Université Badji Mokhtar Annaba . Algérie

Direction des Publications

e-ISSN : 2352-9717

Revue des Sciences et de la Technologie

Synthèse

Volume 30
Numéro 01, Juin 2025

ISSN - 1111 - 4924

Université Badji Mokhtar, Annaba
Direction des Publications

Revue des Sciences et de la Technologie
Synthèse

جامعة بـــاجـــي مختـــار - عنـــابـــة -
مديـــرية النشـــر

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Section A (Maths, Chemistry, Physics, Computer Science)
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Rédaction et Administration
Direction des Publications,
University Badji Mokhtar,
BP 12, Annaba, 23000, Algeria.
Tel/Fax : 213 (0) 38 57 00 04
E-mail : revue.synthese@gmail.com

Dépôt légal : 414 – 1995 **ISSN – 1111 – 4924** **e-ISSN : 2352-9717**

كلمة مديرية النشر – جامعة باجي مختار عنابة

في إطار استراتيجيتها الجديدة، تعمل مديرية النشر على استقطاب الأعمال العلمية المنتَجة في الجامعة، وتكريم الأساتذة الذين يُسخّرون جهودهم لإنجازات علمية نوعية، بهدف تثمينها وإبراز أثرها في التكوين والبحث.

في هذا السياق، طلبت المديرية من زملاء الأستاذ الدكتور **بلعقود محمد الصالح** (رحمه الله) إعداد مجموعة من المقالات العلمية التي تُبرز إسهامات الفقيد وآثار أعماله على الطلبة المتخرّجين من المخبر والجامعة التي كان ينشط بها.

وقد استجاب عدد من الأساتذة مشكورين لهذا النداء، وشاركوا بمقالات علمية قيّمة تعكس خبراتهم وتقديرهم للفقيد. وتمت مراجعة هذه المقالات وتنسيقها وتحضيرها للنشر في إطار إصدار علمي خاص. نشكرهم على مساهمتهم وانخراطهم في هذا المشروع الهادف.

تهدف مديرية النشر، من خلال هذه الخطوة، إلى تشجيع الأساتذة على الإسهام في النشر العلمي في مجلّات الجامعة، سواء بإعداد أعداد خاصة أو دورية، وكذا تثمين الأعمال العلمية لطلبة الماستر والدكتوراه. هذان الهدفان يعكسان جوهر عملية النشر والتكوين الجامعي، ويساهمان في تعزيز روابط الانتماء بين الأساتذة والمؤسسة الجامعية العريقة.

نحن في انتظار مبادرات مماثلة في المستقبل، تُخلّد ذكرى الأساتذة المتميّزين الذين ساهموا ، وما زالوا يساهمون، بجهودهم في خدمة الجامعة والطلبة والعلم بصفة عامة.

الأستاذ المميز كمال شاوي،

مدير النشر الجامعي.

From The University Publishing Department…

As part of its new strategy, the Publishing Department of Badji Mokhtar University – Annaba (DPU-UBMA) is committed to promoting scientific output and encouraging academic publishing. The DPU-UBMA aims to highlight high-quality scientific work and honor professors who dedicate their efforts to research and academic development, thereby enhancing the value and impact of these contributions.

In this context, the Department invited colleagues of the late Professor *Mohamed Salah Boulakoud,* to prepare scientific articles that shed light on his academic contributions and the influence he had on students, the research laboratory, and the university. Many professors responded positively to this call and submitted valuable articles that have been reviewed, edited, and prepared for publication in a special scientific edition of SYNTHESE Journal, in tribute to the late professor and his academic legacy.

This initiative aims to strengthen a culture of scientific publishing and to encourage faculty members to contribute to the university's journals — whether through special or regular issues — and to promote the work of Master's, PhD students and assistant professors. Such efforts reflect the core mission of academic publishing and university training, while also reinforcing the sense of belonging between faculty and the university.

The DPU-UBMA looks forward to similar initiatives in the future, to honor the memory of distinguished professors who have made—and continue to make—significant contributions to the university, its students, and the scientific community.

The Publishing Department of Badji Mokhtar University – Annaba

Kamel Chaoui,

Emeritus Professor, Engineering Faculty,

Head of DPU – UBMA.

2016 – 1962

سيرة ذاتية للأستاذ الدكتور بولعقود محمد صالح

نود أن نعرب عن خالص امتناننا لاهداء هذا العدد الخاص من مجلة العلوم و التكنولوجيا"SYNTHESE" تخليداً لذكرى الأستاذ محمد صالح بولعقود، رحمه الله، المولود في 10 ديسمبر 1962 في القل ولاية سكيكدة و الذي توفي 14 نوفمبر 2016.

المقالات المقترحة في هذا العدد الخاص ما هي إلا قطرة أخذت من بحر المخبر الذي كان يرأسه الفقيد "الفيزيولوجيا البيئية الحيوانية"، وهي تعبر عن امتنان وتقدير زملائه بالمخبر والدكاترة المتخرجين منه ساهم الفقيد مع زملائه في تكوين عددا هائلا من الإطارات العلمية وأنجز العشرات من مشاريع البحوث ونشر مئات المقالات في دوريات مختلفة. كما يعبر هذا الإهداء عن احترام الأسرة العلمية لشخصه ولانجازاته العلمية المتميزة و مسيرته في الإدارة

الدكتور محمد صالح بولعقود :أستاذ دكتور في علم الأحياء بقسم البيولوجيا، كلية العلوم بجامعة باجي مختار-عنابة ومدير مخبر الفيزيولوجيا البيئية الحيوانية من سنة 2002 إلى 2016 أين وافته المنية أثناء إجراء عملية جراحية على قلبه بعد سكتة دماغية وعائية أصابته وهو يمارس واجبه في الجامعة.
الدكتور محمد صالح بولعقود حاصل على شهادة الدكتوراه تخصص فيزيولوجيا الحيوان من جامعة بريسطول، انجلترا سنة 1990 نتيجة استفادته من منحة دراسية من وزارة التعليم العالي والبحث العلمي، وهذا بعد حصوله على شهادة الدراسات العليا في علم الحيوان من جامعة قسنطينة سنة 1985.

الدكتور محمد صالح بولعقود كان أستاذا و باحثا متميزا، حيث ألقى الكثير من المحاضرات النظرية والتطبيقية طيلة عمله في الجامعة، كما قدم الكثير من المداخلات في المؤتمرات والملتقيات العلمية الوطنية والدولية، وشارك في لجانها العلمية والتنظيمية. اشرف على الكثير من أطروحات الماجستر والدكتوراه، كما اقترح العديد من مشاريع البحوث العلمية المتعلقة بصحة الإنسان والمحيط ومشاكل التكاثر في عالم الحيوان.، في الوقت الذي عمل فيه كخبير لمشاريع وطنية ودولية. لديه أكثر من ثمانين منشورا علميا في مختلف الدوريات العلمية المحكمة.

