino group from aspartate to be transferred to oxoglutarate, generating glutamate and oxalacetate. Malate dehydrogenase uses the reducing agent nicotinamide adenine dinucleotide to convert oxaloacetate to malate. The activity of the enzyme, based on kinetic measurement, is proportional to the rate of reduction in absorbance caused by the oxidation of NADH to its oxidized form, NAD, at 340 nm.

The principle of measurement of ALT/GPT (Kit Cat No. REF4194, Giesse Diagnostics, Italy) based on the enzymatic removal of an amino group from alanine to be transferred to oxoglutarate, generating glutamate and pyruvate. Pyruvate is converted to lactate-by-lactate dehydrogenase, which is provided with the reducing agent nicotinamide adenine dinucleotide. The activity of the enzyme, based on kinetic measurement, is proportional to the rate of reduction in absorbance caused by the oxidation of NADH to its oxidized form at 340 nm.

Measurement of renal function tests:

Urea: According to the manufacturer's instructions for the urea measurement kit (supplied by BioSystem, Spain, catalog no. 11537), urea is converted to carbon dioxide and ammonia by urease. The generated ammonia interacts with sodium salicylate and hypochlorite, catalyzed by the presence of nitroprusside, producing a green-colored solution that can be quantified at a wavelength of 600 nm using a spectrophotometer.

Creatinine: The process of measuring creatinine levels in a sample can be done using a creatinine measurement kit such as the one supplied by BioSystem in Spain. The kit, catalog number 12502, uses a principle based on the interaction between creatinine and picrate ions to create a reddish complex. This complex formation occurs at a rate that is proportional to the concentration of creatinine in the sample being tested. To measure the concentration of creatinine, the rate of complex formation is assessed by the increase in absorbance over a predetermined time interval. By following the manufacturer's instructions for the kit, one can accurately measure the concentration of creatinine in a sample using this method. This type of measurement is commonly used in medical settings, as creatinine is a waste product that is filtered out of the body by the kidneys. Elevated levels of creatinine in the blood can indicate kidney dysfunction or other medical conditions, making accurate measurement of creatinine levels an important diagnostic tool. By using the creatinine measurement kit and following the manufacturer's instructions, healthcare professionals can obtain reliable and accurate results to aid in the diagnosis and treatment of patients.

Histological study: After the administration of the drug, a time interval of four hours was allowed to pass before the animals were sacrificed. The organs were removed and washed with normal saline to remove any residual drug or impurities. The organs were then fixed in 4% formalin overnight to preserve their structure. The next day, the paraffin-embedded blocks were sectioned at 5 µm and placed on slides to be stained with hematoxylin-eosin. The slides were then examined under a light microscope at 400X magnification by a blinded pathologist who was unaware of the treatment group or dose. The results obtained from this process provide valuable insights into the safety and efficacy of the drug being tested.

Statistics: In this study, five different conditions were analyzed using a one-way ANOVA with post hoc Bonferroni tests to identify the different groups. The statistical software used for this analysis was GraphPad Prism. The results of the ANOVA analysis were then displayed using a histogram, which is an effective way of visually representing data as the mean±standard deviation. To ensure that the sample sizes were sufficient to detect any differences between the conditions, a power of 80% or greater was used. Additionally, the alpha error level (P) in all experiments had to be 0.05 to be statistically significant, which is a standard requirement in statistical analysis.

Results.

Analysis of the results of renal function tests (Figure 2) revealed that serum creatinine concentrations (in mg/dl) in the high dose (2000 mg/kg/day) group of CM I (13.7±1.1) and CM II (12.7±0.4) were significantly higher than those in the control group (7.7±1.8) or low dose (1000 mg/kg/day) CM I (10.8±2.4) and CM II (10.7±4). Similarly, blood urea (in mg/dl) has been significantly elevated in the high dose (2000 mg/kg/day) group of CM I (16.2±3) and CM II (22±8) compared to the control group (6±1.2) or low dose (1000 mg/kg/day) CM I (10.8±2.4) and coumacine II (9.7±2.9).

