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## CARBON DOT DRESSING AS A TREATMENT OF ALKALI-INDUCED SKIN BURNS

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**Background.** Chemical burns, comprising 5–10 % of total burns but causing 30 % of burn-related deaths, are now a notable concern in Ukraine. Current clinical protocols lack specific approaches for chemical burns, and research on this type of burn is limited. Carbon-based nanoparticles show promise for wound healing because of anti-inflammatory, antioxidant, and antibacterial activities. So, the ability of carbon dots obtained from citric acid and urea (further called CD) to improve the healing of alkali-induced skin burn was aimed to be discovered.

**Materials and Methods.** The study was conducted on male Wistar rats. Burn was modeled by application of gauze disc soaked with 3 M NaOH solution on shaved skin of anesthetized rats for 10 min. A CD dressing, consisting of a CD solution (1 mg/mL) mixed with cellulose-based hydrogel that served as a vehicle, was applied to burned skin daily during a 7-day period. There were following groups: control (healthy rats), a burn-only group (rats that received no dressing), a burn + vehicle group (rats that received vehicle dressing), and a burn + CD group (rats that received CD dressing). The study involved monitoring of burn areas, conducting skin histopathology, and performing blood biochemical analyses.



**Results.** The daily CD dressing significantly decreased alkali-induced burn area (by 76 % compared to 40 % in burn-only group) after seven daily dressings. The level of inflammation in the burn site was also less expressed in CD-treated animals, compared to respective controls (non-treated animals and animals treated with Vehicle). There was no substantial systemic toxicity of the burn (of such area) and its healing, manifested by absence of body weight loss, and absence of dramatical changes in serum biochemical parameters (indicators of liver and kidney function). However, animals of all the groups that experienced burns had a significantly lower body weight gain and mesenteric lymph nodes weight compared to healthy rats.

**Conclusions.** So, the application of carbon dots mixed with hydrogel speeded up alkali-induced burn healing without negative impact on the organism.

**Keywords:** carbon dots, alkali-induced burn, skin inflammation

## INTRODUCTION

According to the World Health Organization, burns constitute a global problem, causing approximately 180,000 deaths annually. Although chemical burns account for only 5–10 % of total burns, they contribute to 30 % of burn-related deaths (Hardwicke, *et al.*, 2012) due to their severe impact on the body, complicated pathogenesis, and the absence of optimal treatment protocols. Until recently, chemical burns were not a significant concern for the healthcare system in Ukraine. However, with the onset of military conflict, this issue has become prominent. Currently, there is a lack of statistical data on the proportion of chemical burns among all types of injuries or the overall prevalence of chemical burns in the Ukrainian population. Nevertheless, these figures are likely to be quite high.

Clinical protocols for providing medical assistance to burn patients and addressing their consequences (Standard of Burns Medical Care, 2023) offer general recommendations, including analgesics, corticosteroids as anti-inflammatory agents, antibiotic therapy, and shock therapy, depending on the severity of the injury. However, specific approaches that consider the nature of chemical exposure are absent. Although there is a fundamental difference in the development and resolution of thermal and chemical burns, research on the latter is scarce (Goertz, *et al.*, 2013).

Nanoparticles of various origins, especially those based on carbon, have attracted researchers' attention as universal tools to influence living organisms. Carbon nanocomposites, doped with hetero-atoms and various functional groups capable of fluorescence (used for bio-visualization) (Yu, *et al.*, 2021), exhibit anti-inflammatory (Lee, *et al.*, 2020), antibacterial, antiviral properties (Ghirardello, *et al.*, 2021), and can suppress or stimulate cell proliferation (Lu, *et al.*, 2019), as well as provide targeted drug delivery (Park, *et al.*, 2021). All these properties provide a basis for considering nanocomposites as potential wound-healing agents (Kasouni, *et al.*, 2021) suitable for application in burn injuries, and as a tool for visualizing the fate of different cell populations during this pathological process.

Based on the above, the aim of the study was to investigate the ability of carbon dots obtained from citric acid and urea to improve the healing of alkali-induced skin burn and to mitigate its harmful effects on the organism.