الدكتور محمد صالح بولعقود اعتلى منصب نائب مدير جامعة باجي مختار-عنابة من 2002 إلى 2010، و رئيسا لقسم البيولوجيا بين سنتي 1999 و 2000.

بقلم الأستاذ الدكتور شريف عبد النور

Biography:

Professor Mohamed Salah BOULAKOUD (1962 – 2016)

We would like to express our sincere gratitude for dedicating the issue N° 1 Vol. 30 of Science and Technology Journal "SYNTHESE" special issue to the memory of Professor Mohamed Salah BOULAKOUD, may God have mercy on him, who was born in 10 December 1962 in Collo , Skikda and he passed away 14 November 2016.

The articles proposed in this issue are just a drop taken from the ocean of the Animal Ecophysiology Research Laboratory, and they express the gratitude and appreciation of his colleagues and the doctors who graduated from this scientific structure. They contributed to the formation of a many researchers completed dozens of research projects and published hundreds of articles in various journals. They also express the respect of the university staff for his person and his distinguished scientific achievements.

Pr BOULAKOUD was a faculty member of the Biology Department affiliated to the Sciences Faculty at Badji Mokhtar University of Annaba. He was also the Director of the Animal Ecophysiology Laboratory from 2002 to 2016. He passed away during a heart surgery after suffering a stroke while he was doing his duties at the university.

He holds a PhD in Animal Physiology from the University of Bristol, England in 1990. He was awarded a scholarship from the Algerian Ministry of Higher Education and Scientific Research. He obtaining the Higher Studies Degree in animal biology from the University of Constantine in 1985.

He was a distinguished professor and researcher, as he gave many theoretical and applied lectures throughout his work at the university. He presented many features in national and international scientific conferences and forums, and participated in scientific and organizational committees. He supervised many master's and doctoral theses, and proposed many scientific research projects related to human and environmental health and reproductive problems in the animal world. He worked also as an expert for national and international projects. He has more than eighty scientific publications in various peer-reviewed scientific journals.

Pr. Mohamed Salah BOULAKOUD held the position of Vice-Rector of Badji Mokhtar University from 2002 to 2010 and was Head of the Biology Department between 1999 and 2000.

Pr. Cherif ABDENOUR

Sommaire

Analysis of selected risk factors associated with cardiovascular diseases : A study on patients living in Tébessa region (Algeria)

Messaadia Amira*, Saka Saad, Aouacheri Ouassila****
*Departement of Applied Biology, Chahid Cheikh Larbi Tebessi University - Tebessa, Algeria
** Departement of Biochemistry, University of Badji Mokhtar - Annaba, Algeria

Article Info

Article history:

Received: 22/11/2024
Revised: 16/12/2024
Accepted: 16/12/2024

Keywords:

Carviovascular diseases, Risk factors, Biochemical markers, Prevention strategies

ABSTRACT

Cardiovascular diseases (CVDs) represent a major public health concern due to their increasing prevalence and significant impact on health outcomes. To prevent the development of such complications, we focused on studying modifiable and non-modifiable factors involved in their progression. This study was conducted on 73 patients diagnosed with various cardiovascular pathologies, living in the Tébessa region (Algeria). Data are collected through a structured questionnaire designed to document behavioral and physiological risk factors. Among these participants, 25 provided blood samples, which are analyzed to perform a biochemical profile and measure the concentrations of reduced glutathione (GSH) and malondialdehyde (MDA). Our findings reveal that men are at a higher risk of CVDs compared to women. Hypertension, diabetes, smoking, and obesity are more prevalent, accompanied by statistically significant disturbances in biochemical metabolism and redox status. In conclusion, our results indicate that cardiovascular diseases are associated with both biochemical and behavioral disruptions. Early screening, regular health monitoring through blood tests, weight management, physical activity, and smoking cessation are critical recommendations to prevent cardiovascular risk.

Corresponding Author: messaadia.amira@univ-tebessa.dz

1. INTRODUCTION

Cardiovascular diseases (CVDs) remain the leading cause of global morbidity and mortality, representing a critical public health challenge. In 2017, an estimated 17.7 million of the 55 million global deaths were attributed to CVDs, with projections suggesting this figure could rise to 23.6 million by 2030 [1]. This alarming trend underscores the urgent need for effective strategies to mitigate the growing burden of these diseases [2].

CVDs encompass a spectrum of conditions, including coronary artery disease, stroke, hypertension, and heart failure, each with distinct pathophysiological mechanisms. Despite this diversity, these diseases share common risk factors that necessitate a comprehensive approach to prevention and management [3]. These risk factors are typically categorized as non-modifiable (e.g., age, sex, race, and genetic predisposition) and modifiable (e.g., diabetes, hypertension, dyslipidemia, smoking, physical inactivity, poor dietary habits, and obesity). Behavioral, environmental, and social determinants further contribute to the complexity of CVD etiology, amplifying the challenge of risk reduction [4].

Among the modifiable factors, elevated blood pressure imposes significant strain on the cardiovascular system, increasing the risk of myocardial infarction and stroke [5]. Similarly, dyslipidemia, marked by elevated low-density lipoprotein (LDL) cholesterol and reduced high-density lipoprotein (HDL) cholesterol levels, accelerates atherosclerosis, a key driver of CVD progression [6]. Lifestyle behaviors, such as smoking, unhealthy diets, and physical inactivity, exacerbate these risks by promoting oxidative stress, inflammation, and endothelial dysfunction [7].

This study aims to analyze the demographic, behavioral, and biochemical characteristics of patients diagnosed with cardiovascular diseases in Tébessa, Algeria. By identifying prevalent patterns and associations, the research seeks to enhance understanding of these factors and their implications for targeted prevention and management strategies.

2. RESEARCH METHOD

2.1. Population

Our study was conducted in two parts. The first part involved 73 patients (both sexes included) suffering from various cardiovascular pathologies. This phase was carried out in the biochemistry laboratories of Bouguera Boulaares Hospital (Bakaria – Tébessa) and Mohammed Chbouki Hospital (Chéria – Tébessa). During a training internship and with the participants' consent, a questionnaire was distributed to gather information on the following factors : age, sex, weight, height, health issues, tobacco and alcohol consumption, and frequency of

physical activity. Exclusion criteria included individuals who declined to participate, young subjects, and those with mental retardation.

2.2. Biochemical and redox status analysis

Out of the 73 patients, 25 volunteers agreed to provide blood samples for the analysis of the following biological parameters : glucose [8], urea [9], creatinine [10], uric acid [11], triglycerides [12], cholesterol [13], LDH and HDL-cholesterol [14,15], GSH [16], and MDA [17]. For accurate interpretation of the obtained results, we recruited a healthy population of 25 volunteers to serve as the control group in our study.

2.3. Statistical Analysis

Descriptive data were presented using Excel (2016). Logistic regression analysis was performed using Python/Statsmodels with a significance threshold of $p<0.05$. The results of the biochemical analyses and redox status were expressed as mean ± standard deviation (M ± SD). These means were compared to the control values using a Student's t-test, performed with the MINITAB Software (ver. 13.31).