Moreover, histopathology analysis of renal tissue (Figure 3) has confirmed that the renal tissue of mice treated with low dose (1000 mg/kg/day) CM I and II has shown mild degeneration with no signs of necrosis or oedema, while the high dose (2000 mg/

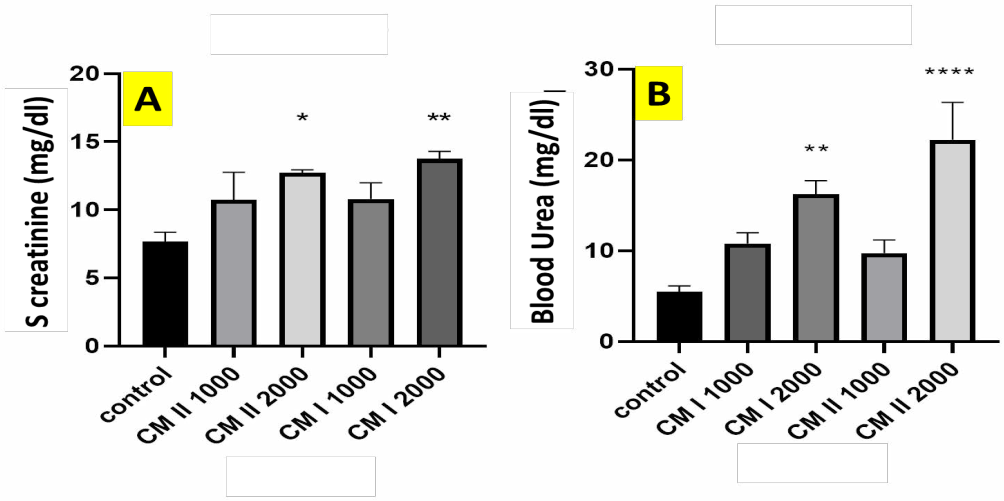

Figure 2. *Renal function tests of CMI and II. (A) Creatinine level in control mice in comparison with mice treated with CM I 1000 and 2000 mg/kg and CM II 1000 and 1000 mg/kg. (B) Urea level in control mice in comparison with mice treated with CM I 1000 and 2000 mg/kg and CM II 1000 and 1000 mg/kg. Data expressed as mean±SD. *P< 0.05, **P< 0.01,**** P<0.001.*

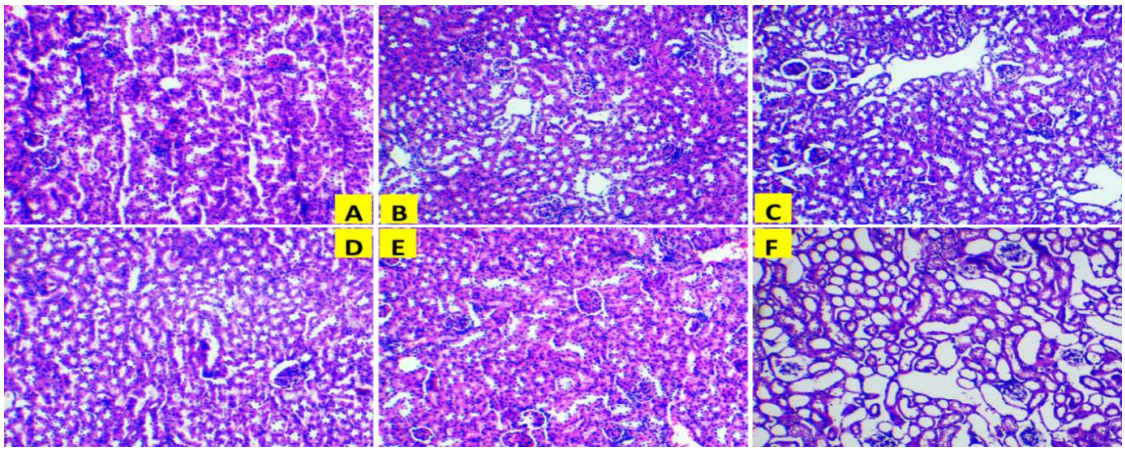

Figure 3. *Histopathological sections of mouse renal tissues in the studied groups. A and D = control groups, B = CM I 1000 mg/kg IP, C = CM I 2000 mg/kg IP, E = CM II 1000 mg/kg IP, F = CM II 2000 mg/kg IP.*

