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ΦΑΡΜΑΚΕΥΤΙΚΗ, 35, IV, 2023 | 57-63 ΕΡΕΥΝΗΤΙΚΗ ΕΡΓΑΣΙΑ

PHARMAKEFTIKI, 35, IV, 2023 | 57-63 RESEARCH ARTICLE

Characterization of the renal safety profiles of coumacines

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KEYWORDS:
Coumacine, coumarin, anticoagulants, renal function.

ARTICLE INFO:
Received: March 21,2023
Revised: May 14, 2023
Accepted: June 17, 2023
Available on line: November 20, 2023

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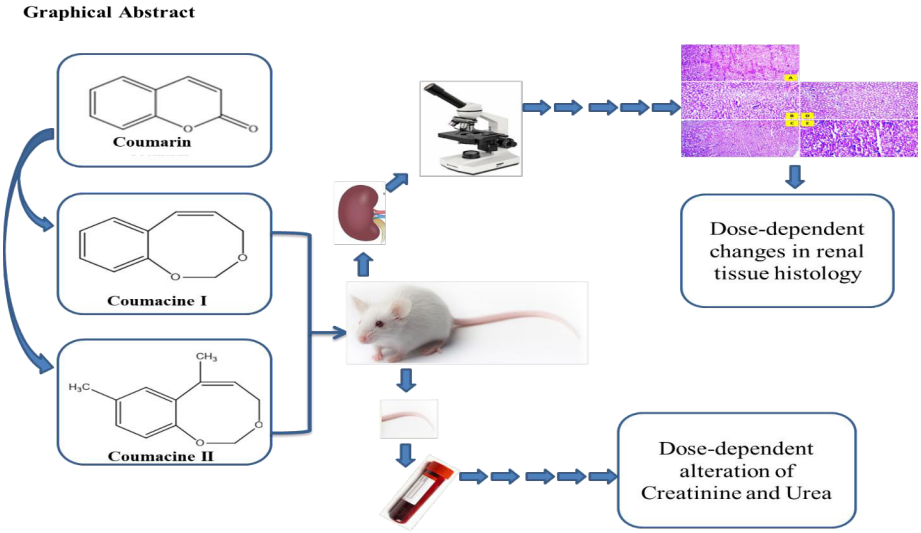
ABSTRACT

The bottleneck step following the synthesis and characterization of the drug in the pharmaceutical setting is their adverse effects in the light of useful therapeutic doses. The kidney as the main clearance organ is the target for metereos of chemicals, xenobiotics, and drug metabolites. The present study aimed at characterizing the renal safety profile of newly synthesized coumarins-coumacine I and II. To do so, a mouse model was used with a total of 30 mice subclassified into 5 groups; Group 1 (Control group): given placebo vehicle IP for five consecutive days, Group 2: given Coumacine I at a dose of 250mg/kg IP, Group 3: given Coumacine I at dose 500mg/kg IP, Group 4: given Coumacine II at dose 250mg/kg IP, and Group 5: given Coumacine II at dose 500mg/kg IP, for each treated group coumacines given for five consecutive days. Blood samples were withdrawn at the end of the study from sacrificed animals and kidneys harvested for histological study. The results confirmed that serum creatinine and urea rose significantly ($p<0.05$) in the high-dose group compared to the control or low-dose group. Histological study revealed that mild degenerative changes are associated with a low dose of coumacine compared to moderate or severe degeneration associated with a high dose of either coumacines. This pilot study provides promising future direction for the discovery of new medication with anticoagulant therapy with improved pharmacokinetics or additional pharmacodynamic properties.



1. **Introduction**

Coumarins are a significant and broad class of oxygen heterocycles that are frequently discovered as secondary metabolites produced by plants[1]. The coumarins can be generally classified into 7 groups; simple coumarins, furanocoumarins, pyranocoumarins, dicoumarins, dihydrofuranocoumarins, isocoumarins, and phenylcoumarins[2]. Coumarines are a widely expanded group of drugs in the last decade several members were introduced in the pipeline of therapeutic applications in clinical settings[3]. The biochemical and pharmacological aspects with wide therapeutic efficacy of coumarin make this group of clinical importance including their application in high protein oedema[4], chronic infections[5], cancer treatment[6], antioxidants[7], anti-inflammatory[8], blood coagulation and anticoagulant[9].