3. RESULTS AND ANALYSIS

3.1. Demographic and clinical Characteristics

The sample consisted of 73 patients with various cardiovascular diseases, of which 30 (41.1%) were female and 43 (58.9%) were male (Table 1). The patients were distributed across three age groups: 5 (6.8%) patients aged 20 to 40 years, 25 (34.2%) aged 41 to 60 years, and 43 (58.9%) aged over 60 years. Regarding lifestyle factors, 32 (43.8%) were non-smokers and 41 (56.2%) were smokers, while 50 (68.5%) were non-drinkers, and 23 (31.5%) consumed alcohol. In terms of physical activity, 28 (38.4%) patients were physically active, and 45 (61.6%) were inactive

Table 1. Distribution of patients based on demographic and clinical characteristics

Sex	Women		Men			
N	30		43			
%	41.1%		58.9%			
Age groups	20-40	41-60		> 60		
N	5	25		43		
%	6.8	34.2		58.9		
Tabacco consumption	Non smoker		Smokers			
N	32		56.2			
%	43.8		41			
Alcohol consumption	Non-drinkers		Drinkers			
N	50		23			
%	68.5		31.5			
Physical activity	Inactive		Active			
N	45		28			
%	61.6		38.4			
Existing health conditions						
	HTN	Diabetes	Anemia	Stroke	CHD	DLD	HT
N	31	22	4	2	2	10	2
%	42.5	30.1	5.5	2.7	2.7	13.7	2.7
	anorexia	underweight	Normal wt.	Over wt.	MO	SO	MBO
BMI	<16	16.5-18.5	18.5-25	25-30	30-35	35-40	> 40
N	0	0	35	26	10	1	1
%	0	0	47.9	35.6	13.7	1.4	1.4

HTN: Hypertension; CHD: Coronary Heart Disease; DLD: Dyslipidemia;
Ht: Hyperthyroidism; MO: Moderate obesity; SO: Sever obesity and MBO: Morbid obesity

The demographic and clinical characteristics of the study population provide a foundational understanding of the factors contributing to cardiovascular disease (CVD) risk. The predominance of male patients (58.9%) is consistent with studies suggesting a higher prevalence of CVD among men, potentially due to hormonal, genetic, and behavioral differences, such as higher rates of smoking and alcohol consumption [18]. The age distribution reveals that a majority of patients (58.9%) are over 60 years old, underscoring the well-established relationship between aging and cardiovascular risk. Age-related changes, such as arterial stiffening and endothelial dysfunction, have been extensively documented as critical contributors to CVD progression [19]. Patients lifestyle habits are among further highlight modifiable risk factors. Smoking prevalence (56.2%) is significantly high, emphasizing its role as a major preventable cause of CVD through mechanisms like oxidative stress and inflammation [20]. Combustion of tobacco products generates two primary forms of smoke: mainstream and sidestream. Mainstream smoke is inhaled and subsequently exhaled by the smoker, whereas sidestream smoke, emitted from the burning tip of a cigarette, is even more hazardous due to its higher concentration of toxic constituents [21]. Among the over 7,000 chemicals identified in cigarette smoke, numerous compounds are implicated in the pathophysiology of cardiovascular diseases (CVD) [22]. Toxic agents such as carbon monoxide, polycyclic aromatic hydrocarbons, nicotine, and heavy metals, along with their

oxides, exert detrimental effects on the vascular endothelium, blood lipids, and coagulation pathways [22]. These disruptions contribute to the development of atherosclerosis and significantly increase the risk of adverse cardiovascular events, including myocardial infarction, stroke, and aortic dissection [23]. Conversely, alcohol consumption was reported in 31.5% of patients, a finding that warrants nuanced interpretation. While excessive alcohol is detrimental, moderate consumption has been linked to a paradoxical protective effect in certain populations [24]. Physical inactivity was prevalent in 61.6% of the patients, reflecting a sedentary lifestyle that significantly exacerbates cardiovascular risk by contributing to obesity, insulin resistance, and hypertension. Encouragingly, evidence suggests that even modest increases in physical activity can lead to substantial reductions in CVD risk [25]. The BMI distribution highlights critical issues related to weight management. A significant proportion of patients (50.7%) were overweight or moderately obese, while 15.1% were classified as severely or morbidly obese. Obesity is a well-documented driver of hypertension, dyslipidemia, and systemic inflammation, which collectively increase CVD risk [26]. Notably, the absence of underweight individuals in the cohort reflects the nutritional transition seen in many regions, characterized by a shift toward high-calorie, low-nutrient diets.

3.2. Logistic regression model

A logistic regression analysis was performed to examine the predictors of hypertension (HTN) among the study population. The results are shown in Table 2.

Table 2: Logistic regression results for predicting hypertnsion (HTN)

Variable	Coefficient	p-value	Odds Ratio
Sex (male)	0.5	0.10	1.65
Age (per year)	0.02	0.001	1.02
Smoking (yes)	1.5	0.03	4.48
Alcohol (yes)	-0.8	0.15	0.45
Physical Activity	-0.3	0.05	0.74
BMI (per unit)	0.2	0.01	1.22

The logistic regression results are presented below:

Sex: Sex did not show a statistically significant relationship with the probability of having HTN (coefficient = 0.5, p = 0.10), suggesting that sex is not a major predictor of HTN in this population.

Age: Age showed a significant association with the likelihood of having HTN (coefficient = 0.02, p = 0.001). For each additional year of age, the probability of having HTN increased by 2% in log-odds terms. This indicates that older patients have a higher risk of HTN.

Smoking: Smoking was identified as a significant risk factor for HTN (coefficient = 1.5, p = 0.03). Smokers were about 4.48 times more likely to have HTN compared to non-smokers.

Alcohol Consumption: Alcohol consumption did not show a significant relationship with HTN (coefficient = -0.8, p = 0.15). Although the negative coefficient suggests a potential reduction in risk, this association was not statistically significant.

Physical Activity: Physical activity showed a significant association with the probability of HTN (coefficient = -0.3, p = 0.05). Physically active patients had a reduced risk of HTN compared to inactive patients, although this effect was relatively modest.

BMI: BMI was also found to be a significant predictor for HTN (coefficient = 0.2, p = 0.01). Each unit increase in BMI was associated with a 20% increase in the probability of having HTN in log-odds terms, highlighting the significant impact of obesity on HTN risk.

The Forest Plot of the logistic regression results for hypertension (HTN) visualizes the odds ratios (OR) for each variable along with their 95% confidence intervals (CI). The red dashed line represents an odds ratio of 1, which would indicate no effect. Variables such as Sex, Smoking, and BMI show significant effects, as their confidence intervals do not cross 1. The Alcohol variable does not show a significant effect, as its confidence interval includes 1.