## MATERIALS AND METHODS

The study was aimed to assess the healing effect of carbon dots obtained from citric acid and urea (further referred to as CD) which have the surface enriched with a large number of oxygen-containing groups (carboxyl and phenol types predominantly)

if applied as a dressing. Carbon dots used in this study were prepared by solvothermal carbonization of a mixture of urea and anhydrous citric acid, taken in a molar ratio of 2:1. CD preparation and characterization was described in detail in (Kuznietsova, *et al.*, 2023b; Mussabek, *et al.*, 2022; Ivanov, *et al.*, 2021; Dubyk, *et al.*, 2022). CD dressing was prepared by mixing CD solution in a saline (1 mg/mL) with commercially available hydrogel Hydrosorb Gel (Hartmann, Germany) that consisted of Ringer solution, carboxyethyl- and carboxymethyl-cellulose, in a 1:1 ratio. Cellulose-based hydrogels are commonly employed as a coating for wound and burn treatment (Alven, *et al.*, 2020; Ribeiro, *et al.*, 2019), so this product was chosen as a vehicle for CD, and as a reference.

The study was conducted on young male Wistar rats with initial body weight  $182.4 \pm 21$  g (CV = 1–1.4 %). Animals were kept in the animal facility of Taras Shevchenko National University of Kyiv under natural lightning at 20–23 °C, and free access to standardized rodent diet and tap water. All experiments were conducted in compliance with bioethics principles, legislative norms and provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, General Ethical Principles for Experiments on Animals adopted by the First National Bioethics Congress (Kyiv, 2001). All *in vivo* protocols were approved by Institutional Animal Care and Use Committee of Taras Shevchenko National University of Kyiv (Protocol No 3, dated 2.05.2019).

For the procedure of inducing chemical burns, rats were anesthetized with telazol (5 mg/kg, intraperitoneally) and xylazine hydrochloride (8 mg/kg intramuscular); fur on their withers was shaved. Gauze discs (2.5 cm diameter, approx. 490 mm<sup>2</sup>) were soaked with 3 M NaOH solution and applied onto the skin for 10 min (Andrews, *et al.*, 2003, modified). An hour after burn induction, either vehicle- or CD-contained dressing was applied on the burn surface, the treatments were repeated daily for seven days.

There were 4 groups of rats (n = 8 in each group): 1) Control (healthy rats with no burns); 2) Burn (rats subjected to burn induction that received no treatment); 3) Burn + Vehicle (rats subjected to burn induction, with a subsequent daily application of hydrogel to the burn for seven days); 4) Burn + CD (rats subjected to burn induction with a subsequent daily application of a CD-containing dressing to the burn for seven days). Animals' body weight was monitored throughout the study period. The animals were sacrificed seven days after burn induction, and blood and tissue samples were collected. Euthanasia was performed after terminal bleeding using cervical dislocation. Then, autopsy was performed, and immune-competent organs were weighed.

To assess burn healing dynamics, burns were photographed daily before the application of dressing. Their area was measured from the photographs using ImageJ software. The burn area change was calculated on a daily basis as a percentage change of the initial burn area (considered as an area of NaOH-soaked disc application) for each individual animal.

For biochemical analysis, blood was collected immediately after the anesthesia from femoral vein, serum was separated after blood centrifugation. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), g-glutamyl transpeptidase (GGT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), urea, and creatinine were determined in the serum using commercial kits (DiagnosticumZrt, Hungary) according to the manufacturer's instructions (<http://www.diagnosticuminc.eu/Products.php>).

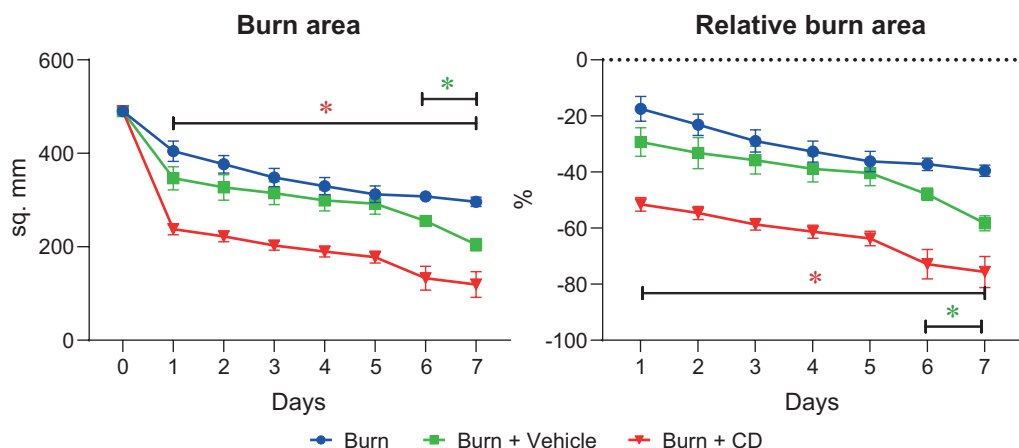
For histopathological analysis, skin samples from the site of burn from each animal were placed into 10 % neutral buffered formalin immediately after harvesting, processed accordingly (Kiernan, 2008) for hematoxylin and eosin (H&E) staining, and examined