Formerly, coumarin has been used as rodenticide due to its vitamin K inhibition properties resulting in internal bleeding and death[10]. The nucleus structure of coumarin enriched the group for the synthesis of new compounds and thereby increased new derivatives in a timely manner[11].

The area of research concerning drug discovery and development was increasingly challenging due to the potential involvement of various parameters in the path of synthesis and experimentation[12], especially in terms of the adverse effect profile of the newly synthesized drugs. Adverse effects on vital organs are the first on the pipeline to be considered with highlighted focuses on the kidney as a major clearance organ[13]. Heterocyclic compounds are increasingly reported as a rich chemical resource for new drug developments[14]. New heterocyclic compounds, under name of coumacines, were synthesized and subsequently characterized for their physicochemical properties by Mustafa Y.F. in 2018 (Figure 1)[15]. The therapeutic potential regarding their antioxidants, anticancer, and other effects have been subsequently characterized in a series of studies[13-15]. Moreover, their adverse effects profile and dosage toxicity were reported using laboratory animals[16].

Coumarines as well-known anticoagulant drugs have potentially been reported to preserve renal functions providing protection against renal damaging compounds[17]. Being excreted though kidney, coumarins need dose adjustment in patients with renal dysfunction[18]. The present study aimed to characterize the safety profile of renal toxicity in experimental animals using the newly derived coumacine compounds, namely coumacine I (CMI), and coumacine II(CMII) as a target for future product release into phase II trial.

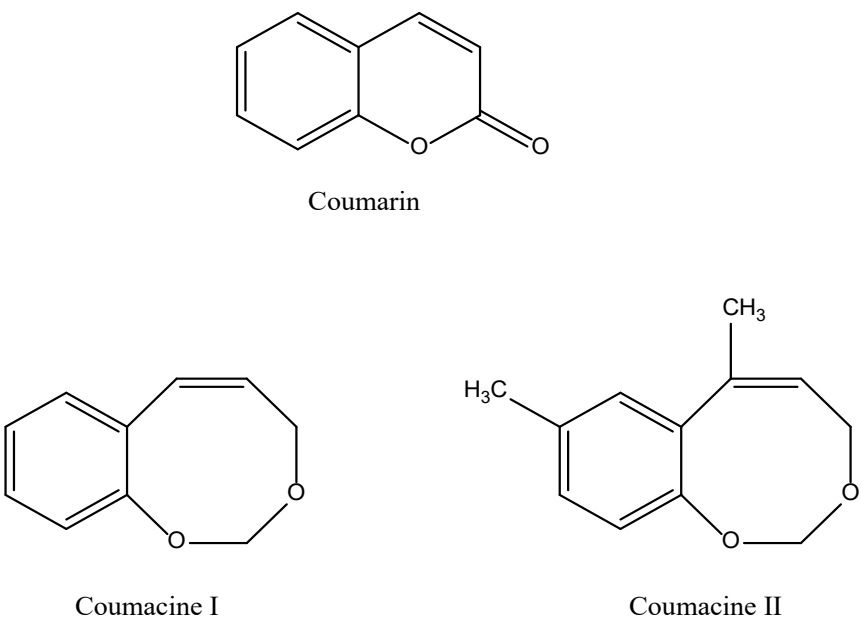


Figure 1. *Chemical backbones of coumarin chemical nucleus, coumacine I (CMI), and coumacine II (CMII)[15].*

2. Materials, and Methods

Chemicals: Coumacine I and II were originally synthesized by Mustafa YF[15] in the College of Pharmacy/university of Mosul. Characterised for physicochemical properties and potentially a candidate for subsequent biological activity. In the present study, Coumacine I and II have been dissolved instantly in a 10% solution of hydroxypropyl beta-cyclodextrin (HPBCD) to enhance their solubility.