The logistic regression analysis underscores the role of modifiable and non-modifiable risk factors. Age emerged as a robust predictor, with a 2% increase in HTN risk per year of age, aligning with prior evidence that aging elevates vascular stiffness and blood pressure [27]. Smoking showed a strong association, with smokers being 4.48 times more likely to develop HTN, corroborating its known vasoconstrictive effects [20]. Conversely, alcohol consumption did not significantly affect HTN, reflecting heterogeneity in alcohol-related HTN mechanisms observed across populations [24]. Physical activity demonstrated a protective effect, reducing HTN risk by 26%, which aligns with studies emphasizing regular exercise in mitigating cardiovascular risks [25]. BMI, another significant predictor, reinforces the critical role of obesity management in HTN prevention, as every unit increase in BMI heightened the odds of HTN by 20% [26].

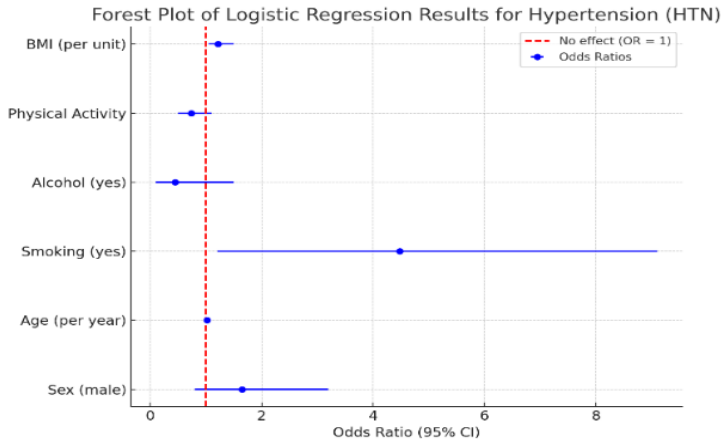

Figure 1. Forest plot of logistic regression results for hypertension (HTN)

3.3. Biochemical study results and redox status

The study findings reveal significant differences between patients with cardiovascular diseases (CVDs) and the control group. A marked increase in fasting blood glucose levels was observed, alongside notable elevations in renal function markers, including urea, uric acid, and creatinine. Lipid profile analysis demonstrated a significant rise in lipid levels among CVD patients. Additionally, the data indicated a significant reduction in GSH levels, accompanied by a highly significant increase in plasma MDA levels in the patient group.

Table 3: Variation of biochemical parameters and oxidative stress biomarkers

Parameters	Control group n=25	CVDs group n=25
Blood glucose (g/L)	1.09 ± 0.43	1.43 ± 0.74 *
Urea (g/L)	0.25 ± 0.04	1.17 ± 0.6 *
Uric acid (mg/L)	56.6 ± 19.3	68.9 ± 32.5 *
Creatinine (Mg/L)	10.71 ± 7.98	15.5 ± 10.6 *
Total Cholesterol (g/L)	1.43 ± 0.328	2.06 ± 0.458***
Triglycerides (g/L)	0.82 ± 0.280	2.05 ± 0.300***
HDL-Cholesterol (g/L)	0.52 ± 0.042	0.44 ± 0.065*
LDL-Cholesterol (g/L)	0.95 ± 0.320	1.64 ± 0.395***
GSH (nmol.10^6/mL)	31.04 ± 5.26	21.78 ± 6.55 *
MDA (nmol/mL)	10.59±6.01	24.63±6.31 **

The study revealed significant disparities in biochemical parameters between patients with cardiovascular disease (CVD) and control subjects. Elevated levels of blood glucose, renal markers, and lipid profiles reflect pronounced metabolic dysregulation and an increased risk of cardiovascular events, consistent with findings reported by [28]. The observed rise in low-density lipoprotein (LDL) cholesterol and triglycerides, coupled with a reduction in high-density lipoprotein (HDL) cholesterol, underscores the critical role of dyslipidemia in atherosclerosis development, aligning with evidence presented by [29]. These findings are further supported by the work of [30], which also documented similar patterns of dyslipidemia in CVD patients.

Numerous studies have consistently shown that diabetes significantly elevates the risk of CVD in diabetic populations compared to non-diabetic individuals. Type 2 diabetes, in particular, disrupts lipid metabolism, leading to atherogenic dyslipidemia characterized by alterations in lipids and lipoproteins [31]. Atherogenic dyslipidemia not only promotes the oxidative modification of lipids, especially LDL, but also facilitates the oxidative modification of proteins. This, in turn, triggers both localized and systemic inflammatory responses, further exacerbating atherosclerotic processes [32].

The findings related to oxidative stress are particularly significant. The observed reduction in glutathione (GSH) levels, coupled with elevated malondialdehyde (MDA) levels in patients with cardiovascular disease (CVD), indicates a disrupted redox balance. This imbalance exacerbates vascular inflammation and endothelial dysfunction, consistent with the mechanisms described by Vekic et al. [33].

Under normal physiological conditions, reactive oxygen species (ROS) production is tightly regulated and balanced by detoxification mechanisms, serving essential roles in cellular signaling and function. However, in pathological states such as atherosclerosis or hypertension, ROS production surpasses the capacity of endogenous antioxidant defenses, leading to oxidative damage and cellular death. At the cardiovascular level, oxidative stress plays a pivotal role in the pathophysiology of conditions such as myocardial infarction, ischemia/reperfusion injury, and heart failure.

These findings align with existing evidence that links oxidative stress markers to both the initiation and progression of cardiovascular diseases, underscoring the critical role of redox homeostasis in maintaining vascular health [33].

4. CONCLUSION

The study highlights that several demographic and lifestyle factors are significantly linked to the risk of hypertension among patients with cardiovascular diseases. Specifically, age, smoking, and body mass index (BMI) emerged as independent risk factors for hypertension in this population. Interestingly, alcohol consumption did not demonstrate a significant association with hypertension, suggesting it may not play a major role as a risk factor in this specific cohort. While physical activity exhibited a protective effect against hypertension, the observed impact was modest, warranting further research to clarify its magnitude and implications.

These findings underscore the necessity for targeted public health strategies that prioritize smoking cessation, promotion of physical activity, and effective obesity management. Additionally, regular screening for comorbid conditions, such as diabetes and hypertension, should be emphasized to mitigate cumulative cardiovascular risks. Tailored health education campaigns focusing on modifiable risk factors are essential to reduce the disease burden in similar populations.

While the study provides valuable insights, its cross-sectional design limits causal inference. Future longitudinal studies should explore the temporal relationships between these factors and cardiovascular outcomes. Additionally, investigating the role of dietary antioxidants in modulating oxidative stress biomarkers could offer novel therapeutic avenues.