under the light microscope. The occurrence and prevalence of cells involved into the immune response (macrophages, neutrophils, lymphocytes), as well as edema, apoptosis, necrosis, hemorrhages, connective tissue accumulation were determined.

Statistical analysis of the data was performed using either two-way (burn area dynamics) or one-way ANOVA (using GraphPad Prism v.9.0.0 software). The data were presented as Mean $\pm$ SEM, the difference was considered statistically significant at  $p < 0.05$ .

## RESULTS AND DISCUSSION

Either absolute or relative burn areas were significantly lower in the Burn + CD group on days 1–7 of the study compared to the Burn group (non-treated). The final burn area decrease (on the terminal day of the study) was 76 % in this group compared to a 40 % decrease in non-treated animals (Burn group). In the Burn + Vehicle group, the wound area was significantly smaller than that of the non-treated rats only on days 6–7 (**Figs. 1, 2**), demonstrating a 58 % decrease, which proves the healing effect of CD in particular.

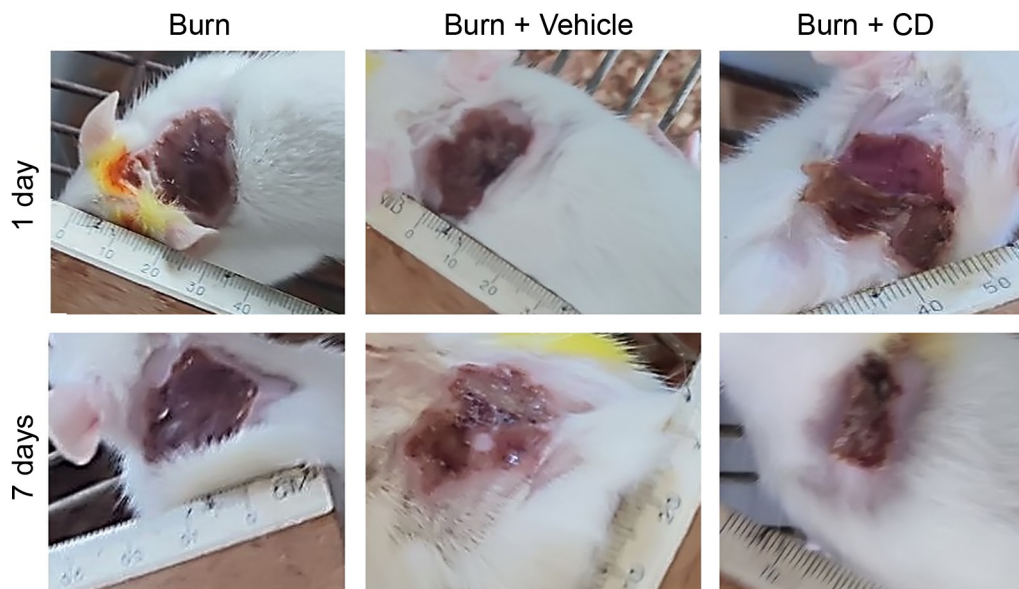


**Fig. 1.** Burn area dynamics during the study; \* $p < 0.05$  compared to non-treated animals (Burn group), color of the asterisk represents the comparison group on the appropriate day of the study