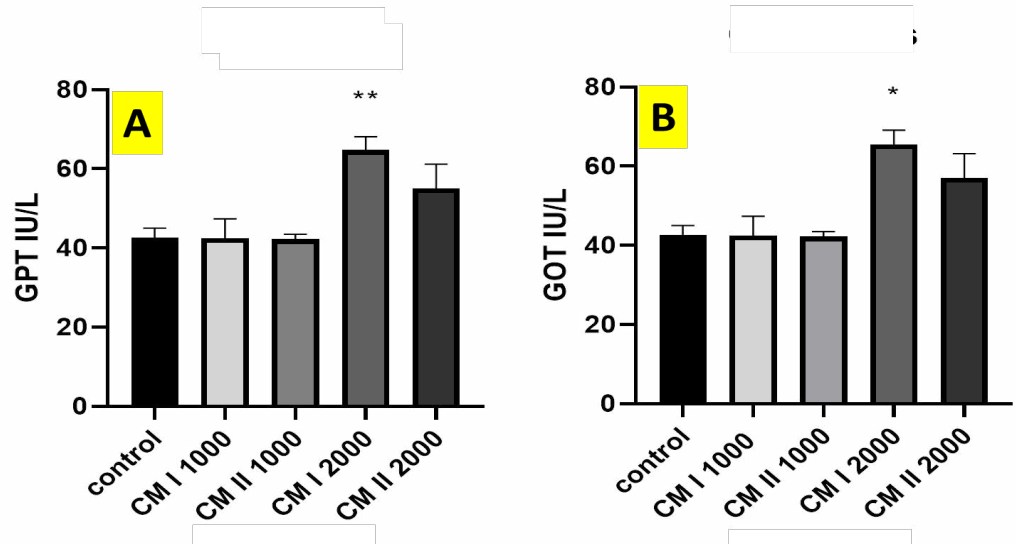

Figure 4. *Liver function tests of CM I and II. (A) GPT level in control mice in comparison with mice treated with CM I 1000 and 2000 mg/kg and CM II 1000 and 1000 mg/kg. (B) GOT level in control mice in comparison with mice treated with CM I 1000 and 2000 mg/kg and CM II 1000 and 1000 mg/kg. Data expressed as mean±SD. *P< 0.05, **P< 0.01.*

kg/day) group of CM I and II has shown moderate degeneration and signs of necrosis and oedema. The control section of renal tissue has shown an intact glomerulus and thick renal tubules, with no sign of degeneration, oedema, or necrosis detected.

Analysis of the results of liver enzymes (Figure 4) revealed that serum GPT activity (in IU/L) in the high dose (2000 mg/kg/day) groups of CM I (62.5±2) and CM II (52.5±0.5) were significantly higher than the control group (42.5±2) or low dose (1000 mg/kg/day) CM I (42.5±8) and CM II (42.5±9). Similarly, GOT activity (in IU/L) has been significantly elevated in the high dose (2000 mg/kg/day) groups of CM I (57±3) and CM II (65.5±7) compared to the control group (42.5±6) or low dose (1000 mg/kg/day) CM I (42.5±2.5) and CM II (42.25±9).

Moreover, histopathology analysis of hepatic tissue (Figure 5) has confirmed that the hepatic tissue of mice treated with low dose (1000 mg/kg/day) CM I and II has shown mild degeneration with no signs of necrosis or oedema, while the high dose (2000 mg/kg/day) group of CM I and II has shown moderate degeneration and signs of necrosis and oedema.

Discussion.

The current study focused on the toxicity of newly derived coumarin-derived heterocyclic compounds, CM I and II, on vital organs like the liver and kidney. The results confirmed that CM I and II are relatively safe, particularly at low doses compared to the high dose, which has demonstrated toxicity at biochemical and histological limits. However, being new compounds, the effects are unpredictable in light of the unavailability of comparative studies, and thereby the results should be considered a pilot study to be further supported by future research.

At high doses, CM I and II caused increased creatinine and urea levels, as well as moderate degeneration and histological signs of necrosis and oedema. Coumarin-induced renal damage has been reported with newly designed derivative compounds of coumarins, such as flourocoumarin, including angelicin and psoralen (Figure 6) [18-20]. Lu et al. reported that cisplatin, together with large doses of psoralen and angelicin, caused tubulointerstitial vascular dilatation and congestion in the renal intercellular space, as well as inflammatory cell recruitment [19].