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Etude de l'hépatotoxicité d'un fongicide chimique et un biofongicide

Mallem Leila*[1, 2], Tektak Mohamed[2], Bououza Fatiha[2, 3]
[1] Département de Médecine dentaire, Faculté de Médecine Annaba, Université Badji Mokhtar Annaba-Algérie
[2] Laboratoire d'Ecophysiologie Animale, Faculté des sciences, Département de Biologie, Université Badji Mokhtar Annaba, Algérie
[3] Laboratoire d'Ecophysiologie Animale, Département des sciences naturelles et de la vie, Faculté des Sciences, Université 20 août 1955, Skikda, Algérie

Info. Article

Historique de l'article

Reçu le : 01/01/2025
Révisé le : 02/03/2025
Accepté le : 02/03/2025

Mots-clés :

Extrait aqueux de l'ail, Azoxystrobine, paramètres hépatiques, foie, rat

RESUME

Cette recherche a pour objectif de comparer l'impact toxique de l'Azoxystrobine, un fongicide chimique largement utilisé dans l'agriculture à l'échelle mondiale, avec celui de l'extrait aqueux d'ail, en se basant sur les indicateurs de la fonction hépatique chez des rats mâles. L'étude a été menée sur 40 rats pubères répartis en 5 groupes de 8 individus. Le groupe T0 a servi de groupe témoin. Les groupes T1 et T2 ont été traités avec l'extrait aqueux d'ail, tandis que les groupes T3 et T4 ont reçu de l'Azoxystrobine via leur alimentation, respectivement aux doses correspondant à (1/15, 1/30) de la DL50 pour chaque pesticide, sur une période de six semaines. Les résultats obtenus révèlent que l'Azoxystrobine a entraîné une augmentation significative de la masse du foie chez les groupes exposés au pesticide chimique, en comparaison avec le groupe témoin. Par ailleurs, les activités enzymatiques des transaminases, notamment l'ALAT, l'PAL ont affiché une élévation notable chez les rats traités au pesticide chimique par rapport aux témoins. À l'inverse, une diminution marquée de la concentration l'ASAT et la concentration de l'albumine chez les groupes traités à la forte dose l'azoxystrobine par rapport au groupe non traité. En conclusion, cette étude a démontré que l'Azoxystrobine provoque des perturbations beaucoup plus importantes des marqueurs de la fonction hépatique que l'extrait aqueux d'ail chez les rats soumis au traitement.

Auteur correspondant : mallemleila04@gmail.com

1. INTRODUCTION

Les pesticides sont des substances utilisées en agriculture qui sont essentielles pour la production alimentaire. Ils aident à maintenir ou à améliorer les rendements agricoles et peuvent aussi augmenter la quantité de cultures produites [1].

Depuis le milieu du XXe siècle, l'agriculture a connu des évolutions considérables pour répondre à une demande alimentaire exponentielle, en raison du triplement de la population mondiale, qui est passée de 2,5 milliards à 7,2 milliards d'habitants entre 1950 et 2015 [2]. Les pesticides se sont imposés comme des outils essentiels pour protéger les cultures des ravages causés par les insectes, les mauvaises herbes, les champignons et d'autres organismes nuisibles.

Malgré ces bienfaits, les pesticides sont remis en cause en raison de leur toxicité pour l'utilisateur, la résistance émergente, et la contamination environnementale par les résidus.

Les systèmes agricoles biologiques sont moins exposés aux infestations par des agents pathogènes que ceux de l'agriculture conventionnelle [3], [4]. Les fongicides sont des produits phytosanitaires spécifiquement élaborés pour protéger les cultures en prévenant ou en combattant les infections fongiques. Ces infections peuvent provoquer des maladies qui détériorent les plantes, ce qui diminue les rendements et la qualité des récoltes. Ils sont capables de freiner la croissance de divers champignons directement ou indirectement. Les fongicides, en plus d'arrêter les processus respiratoires du système énergétique des cellules fongiques, affectent également la division cellulaire après avoir pénétré dans la plante [5].

Des recherches ont révélé que ces pesticides sont souvent utilisés pour limiter l'usage de pesticides chimiques en agriculture, tout en favorisant l'emploi de produits phytosanitaires d'origine biologique [6].

Ces dernières années, l'utilisation croissante de solutions naturelles s'est affirmée comme une approche prometteuse pour protéger la santé humaine et améliorer le stockage et la défense des cultures agricoles. Il est important de noter que l'idée de « biopesticides » ne date pas d'aujourd'hui. Dès le VIIe siècle avant notre ère, les agriculteurs chinois recouraient à des plantes telles que Illicium lanceolatum pour se défendre contre les infestations d'insectes [7]. Des avancées scientifiques récentes ont confirmé l'efficacité des composés dérivés des plantes dans les mécanismes de protection des cultures, offrant ainsi des alternatives biologiques sans toxicité notable.

Parmi les méthodes de lutte contre les ravageurs agricoles, les producteurs privilégient souvent, parfois de manière excessive et inappropriée, les traitements aux pesticides chimiques [8].

Il est également important de rappeler que le foie joue un rôle crucial dans l'élimination des substances toxiques circulant dans le sang. Cet organe assure de nombreuses fonctions essentielles, notamment le métabolisme, la détoxification et la sécrétion/excrétion biliaire. Il est responsable du métabolisme de tous les produits chimiques rencontrés par l'organisme, y compris les pesticides [9]. Ainsi, le foie est un organe clé dans le métabolisme des xénobiotiques. L'hépatotoxicité est un indicateur important pour évaluer l'impact d'un xénobiotique sur la santé [10]. La santé du foie est primordiale, car tout ce qui l'affecte à des répercussions rapides sur d'autres organes [11]. En outre, la vulnérabilité des tissus hépatiques aux effets du stress dû à l'exposition aux pesticides dépend de l'équilibre entre le stress oxydatif et les capacités antioxydantes.

Dans ce contexte, ce travail vise en premier lieu de confirmer la toxicité du fongicide chimique utilisé puis, en second lieu les comparer ces effets toxiques aux biopesticides comme à l'extrait aqueux de l'ail, sur la fonction hépatique chez le rat mâle.

Le bon fonctionnement du foie est primordial, car tout atteinte à cet organe peut entraîner des effets immédiats sur l'ensemble du corps [11]. De plus, la susceptibilité des tissus hépatiques aux dommages liés à l'exposition aux pesticides dépend d'un équilibre délicat entre la production de stress oxydatif et l'efficacité des systèmes antioxydants de l'organisme pour le neutraliser.

Dans ce contexte, ce travail vise en premier lieu à confirmer la toxicité du fongicide chimique utilisé puis, en second lieu les comparer ces effets toxiques aux biopesticides comme à l'extrait aqueux de l'ail, sur la fonction hépatique chez le rat mâle

2. METHODOLOGIE DE RECHERCHE

2.1. Traitement et dosage des paramètres hépatiques

Quarante rats mâles pubères de souche *Wistar*, pesant entre 230 et 260 g en moyenne, ont été utilisés dans cette étude. Dès leur arrivée dans l'animalerie du département de biologie, les animaux ont été logés dans des cages adaptées à leur taille. Leur alimentation consistait en un régime commercial présenté sous forme de granulés, tandis que l'eau du robinet leur était fournie en libre accès à l'aide de biberons. Les rats ont été maintenus dans un environnement contrôlé offrant des conditions optimales de température, d'éclairage et d'humidité.