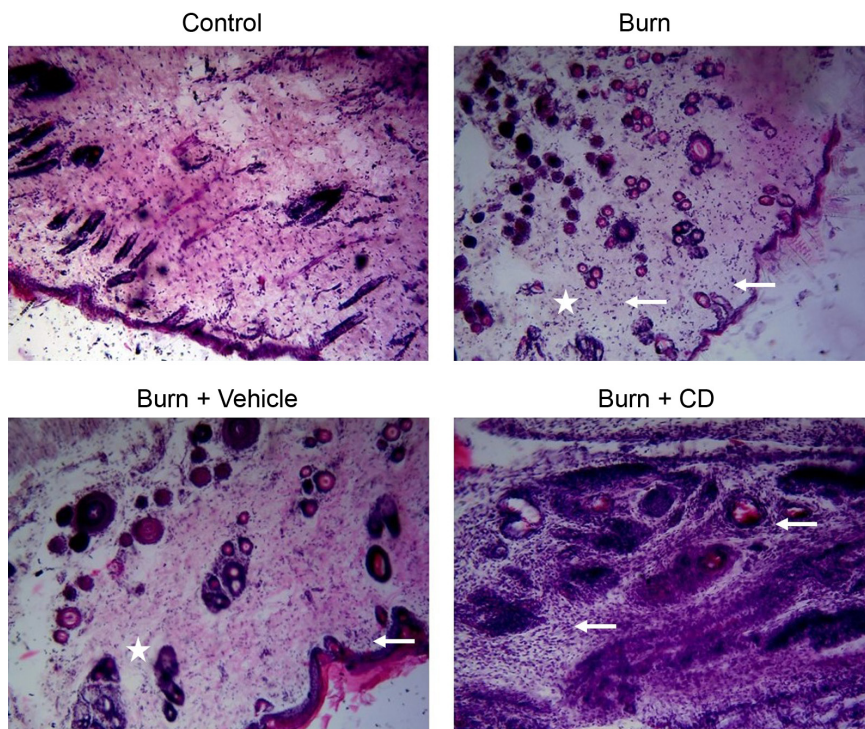
CDs were demonstrated to exhibit antimicrobial and wound-healing properties in the context of infectious wounds. Due to the presence of surface defects and unpaired electrons, CDs can efficiently eliminate surplus free radicals, thereby mitigating oxidative stress damage. This process facilitates the swift transition from wound inflammation to proliferation and expedites the overall wound-healing process (Qu, *et al.*, 2023). Additionally, the treatment with CDs significantly reduced the production of reactive oxygen species by macrophages in infectious wounds, concurrently prompting macrophage polarization towards an anti-inflammatory phenotype (M2). This polarization results in decreased inflammation and expedites pace of wound healing (Gujju, *et al.*, 2023).

According to histopathological analysis, rats which had burns seven days after burn induction expressed substantial inflammation and edema, with tissue populated with different inflammatory cells with the prevalence of neutrophils (macrophages and lymphocytes were also widely presented). The same features were observed in Vehicle-treated animals (Burn + Vehicle group). Rats treated with CD (Burn + CD group) also revealed similar signs but less expressed than non-treated animals (Burn group), which is in line with the burn area dynamics (**Fig. 3**). No edema was observed in this group. It should be