Nevertheless, a coumarin analogue obtained from the Daphne species, daphnetin (Figure 6), is a physiologically active phytochemical with a wide range of bioactivities [21]. Daphnetin has been reported to provide a reno-protective effect against cisplatin-induced renal injury by reducing creatinine and blood urea nitrogen alongside improved inflammation, oxidative stress, and apoptosis [22]. Simple coumarin derivatives, including umbelliferone, esculetin, scopoletin, and osthole (Figure 6), are coumarin derivatives that have undergone hydroxylation and alkoxylation [23]. Esculetin has been reported to improve renal function and nephropathy in a diabetic patient via increasing antioxidant enzymes and reducing reactive oxygen species [24]. Imperatorin, as a naturally occurring furocoumarin (Figure 6), is most prevalent in medicinal plants, and imperatorin has therapeutic effects and could be used for chronic diseases [25]. Imperatorin has been shown in cell lines and animal models to be toxic to the renal system and to induce ionic transporters. Kun-Ming mice [26,27].

Low doses of CM I and II had no effect on plasma GOT and GPT levels, whereas high doses increased liver enzyme activities and caused histopathological changes such as oedema, necrosis, and moderate degeneration when compared to control or low doses. Psoralen, a structural derivative of coumarin, has been shown to have no negative effects on liver architecture [19]. Angelicin has been reported to induce no impairment or liver toxicity whether used alone or together with psoralen [18]. Moreover, bergapten, or 5-methoxypsoralen (Figure 6), is a furocoumarin compound extracted from bergamot essential oil; this compound undergoes quick and extensive hepatic biotransformation, and thereby hepatic injury is expected with this herbal-derived coumarin [28]. However, daphnetin provides hepatoprotection against lipopolysaccharide/D-galactosamine-induced liver failure in mouse models, as reflected by mitigated oxidative stress, proinflammatory markers, and liver enzymatic activity

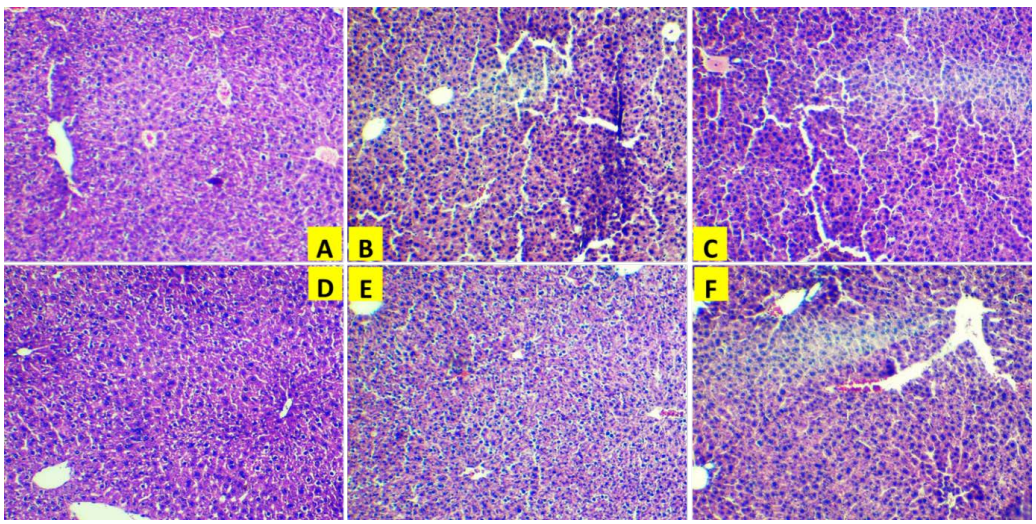

Figure 5. *Histopathological sections of mouse hepatic tissues in the studied groups. A and D = control groups, B = CM I 1000 mg/kg IP, C = CM I 2000 mg/kg IP, E = CM II 1000 mg/kg IP, F = CM II 2000 mg/kg IP.*