Après une période d'adaptation de 15 jours, les animaux ont été divisés en 5 groupes de 8 rats chacun. Pour cette étude, nous avons testé le fongicide Azoxystrobine qui est très utilisé dans l'agriculture même au niveau mondial. Il appartient à la famille des strobilurines. Le 2ème produit est l'extrait aqueux de l'ail préparé selon la méthode de [12].

L'alimentation est broyée sous forme de poudre pour faciliter l'accès des pesticides. En prenant en considération que chaque rat consomme en moyenne 20 g d'aliment par jour. Une quantité de 160g d'aliment a été distribué par jour et par cage pendant toute la période de traitement. Le traitement s'est fait par voie alimentaire pendant 6 semaines.

T0 : Rats témoins
T1 : Rats traités par l'Ail à la dose de 1/30 de DL50.
T2 : Rats traités par l'Ail à la dose de1/15 de DL50.
T3 : Rats traités par l'Azoxystrobine à la dose de 1/30 de DL50
T4 : Rats traités par l'Azoxystrobine à la dose de 1/15 de DL50.

A la fin de la période de traitement, tous les animaux ont été sacrifiés. Le foie de chaque animal a été prélevé, puis pesés à l'aide d'une balance de précision.

Le sang a été récupéré pour réaliser le dosage des marqueurs d'exploration de la fonction hépatique (TGO, TGP, PAL, Albumine). Le sang est prélevé dans des tubes héparinés, puis centrifugés pendant 15 minutes à 3500 t/mn pour récupérer le plasma. Les échantillons plasmatiques sont conservés à une température de -20 °C pour la détermination des de l'activité enzymatique des paramètres de la fonction hépatique étudiés.

2.2. Dosage des principaux marqueurs hépatiques

Le Dosage quantitatif de l'activité alanine aminotransférase (ALAT) et l'activité aspartate aminotransférase (ASAT) ont été mesurés en suivant la méthode de [13].

Le Dosage quantitatif la phosphatase alcaline (PAL) et la concentration de l'albumine ont été mesuré selon les méthodes de [14**]** respectivement.

2.3. Etude statistique :

Les résultats de chaque paramètre évalué sont exprimés sous forme de moyenne avec l'écart-type (Moyenne ± SEM). L'analyse statistique a été effectuée en utilisant le test t de Student via le logiciel Prism. Les comparaisons des moyennes ont été réalisées entre le groupe témoin et les groupes traités. Les différences ont été interprétées selon les seuils suivants :

Significatif : ($p<0,05$)
Hautement significatif : ($p<0,01$)
Très hautement significatif : ($p<0,001$)

3. ANALYSES ET RESULTATS

3.1 Variation de la masse absolue du foie

Nous constatons dans la (Fig. 1) en ce qui concerne la variation de la masse hépatique chez les différents groupes de rats traités par rapport au groupe témoin, une augmentation notable de la masse du foie a été observée chez les deux groupes exposés à l'azoxystrobine. Par ailleurs, une augmentation particulièrement significative a été relevée uniquement chez le groupe ayant reçu une forte dose d'extrait d'ail. Les chercheurs [15] ont observé les mêmes résultats après traitement au même fongicide chimique utilisé dans notre expérimentation. Ils ont enregistré une hépatotoxicité se traduisant par une augmentation de la masse du foie et une modification des indicateurs biochimiques et enzymatiques du la fonction hépatique. D'autres résultats similaires ont été observés dans l'étude de [16], signalé toujours une augmentation de la masse du foie lors d'une exposition orale des groupes de quatre chiens beagle ayant reçu des gélules contenant de l'azoxystrobine (pureté, 96,2 %) à une dose de 0, 3, 25 ou 200 mg/kg pc par jour pendant 52 semaines. Cette augmentation est due probablement à la sécrétion massive des enzymes au cours de la détoxification [17].

Figure (1) : Changement de la masse absolue du foie (g) chez les divers groupes de rats après 6 semaines de traitement (Moyenne ± SEM, n=8).

** Hautement significatif : (p < 0,01), *** Très hautement significatif : (p < 0,001)

3.2. Variation de l'activité enzymatique des transaminases ASAT, ALAT et PAL

Les données relatives à l'activité des transaminases (aminotransférases) et de la phosphatase alcaline (PAL) chez les divers groupes de rats sont indiquées dans le Tableau 1.

3.3. L'activité enzymatique des transaminases

Les analyses de l'activité enzymatique de l'ASAT ont mis en évidence une augmentation marquée, très significative et hautement significative, chez les groupes exposés à l'Azoxystrobine (T3, T4) comparativement au groupe témoin. En ce qui concerne les groupes ayant reçu l'extrait aqueux, une diminution significative de l'ASAT a été constatée uniquement dans le groupe soumis à la dose la plus élevée, par rapport aux rats non traités.

Aucun changement n'a été observé dans les résultats L'activité enzymatique de l'ALAT chez les deux groupes traités par l'extrait aqueux de l'ail. En revanche, une augmentation très hautement significative et très significative chez les groupes T3 et T4 respectivement toujours comparés au groupe témoin. Les transaminases,

Tableau 1. Évolution de l'activité enzymatique des transaminases (U/L) dans les divers groupes après une période de 6 semaines de traitement

Groupes	ASAT(U/L)	ALAT(U/L)	PAL(U/L)
T0 : Témoin	133.30± 1.109	21.75 ± 0.854	99.5 ± 2.500
T1 : Traité à l'ext. aq 1/30 LD_{50}	128.00± 3.367	21.50 ± 0.866	**99.0 ± 2.944**
T2 : Traité à l'ext.aq 1/15 LD_{50}	114.00± 5.180 *	21.25 ± 0,946	**98.25 ± 2.217**
T3 : Traité à l'azoxy 1/30 LD_{50}	114.00± 6.014 *	34.00 ± 1.683 **	239.0 ± 8.175 ***
T4 : Traité à l'azoxy 1/15LD50	85.90± 8.995 ***	43.25 ± 5.506***	275.0 ± 2.858 ***

(Moyenne ± SEM, n=8).

significatif (p<0,05), ** Hautement significatif : (p<0,01), * Très hautement significatif : (p<0,001)*

également appelées aminotransférases, sont des enzymes qui interviennent dans le transfert d'un groupe amine d'un acide aminé à un autre. Ce transfert peut se faire depuis l'acide aspartique ou l'alanine vers l'acide α-cétoglutarique [18]. Ces enzymes jouent un rôle central dans le cycle de Krebs. L'alanine aminotransférase (ALT), connue aussi sous le nom de sérum glutamate-pyruvate transaminase (SGPT), est une enzyme localisée exclusivement dans le cytoplasme des cellules. Elle est principalement présente dans les hépatocytes, ce qui en

fait un indicateur hautement spécifique des lésions des cellules hépatiques. Par ailleurs, il convient de souligner que parmi les pesticides utilisés en agriculture, 59 substances (soit 55 %) et 69 produits formulés (soit 75 %) sont reconnus pour leur toxicité hépatique [19]. Lorsque les membranes des hépatocytes sont endommagées, les enzymes alanine aminotransférase (ALAT) et aspartate aminotransférase (ASAT) sont libérées dans la circulation sanguine. Des recherches antérieures ont démontré que des concentrations élevées de ces enzymes, après une exposition aux pesticides, peuvent être associées à des lésions hépatiques, telles que la stéatose hépatique [20]. Une augmentation des transaminases reflète également des dommages au niveau des cellules hépatiques [21].