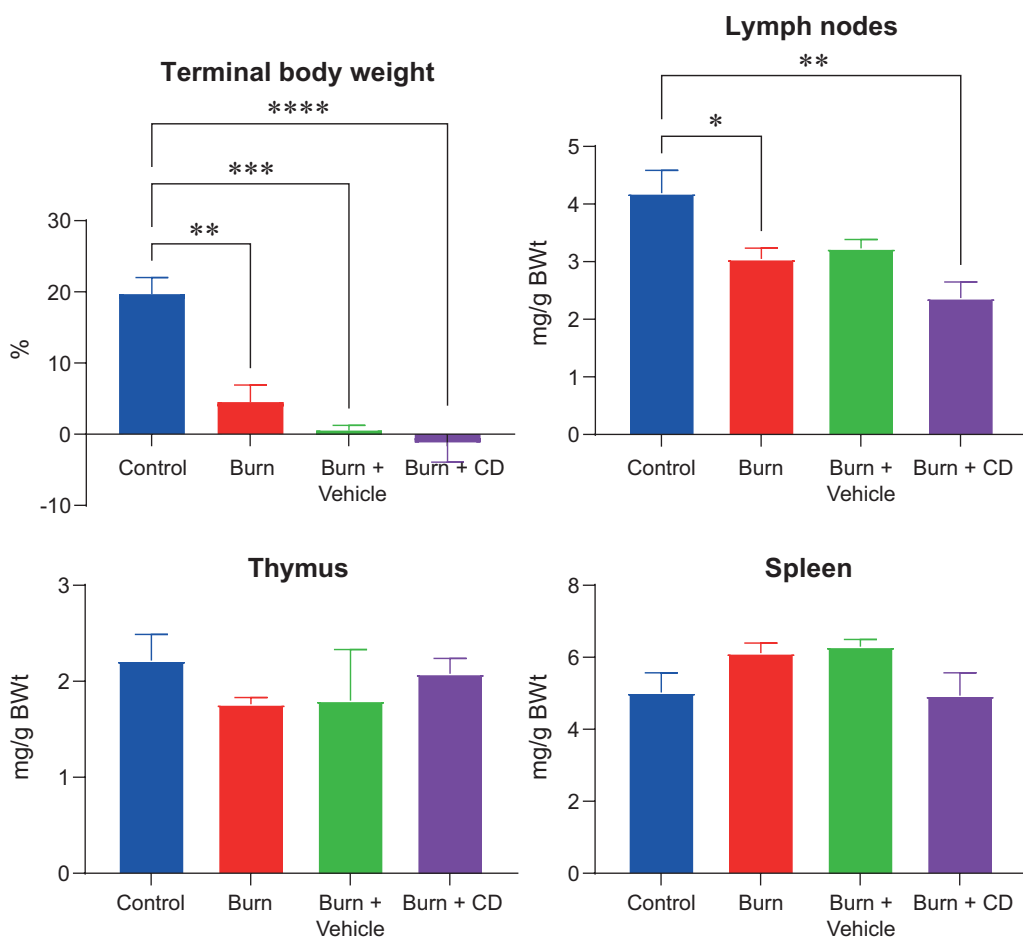
**Fig. 2.** Photographs of burns of rats that experienced burns and were treated with Vehicle or CD on the 1st and the last (7th) days of the study



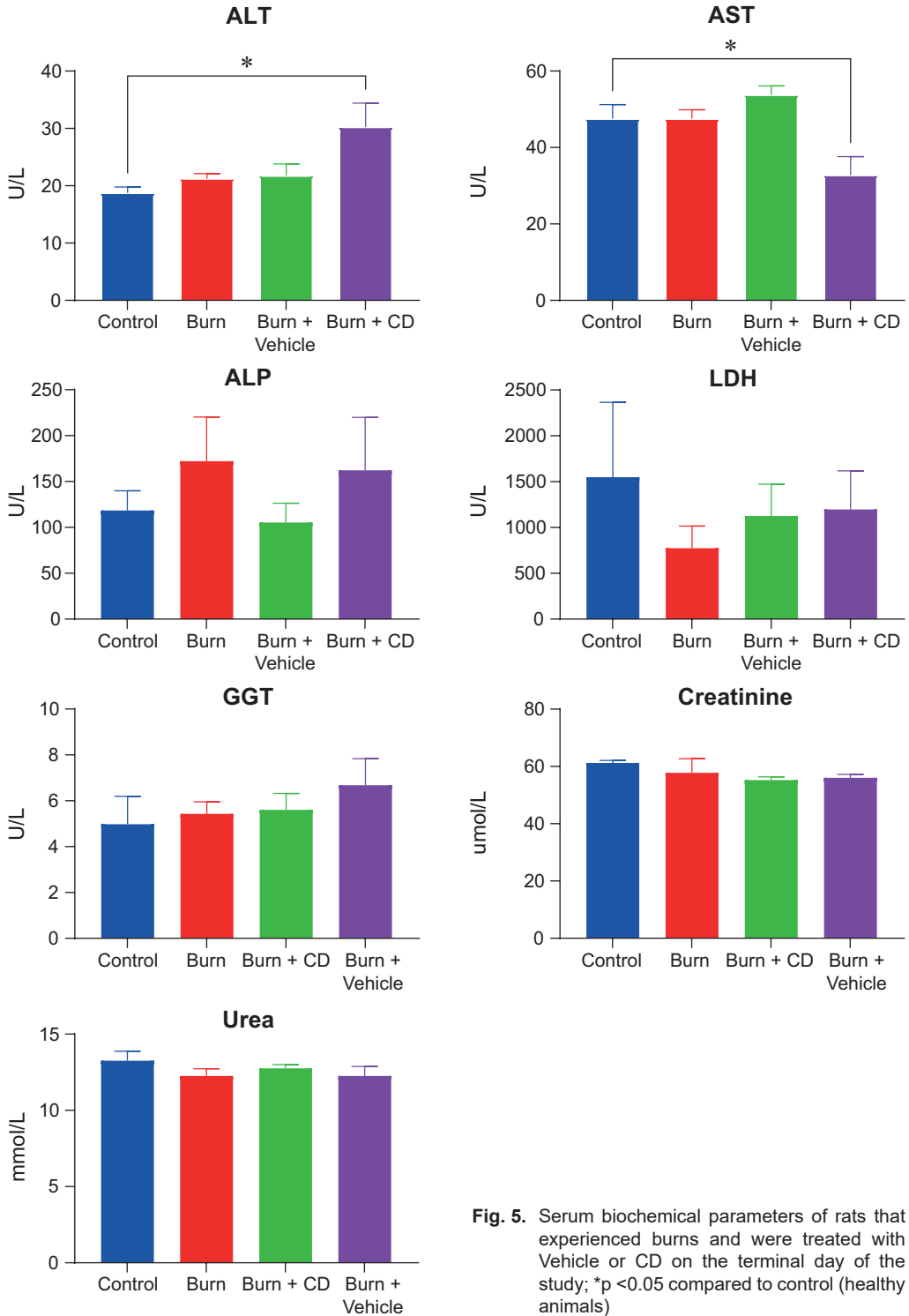
**Fig. 3.** Microphotographs of skin samples from the site of burn of rats that experienced burns and were treated with Vehicle or CD on the terminal day of the study. Asterisks indicate edema regions, arrows – inflammatory cells accumulations. H&E,  $\times 400$