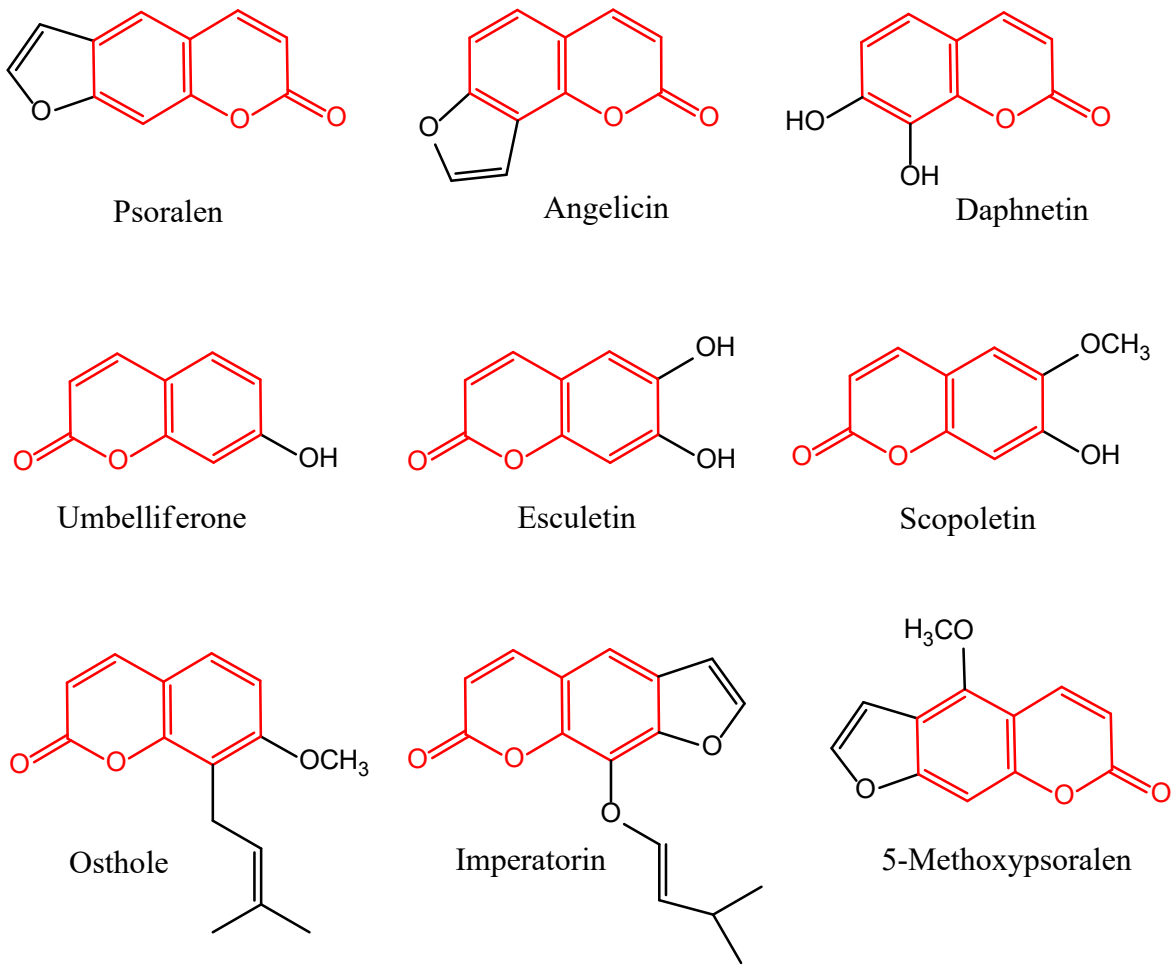

Figure 6. *Structural formulas of psoralen, angelicin, daphnetin, umbelliferone, esculetin, Scopoletin, osthole, imperatorin, and 5-methoxypsoralen. The coumarin backbone is represented in red.*

[21]. According to a report by Tien et al., esculetin therapy at doses of 100 and 500 mg/kg can reduce the levels of antioxidant enzymes, including catalase (CAT) and superoxide dismutase (SOD), which help combat free radicals produced throughout lipid peroxidation. Concurrently, esculetin administration decreased the activity of liver function tests such as alanine transaminase (ALT) and aspartate transaminase (AST) [29]. Imperatorin increases liver enzymatic activities in Kun-Ming mice exposed to a dose of 40 mg/kg/day [28]. Psoralen-induced liver damage is more likely to be linked to endoplasmic reticulum dysfunction, mitochondrial damage, and oxidative stress [20]. Alternatively, psoralen, which is a coumarin derivative, has been studied extensively for liver damage using mice and L02 hepatic human cell line models. The outcome has revealed that psoralen elevated liver enzymes in a dose-dependent manner, leading to an increase in the levels of alkaline phosphatase, AST, alanine aminotransferase, albumin, total plasma protein, and bilirubin [30]. Moreover, a cell line model has revealed that psoralen inhibits cell viability [30,31]. Umbelliferone is present in fruits and root plants, such as the golden apple, the bitter orange, and the carrot [32-34]. Umbelliferone provides hepatic protection against alcohol-induced liver damage [35].