Les résultats obtenus montrent que le traitement des rats avec de l'Azoxystrobine a provoqué une augmentation notable de l'activité enzymatique de la phosphatase alcaline (PAL), comme indiqué dans le tableau 1. Cette élévation est particulièrement marquée et hautement significative dans les groupes T3 et T4, exposés à l'Azoxystrobine. Une diminution non significative a été observée chez les groupes T1 et T2 traités aux deux doses d'extrait aqueux d'ail par rapport au groupe témoin non traité.

L'augmentation de l'activité enzymatique du PAL enregistrée chez les animaux traités dans l'alimentation par l'Azoxystrobine, peut être expliquée par l'endommagement du foie [22]. Des études similaires ont été rapportées après l'utilisation de fongicides, suggérant qu'un usage excessif des pesticides pourrait causer des dommages importants au foie. Cela pourrait entraîner une altération de la perméabilité de la membrane plasmique, facilitant ainsi le passage des enzymes du foie vers le plasma [23].

Les mêmes résultats ont été enregistré chez les chiens traités par l'azoxystrobine (96,2 %) aux doses de 0, 10, 50 et 250 mg/kg pc par jour pendant une période plus prolongée de 93 jours [24].

Concernant les résultats enregistrés chez les groupes recevant l'extrait aqueux de l'ail, nos résultats montrent une diminution non significative comparé au groupe non traité. Les recherches de **[25]** ont révélé que l'ail cru atténuait les dommages au foie, Cela a été signalé par une diminution des niveaux sériques d'ALAT et d'ASAT chez 20 patients alcooliques qui ont été recrutés et ont pris environ 2,4 g de bulbes d'ail crus chaque matin pendant 45 jours [26]. Le même résultat a été montré par une autre recherche le traitement des rats mâles *Wistar* par l'extrait aqueux de l'ail à la dose (p/p à 2%) pendant 4 semaines, a provoqué une diminution de la phosphatase alcaline sérique [27]. En revanche, dans une étude en traitant des rats quotidiennement par voie orale avec de l'extrait d'ail à deux doses différentes (250 mg et 500 mg/kg du poids corporel), les résultats ont montré une augmentation des activités des transaminases (ALAT, ASAT) ainsi que de la phosphatase alcaline (PAL).

3.6. Concentration de l'Albumine (g/dl)

Les résultats de la concentration d'albumine sérique montrent une réduction significative uniquement dans le groupe T4, traité avec la dose maximale d'Azoxystrobine, comparé au groupe témoin non traité. En revanche, aucune différence notable n'a été observée dans les groupes ayant reçu l'extrait aqueux d'ail (T1 et T2), ni dans le groupe traité avec la faible dose de fongicide chimique (T3), par rapport au groupe témoin.

Une diminution très hautement significative de la concentration de l'albumine a été enregistrée chez les groupes T4 traité à l'Azoxystrobine aux doses 1/15 de la DL50 par rapport au groupe des animaux non traités. Sachant que l'albumine est une protéine sérique synthétisée par le foie et son niveau sérique dépend de certains facteurs tels que l'état nutritionnel, la fonction hépatique [24].

Table 2. Variations de l'albumine (g/dl) les rats des différents groupes après 6 semaines de traitement

Groupes	T0	T1 :1/30 de DL_{50} (Ext aq)	T2 : 1/15 de DL_{50} (Ext aq)	T3 :1/30 de DL_{50} (Azx)	T4: 1/15 de DL_{50} (Azx)
Concentration de l'albumine	39,75 ± 3,092	36,50 ± 1, 323	36,75 ± 0,854	39,75 ± 3, 092	21,75 ±2,562 **

*($\bar{X}$±SEM, n=8) ; ** Hautement significatif : (p<0,01)*

Plusieurs études ont montré que la réduction des protéines plasmatiques était liée à l'intoxication par les différents résidus de pesticides [29]. Les pesticides sont métabolisés dans le foie et ils contribuent à provoquer des altérations de l'adhésion cellulaire**.** D'autre part, les résultats de cette hypo albuminémie chez les animaux traités par la forte dose de l'Azoxystrobine est peut-être due à un désordre hépatique, qui a eu pour conséquence la perturbation de la synthèse hépatique de l'albumine [24].

CONCLUSION

Les résultats montrent clairement que l'utilisation de l'extrait aqueux de l'ail dans l'alimentation dans les conditions expérimentales utilisées parait moins toxique que le pesticide chimique, Azoxystrobine. Donc, l'exposition au pesticide chimique l'Azoxystrobine aux doses et la période utilisée peut provoquer une perturbation et un déséquilibre au niveau des paramètres de la fonction hépatique. Ceci est toujours considéré comme une

preuve que les produits naturels à effet pesticide comme l'extrait aqueux de l'ail sont à la fois efficaces et surtout non toxiques pour les êtres vivants et l'environnement.

Remerciements
Je tiens à adresser mes sincères remerciements à tous les chefs d'équipes et les membres du Laboratoire de Recherche d'Ecophysiologie Animale. Mes remerciements vont également au Chef de Laboratoire, le Professeur CHERIF Abdenour de nous avoir donné l'occasion de rendre hommage à notre défunt Professeur, BOULAKOUD Mohamed Salah.

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تنقية أنزيم تحويل الأنجيوتنسين I (ECA I) من رئة الفأر من سلالة Wistar بإستخدام تقنيات الكروماتوغرافيا : دراسة بيوكيميائية و أنزيمية

مصارحم.، طهراوي ك. ، بوزرنة ن ، بولقودم.ص.
كلية العلوم، جامعة باجي مختار - عنابة

Article Info.

Article history:

Received: 18/11/2024
Revised: 17/04/2025
Accepted: 08/04/2025

الكلمات الدالة : أنزيم تحويل الأنجيوتنسين، التنقية، الكروماتوغرافيا، PAGE-SDS، مواصفات الأنزيم

keywords: Angiotensin - converting enzyme; Purification ; chromatography; PAGE-SDS; Enzyme characterizations

ملخص

يقوم أنزيم تحويل الأنجيوتنسين I أ و Peptidyl dipeptide hydrolase بدور هام في النظام رينين- أنجيوتنسين - ألدوستيرون، و ذلك بتحويل الأنجيوتنسين I إلى مركب فعال و هو الأنجيوتنسينI . الذي يمتلك فعالية كبيرة في تقلص الأوعية الدموية, كما يساهم في تشكل الأنجيوتنسينIII وهو هرمون يحث على إنتاج الألدوسترون بالإضافة إلى أدواره المتعددة الأخرى. ECAI هو جليكوبروتين يتكون من سلسلة ببتيدية واحدة. يضم عنصر الزنك في مركزه الفعال.