noted, that control samples (skin from withers of healthy animals) demonstrated slight population of derma with tissue macrophages and lymphocytes, and slight connective tissue accumulation (**Fig. 3**), which is typical for skin.

Animals did not lose weight during the study, however, seven days after burn induction relative body weight gain was significantly lower in all groups that experienced burns compared to the control group (healthy animals), which might evidence a systemic effect of burn on the organism. According to autopsy findings, the relative weight of lymph nodes decreased (significantly or as a tend) seven days after burn induction in all groups that experienced burns (either treated or non-treated animals) (**Fig. 4**). According to the literature, burn injury causes diminished gut immunity, hypoperfusion and gut dysmotility, which leads to increase in intestinal permeability (due to hypoperfusion and subsequent tissue inflammation and damage) and enables the gut intestinal bacteria and their products (such as endotoxin) to cross the intestinal epithelial barrier



**Fig. 4.** Relative terminal body weight expressed as percentage change compared to the initial one, and weights of immune-competent organs of rats that experienced burns and were treated with Vehicle or CD on the terminal day of the study; \* $p < 0.05$  compared to control (healthy animals)



**Fig. 5.** Serum biochemical parameters of rats that experienced burns and were treated with Vehicle or CD on the terminal day of the study; \*p < 0.05 compared to control (healthy animals)

into the systemic or lymphatic circulation and contribute to the pathology after burn injury (Jeschke, *et al.*, 2020). So, the lymph node weight decrease might be caused by hypoperfusion of the intestinal tissues. It should be noted that the relative weights of other immune-competent organs like spleen and thymus did not change in animals exposed to burns compared to the healthy ones.

According to biochemical analysis (**Fig. 5**), there was an increase in ALT and a decrease in AST activities in CD-treated rats, which might evidence some toxicity against the liver, not because of burn experience but rather as a result of a combined action of burn and CD burden.

The potential of carbon dots to be accumulated in the liver and affect it is known (Hong, *et al.*, 2018), but liver function impairment is barely observed (Zhang, *et al.*, 2020; Kuznietsova, *et al.*, 2023a, 2023b). As the skin of burned animals is damaged, so absorption through wound surface is possible which leads to the entry of CD into the systemic circulation, and its eventual accumulation in the liver. However, even statistically significant changes in the activity of these enzymes were not dramatic and did not suggest liver toxicity or some tendencies in its functional state during burn experience. Creatinine and urea levels were not changed in any of the experimental groups, indicating no impact on kidney functional state during burn injury resolution (**Fig. 5**).