The limitation of the present study is that these aforementioned studies have provided an inconclusive statement regarding coumarin derivatives and their possible liver and renal damage, therefore, these newly introduced coumarin derivatives, CM I and II, could be a better template for designing new synthetically derived coumarins that might be safer than these aforementioned coumarins, perhaps using even much lower therapeutic doses with a deleterious impact on liver and kidney, thereby making the newly introduced coumacines considered safer than commercially available coumarins. Limitation is also represented in the minute availability of data about the newly synthesized compound [36].

Conclusion.

The newly synthesized CM I and II induced acute liver and kidney damage, particularly at a high dose that was much higher than that of the therapeutically used concentration for coumarins in clinical settings.

REFERENCES

1. Mustafa YF. Synthesis, characterization, and antibacterial activity of novel heterocycle, coumacine, and two of its derivatives. Saudi Pharm J. 2018;26:870-875.
2. Mustafa YF, Abdulaziz NT. Hymecromone and its products as cytotoxic candidates for brain cancer : A brief review. NeuroQuantology 2021;19:175-186.

3. Mustafa YF. Classical approaches and their creative advances in the synthesis of coumarins: A brief review. J Med Chem Sci. 2021;4:612-625.
4. Mustafa YF. Synthesis, characterization, and biomedical assessment of novel bisimidazole–coumarin conjugates. Applied Nanoscience. 2021:1-2.
5. Mustafa YF, Mohammed NA. A promising oral 5-fluorouracil prodrug for lung tumor: Synthesis, characterization, and release. Biochem Cell Arch. 2021;21:1991-1999.
6. Fakri Mustafa Y, Riyadh Khalil R, Tareq Mohammed E, et al. Effects of structural manipulation on the bioactivity of some coumarin-based products. Archives of Razi Institute. 2021;76:1297-1305.
7. Mustafa YF, Mohammed ET, Khalil RR. Synthesis, characterization, and anticoagulant activity of new functionalized biscoumarins. Egypt J Chem. 2021;64:4461-4468.
8. Mustafa YF, Khalil RR, Mohammed ET. Synthesis and antitumor potential of new 7-halocoumarin-4-acetic acid derivatives. Egypt J Chem. 2021;64:3711-3716.
9. Mohammed ET, Khalil RR, Mustafa YF. Phytochemical analysis and antimicrobial evaluation of quince seeds' extracts. J Med Chem Sci. 2022;5:968-979.
10. Shah D, Iqbal A, Alshehri FS, et al. The neuroprotective propensity of organic extracts of Acacia Stenophylla Bark and their effectiveness against scopolamine-/diazepam-induced amnesia in mice. Journal of Inflammation Research. 2022:4785-4802.
11. Al-Shakarchia W, Abdulazizb NT, Mustafaa YF. A review of the chemical, pharmacokinetic, and pharmacological aspects of quercetin. 2022;4:645-656.
12. Mustafa YF. Synthesis, characterization, and preliminary cytotoxic study of sinapic acid and its analogues. J Glob Pharma Technol. 2019;11:1-10.
13. Nejres AM, Ali HK, Behnam SP, et al. Potential effect of ammonium chloride on the optical physical properties of polyvinyl alcohol. Systematic Reviews in Pharmacy. 2020;11:726-732.
14. Roomi AB, Widjaja G, Savitri D, et al. SnO2: Au/carbon quantum dots nanocomposites: synthesis, characterization, and antibacterial activity. Journal of Nanostructures. 2021;11:514-523.
15. Mustafa YF. Chemotherapeutic applications of folate prodrugs: A review. NeuroQuantology. 2021;19:99-112.
16. Kasim SM, Abdulaziz NT, Mustafa YF. Synthesis and Biomedical Activities of Coumarins Derived From Natural Phenolic Acids. Journal of Medicinal and Chemical Sciences. 2022;5:546.