Animal treatment: Replicates of 6 mice per group were used in the present study (30 mice in total, 8 weeks age, 2-3 mice per cage, temperature 23-25 ˚C and humidity of 50-55%). Standard food pellets *ad libitum* and free access to water. The 30 mice were divided as follows:

Group 1 (Control group): given 10% HPBCD solution IP for five consecutive days

Group 2: given Coumacine I at a dose 250mg/kg IP for five consecutive days

Group 3: given Coumacine I at a dose 500mg/kg IP for five consecutive days

Group 4: given Coumacine II at a dose 250mg/kg IP for five consecutive days

Group 5: given Coumacine II at a dose 500mg/kg IP for five consecutive days

Blood samples were withdrawn from all mice on day 6, serum was isolated, and samples were frozen for further analysis.

Renal function tests measurements: As per manufacturer instructions creatinine (kit supplied by Biolabo Cat No. 90107, France) was measured by Enzymatic Method. The principle of this assay is based on the lysis of endogenous creatinine in samples through two consecutive steps, which culminate in the formation of hydrogen peroxide. The amount of hydrogen peroxide produced is reciprocally related to the concentration of creatinine in the samples. The colourless hydrogen peroxide was converted to a chromogenic compound via n-ethyl-n-sulphopropyl-m-toludine and 4-amino antipyrin to the coloured compound to be detected at an optical density of 545 nm[19].

As per manufacturer instructions blood urea (kit supplied by Biolabo Cat No. 90107, France) was measured by Enzymatic Method. The principle of as-

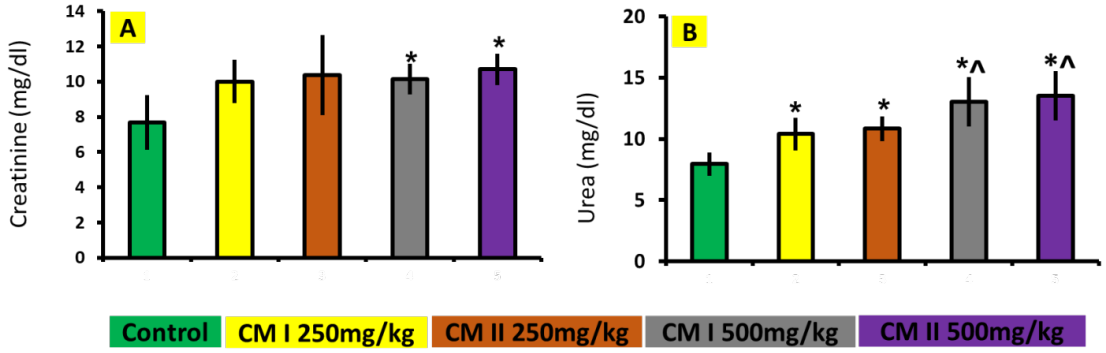


Figure 2. *Renal function tests of coumacine I and II at different doses (A) creatinine, (B) Urea. Data expressed as mean±SD. *^P< 0.05, *significant as compared to control group, ^significant as compared to coumacine I and coumacine II group at low dose.*

say based on lysis of endogenous creatine through two consecutive steps ending with the formation of hydrogen peroxide reciprocally related to creatine in the samples. The colourless hydrogen peroxide were converted to a chromogenic compound via n-ethyl-n-sulphopropyl-m-toludine and 4-aminoantipyrin to coloured compound to be detected at an optical density of 545nm[20].

Histological study: Kidney harvested from sacrificed mice immediately, were quickly washed with normal saline and fixed overnight in formalin. The next day, samples were embedded in paraffin blocks. These tissue blocks were sectioned (5 µm) stained with eosin-hematoxyline, and examined under a light microscope[21].

Statistics: Analysis of parametric data was conducted using IBM SPSS statistics 25 software. To compare groups, one-way ANOVA with Bonferroni tests as a post hoc test was performed to determine variations among groups. Data were expressed as mean values ±SD. Using a power of 80% or greater to determine sample sizes and an alpha error level (P) of ≤ 0.05 in all experiments is necessary for statistical significance.