## CONCLUSION

Summarizing, the daily application of CD dressings significantly decreased the alkali-induced burn area and the level of inflammation in burn sites during the study, compared to respective controls (non-treated animals and animals treated with Vehicle). In addition, there was no substantial systemic toxicity of the burn (of such area) and its healing, manifested by the absence of body weight loss, and the absence of dramatical changes in serum biochemical parameters (indicators of the liver and kidney function).

Thus, the application of carbon dots speeded up alkali-induced burn healing without negative impact on the organism.

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## COMPLIANCE WITH ETHICAL STANDARDS

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Human Rights:** This article does not contain any studies with human subjects performed by any of the authors.

**Animal studies:** All international, national, and institutional guidelines for the care and use of laboratory animals were followed.



## AUTHOR CONTRIBUTIONS

Conceptualization, [H.K.; N.D.]; methodology, [H.K.; N.D.]; validation, [H.K.; N.D.]; formal analysis, [H.K.; N.D.]; investigation, [H.K.; N.D.; A.I.; R.B.; B.B.; M.K.; I.L.; T.L.; O.O.; T.R.; Y.R.; A.Z.]; resources, [H.K.; N.D.; Y.R.]; data curation, [N.D.; T.L.]; writing – original draft preparation, [H.K.]; writing – review and editing, [H.K., N.D.]; visualization, [H.K.] supervision, [N.D.]; project administration, [N.D.]; funding acquisition, [H.K.; N.D.; Y.R.].

All authors have read and agreed to the published version of the manuscript.

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## ВУГЛЕЦЕВІ ТОЧКИ ЯК ЗАСІБ ЗАГОЄННЯ ЛУЖНО-СПРИЧИНЕНИХ ОПІКІВ ШКІРИ

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**Вступ.** Хімічні опіки, які становлять 5–10 % від загальної кількості опіків і спричиняють 30 % смертей, пов'язаних з опіками, зараз є значущою проблемою в Україні. Діючі клінічні протоколи не мають конкретних підходів до лікування хімічних опіків, а дослідження цього типу опіків обмежені. Наночасткам на основі вуглецю притаманна ранозагоювальна здатність завдяки протизапальним, антиоксидантним і антимікробним властивостям. Отже, метою роботи було вивчити можливість використання вуглецевих точок, отриманих із лимонної кислоти й сечовини (далі CD), для поліпшення загоєння шкірних опіків, спричинених лугом.

**Матеріали і методи.** Дослідження проводили на щурах-самцях лінії Вістар. Опік моделювали за допомогою аплікації марлевого диска, змоченого 3 М розчином NaOH, на голену шкіру наркотизованих щурів на 10 хв. Розчин CD (1 мг/мл) змішували з гідрогелем на основі целюлози та наносили на уражену шкіру щоденно протягом 7 днів, починаючи з дня моделювання опіку. Було сформовано групи: контроль (здорові щури), група опік (щури, які не зазнавали обробки), група опік + носій (щури, які зазнавали обробку лише гідрогелем), і група опік + CD (щури, які зазнавали обробку CD). Вимірювали площу опікового ураження, термінально здійснювали гістопатологічний аналіз шкіри та біохімічний аналіз крові.

**Результати.** Щоденна обробка CD значно зменшувала площу опікового ураження, спричиненого лугом (на 76 %, порівняно з 40 % у групі опіку), після 7 обробок. Рівень запалення на місці опіку також був менш вираженим у тварин, які обробляли CD, порівняно з відповідними контролями (тваринами з необробленим опіком і такими, яких обробляли тільки гідрогелем). Суттєвої системної токсичності,

спричиненої опіковою травмою такої площі та її загоєнням, виявлено не було, про що свідчить відсутність втрати маси тіла та відсутність значних змін у біохімічних параметрах сироватки (показників функції печінки та нирок). Однак у всіх груп тварин з опіками спостерігали суттєво менші приріст маси тіла і масу мезентеріальних лімфатичних вузлів порівняно зі здоровими щурами.

**Висновки.** Отже, застосування вуглецевих точок прискорювало загоєння лужних опіків без негативного впливу на організм.

**Ключові слова:** вуглецеві точки, лужний опік, запалення шкіри