17. Chupradit S, Km Nasution M, Rahman HS, et al. Various types of electrochemical biosensors for leukemia detection and therapeutic approaches. Anal Biochem. 2022.
18. Mahendra CK, Tan LT, Lee WL, et al. Angelicin—A Furocoumarin compound with vast biological potential. Frontiers in pharmacology. 2020;11:366.
19. Lu H, Zhang L, Liu D, et al. Isolation and purification of psoralen and isopsoralen and their efficacy and safety in the treatment of osteosarcoma in nude rats. African Health Sciences. 2014;14:641-647.
20. Ren Y, Song X, Tan L, et al. A review of the pharmacological properties of psoralen. Frontiers in pharmacology. 2020;11:571535.
21. Javed M, Saleem A, Xaveria A, et al. Daphnetin: A bioactive natural coumarin with diverse therapeutic potentials. Frontiers in Pharmacology. 2022;13.
22. Zhang L, Gu Y, Li H, et al. Daphnetin protects against cisplatin-induced nephrotoxicity by inhibiting inflammatory and oxidative response. International Immunopharmacology. 2018;65:402-407.
23. Garg SS, Gupta J, Sahu D, et al. Pharmacological and Therapeutic Applications of Esculetin. International Journal of Molecular Sciences. 2022;23:12643.
24. Kadakol A, Malek V, Goru SK, et al. Esculetin ameliorates insulin resistance and type 2 diabetic nephropathy through reversal of histone H3 acetylation and H2A lysine 119 monoubiquitination. Journal of Functional Foods. 2017;35:256-266.
25. Deng M, Xie L, Zhong L, et al. Imperatorin: a review of its pharmacology, toxicity and pharmacokinetics. European Journal of Pharmacology. 2020;879:173124.
26. Wang X, Lou YJ, Wang MX, et al. Furocoumarins affect hepatic cytochrome P450 and renal organic ion transporters in mice. Toxicology letters. 2012;209:67-77.
27. Cao YJ, He X, Wang N, et al. Effects of imperatorin, the active component from Radix Angelicae (Baizhi), on the blood pressure and oxidative stress in 2K, 1C hypertensive rats. Phytomedicine. 2013;20:1048-1054.
28. Quetglas-Llabrés MM, Quispe C, Herrera-Bravo J, et al. Pharmacological properties of bergapten: mechanistic and therapeutic aspects. Oxidative medicine and cellular longevity. 2022;2022.
29. Tien YC, Liao JC, Chiu CS, et al. Esculetin ameliorates carbon tetrachloride-mediated hepatic apoptosis in rats. International journal of molecular sciences. 2011;12:4053-4067.
30. Zhou W, Chen X, Zhao G, et al. Psoralen induced liver injury by attenuating liver regenerative capability. Frontiers in Pharmacology. 2018;9:1179.
31. Yang L, Yu YL, Cheng LY, et al. The accumulation of psoralen contributes to its hepatotoxicity revealed by pharmacokinetic and toxicokinetic study after repeated administration. ACS omega. 2020;5:7307-7315.
32. Kanimozhi G, Prasad NR, Ramachandran S, et al. Umbelliferone modulates gamma-radiation induced reactive oxygen species generation and subsequent oxidative damage in human blood lymphocytes. Eur J Pharmacol. 2011;672:20-29.
33. Jung HA, Park JJ, Islam MN, et al. Inhibitory activity of coumarins from Artemisia capillaris against advanced glycation endproduct formation. Arch Pharm Res. 2012;35:1021-1035.
34. Zhao D, Islam MN, Ahn BR, et al. In vitro antioxidant and anti-inflammatory activities of Angelica decursiva. Arch Pharm Res. 2012;35:179-192.
35. Sim MO, Lee HI, Ham JR, et al. Anti-inflammatory and antioxidant effects of umbelliferone in chronic alcohol-fed rats. Nutrition research and practice. 2015;9:364-369.
36. Al-Shakarchi W, Saber Y, Merkhan MM, et al. Sub Chronic Toxicity Study of Coumacines. Pharmacognosy Journal. 2023;15.