3.Results

Analysis of the results concerning the renal function tests has revealed that the level of serum creatinine rose significantly ($p<0.05$) in high-dose of CMI (10.1±0.9 mg/dl) and CM II (10.7±0.9 mg/dl) compared to the control group (7.7±1.5 mg/dl). However, serum creatinine levels in low dose of CMI (10±1.2 mg/dl) and CMII (10.4±2.3 mg/dl) have shown non-significant differences when compared to the control group (7.7±1.5 mg/dl) (Figure 2A).

Analysis of the results concerning the level of serum urea revealed significant increase ($p<0.05$) in high-dose of CM I (13±2 mg/dl) and CMII (13.5±2 mg/dl) compared to the control group (8±1 mg/dl) or low-dose CMI (10.4±1.3) and CMII (10.8±1), (Figure 2B).

The histological study of the exposed tissue to coumacines was associated with oedematous changes, tissue degeneration, vacuole formation, and angioedema. However, these changes were represented as severe damage with high doses of coumacine I and II compared to low-dose coumacines or normal histology of the control group (Figure 3)

4. Discussion

The present study confirmed that the newly derived compounds from coumarins namely coumacine I and II are relatively safe particularly at low doses, inducing minimal renal toxicity at 250mg/kg whether CMI or CMII. This assumption was confirmed by measured

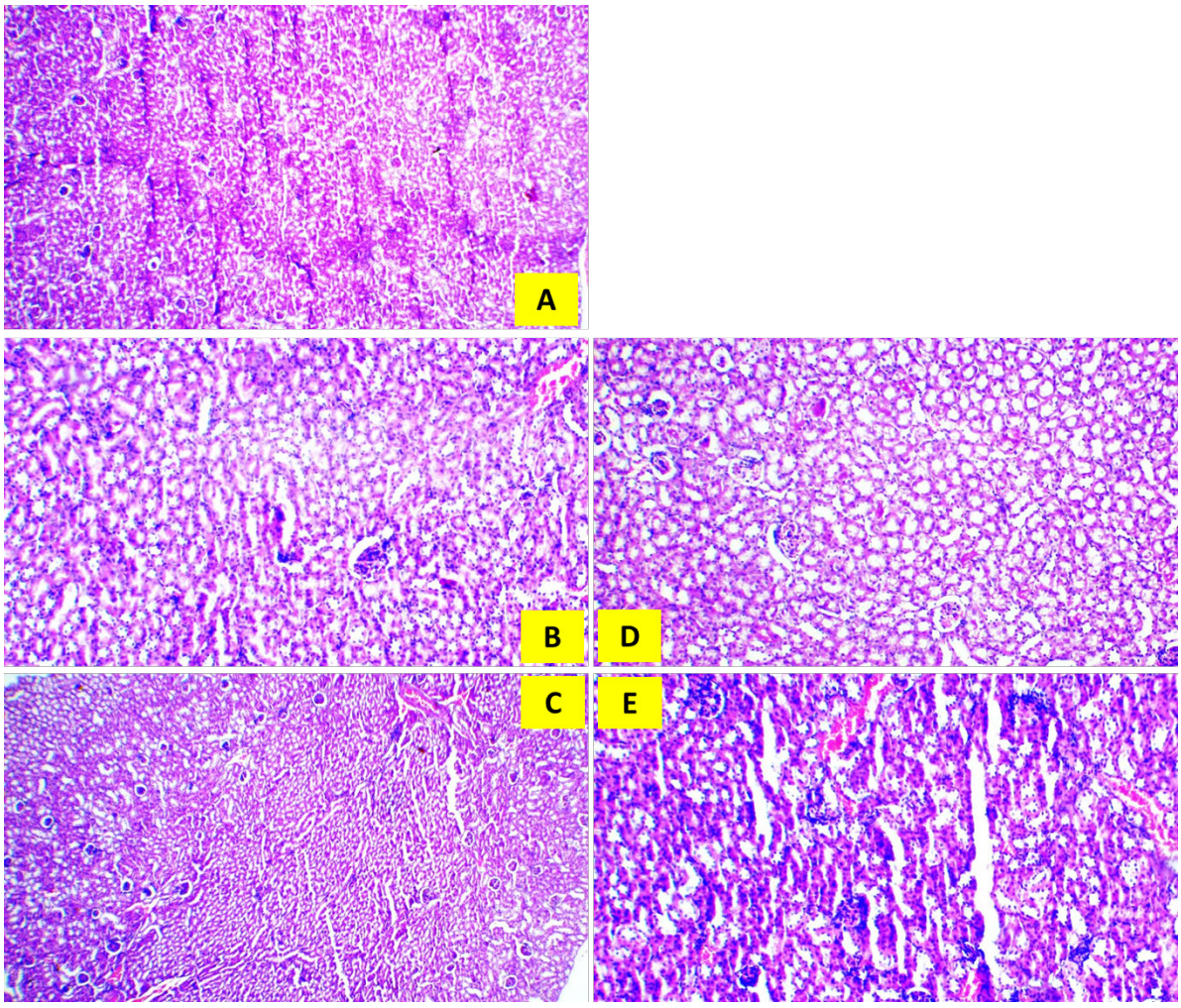


Figure 3. *Shows renal tissue morphology A) control group showed intact renal collecting tubules, glomeruli, and renal tubules B) renal tissue exposed to a subchronic dose of coumacine I at 250 mg/kg which shows no clear difference from the control C) coumacine II at 250 mg/kg which shows oedema and signs of degeneration D) coumacine I at 500 mg/kg renal tissue shows mild changes and degenerations E) coumacine II 500 mg/kg show dramatic signs of degeneration, necrosis, addition to in nephrons units.*

renal function tests and histological kidney characterization. The outcome revealed that low doses induced minimal elevation in urea and creatinine alongside minimal histological changes compared to high-dose of either coumacines. Most of the available literature was directed towards a well-established and clinically used coumarin (warfarin). Therefore, the present study could be considered a pilot study.

The standards of coumarin toxicity have recently been reported as a part of structural variations since fluorocoumarins (e.g. psoralen and angelicin) have been reported as a renal damaging entity. Several studies have shown that psoralen and angelicin can cause severe kidney damage in animals and humans. Fluorocoumarins are a type of coumarin that contain a fluorine atom. This structural variation makes

them more toxic than other coumarins. Psoralen and angelicin are two of the most commonly studied fluorocoumarins. They are often used in the treatment of skin disorders such as psoriasis and vitiligo. However, they have been found to cause kidney damage in some patients. The mechanism by which psoralen and angelicin cause kidney damage is not yet fully understood. However, it is believed that they may interfere with the normal functioning of the kidneys. They may also cause inflammation and oxidative stress within the kidneys, leading to damage and dysfunction[22-24.] Renal vascular congestion, inflammatory cell infiltration, and renal capillary dilatation has been reported with a combination of cisplatin with psoralen or angelicin[25.] Conversely, daphentin (a herbal-derived coumarin analogue) blocked cisplatin-induced renal toxicity, as confirmed by measured renal function tests, reduced pro-inflammatory parameters, shifted the balance toward antioxidant activity, and blocked apoptosis[26.]

On the other hand, simple coumarin might induce no harmful effects, and perhaps some of them might paradoxically induce beneficial effects, such as esculetin has shown that it improved the prognosis of patients with diabetic nephropathy mainly inducing antioxidant enzymes imparting tissue protection[27], however, imperatorin as a simple coumarin has reported renal toxicity in mice models, thereby the coumarin toxicity is structural rather than group-based[28-30].

5. Conclusion

Low doses of newly discovered coumacines have greatly shown safe or mild effects on the kidney compared to high doses providing a template for the discovery of new derivatives with improved pharmacokinetic and pharmacodynamics profiles. □

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