يهدف هذا البحث إلى تنقية أنزيم تحويل الأنجيوتنسين I من رئة الفأر Wistar بإستخدام تقنيات الكروماتوغرافيا من جهة ودراسة خصائصة البيوكيميائية والأنزيمية من جهة أخرى. وذلك أنطلاقا من المستخلص الرئوي : الإستخلاص بإستعمال منظف غير أيوني Nonidet P-40 ثم تطبيق كروماتوغرافيا التبادل الأيوني على هلامة DEAE - Spherodex M ، كروماتوغرافيا الألفة على هلامة Sepharose وذلك بإستخدام مثبط نوعي للأنزيم (Lisinopril MK-521) مثبت على الهلامة (MK-521/ Sepharose CL-4B) . حيث وصل النشاط الأنزيمي النوعي والمردودية 11.97 U/mg و 214.643 على الترتيب. كما تبين نتائج الهجرة الكهربائية على هلامة الأكريلاميد (PAGE- SDS) وجود شريط واحد مما يدل على نقاوة الأنزيم مع وجود بعض الشوائب. والتي تم التخلص منها بتطبيق تقنية كروماتوغرافيا السائل ذات الأداء العاليHPLC على هلامة Superose 12 حيث أزداد النشاط الأنزيمي النوعي والمردودية وبكيفية معتبرة و بلغ 33.43 U/mg و 596.96 على الترتيب مع ظهور شريط واحد فقط على هلامة الهجرة الكهربائية. تم تقدير تأثير بعض المثبطات على نشاط الأنزيم حيث يمتلك Enalaprilate قدرة تثبيط تفوق بـ 4 مرات قدرة Lisinopril أما EDTA فيمتلك قدرة تثبيط ضعيفة بإعتباره مثبط غير نوعي مقارنة بالمثبطين السابقين. تم تعيين كذلك الوزن الجزيئي للأنزيم والذي بلغ 170000دالتون. بينت هذه الدراسة على إمكانية تنقية أنزيم تحويل الأنجيوتنسين I من رئة الفأر Wistar بإستخدام تقنيات الكروماتوغرافيا.

Abstract

The angiotensin I - converting enzyme (ACE), or peptidyldipeptide hydrolase (EC. 3.4.15.1), is an enzyme, which plays an important role in the renine- angiotensin- aldosterone system. In other words, this enzyme transfers the angiotensin I to the angiotensin II. The latter has a high capacity and effeteness in the concentration of blood vessels and it contributes in the formation of angiotensin III. Angiotensin II stimulate also the production of aldosterone hormone. In addition, the previous enzyme has a number of other different functions. ECA I is a glycoprotein, contains one peptide chain and zinc element in its active site.

This research aims to purify the angiotensin I-converting enzyme (ACE) from the lungs of Wistar rats using chromatography techniques on one hand, and to study its biochemical and enzymatic properties on the other. Starting from the lung extract : the purification was dependent on the extraction by a nonionic detergent (Nonidet P-40) and the utilization of DEAE- spherodex M in ion exchange chromatography. However, the sepharose was used in the affinity chromatography with lisinopril (MK-521) as a specific inhibitor, which was bounded to the MK-521 sepharose CL-4B. the

result indicates that the specific enzyme activity and the yield was 11.97 u/mg and 214.643 u/mg respectively. On the other hand, the polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (PAGE-SDS) resulted in only a single band, which indicates for the purity of the enzyme and a few contaminants substances. The later can be excluded by the application of HPLC on gel filtration superose 12. In which there was an increase of specific enzyme activity and the yield in considerable quantity : 33.42u/mg and 596.96u/mg respectively and the appearance of one band by the polyacrylamide electrophoresis. In this investigation was also measured the effect of some inhibitors on enzyme activity. The findings indicated also that enalaprilate inhibitor has capacity four times of lisinopril whereas EDTA has a weak inhibition. In other words, EDTA is not a specific an inhibitor. The enzyme presents a molecular weight of 170000.

Corresponding Author: *mmessarahdz@yahoo.fr*

مقدمة

نزيم تحويل الأنجيوتنسين I (ECAI) [E.C3.4.15.1] **أو** Peptidyl dipeptide hydrolase. يسمى كذلك Kininase II **هو عبارة عن** dipeptidyl carboxypeptidase . يتواجد بكميات كبيرة في الخلايا البطانية Endothéliales المكونة للشعيرات الدموية، **وفي قشرة الرئة وكذلك** على مستوى خلايا الأنابيب المجاورة للكلية وبكميات متغيرة في الأنسجة الأخرى [1] . يلعبECA دور كبير في النظام رينين – أنجيوتنسين، حيث يقوم بتحويل الأنجيوتنسين I إلى مركب فعال وهو الأنجيوتنسين II له قدرة كبيرة في تقلص الأوعية الدموية Vasoconstriction [2-5] .

ينقسم أنجيوتنسين I **هو غير نشِط أو فعّال نسبيًا، إلى أجزاء بواسطة إنزيم تحويل الأنجيوتنسين وهناك جزء آخر هو** الأنجيوتنسين II، وهو هرمون شديد الفعالية. يتسبَّب الأنجيوتنسين II بتقلُّص عضلات جدران الشرايين الصغيرة (الشُّرينات) وارتفاع ضغط الدم.كما يقوم أنجيوتنسين II بتنبيه تحرير هرمون الألدوستيرون من الغدّة الكظرية، والفازوبريسين (الهرمون المضاد لإدرار البول) من الغُدَّة النُّخامِيَّة. يؤدي الألدوستيرون Aldosterone**والفا**زوبريسين إلى احتباس الصوديوم (الملح) من قبل الكلى.كما يؤدي الألدوستيرون إلى طرح البوتاسيوم عن طريق الكلى.تؤدي زيادة الصوديوم إلى احتباس الماء، ومن ثمّ زيادة حجم وضغط الدَّم[6-9] .

فأنزيم ECA هو **عبارة عن** جليكوبروتين يضم عنصر Zn++ **في مركزه الفعال** [10-11] .**وبالتالي فإن معرفة تركيب** المركز الفعال للأنزيم يسمح بتركيب مثبطات نوعية تستخدم بشكل واسع في معالجة ارتفاع الضغط الدموي Hypertension، كما يستخدم تقدير نشاط ECA **البلازمي** كدليل على **الإصابة بمرض** Sacroïdose وهو مرض مناعي يسبب نمو التجمعات الدقيقة للخلايا الالتهابية أو الأورام الحُبيبية في أيّ **جزء من الجسم، وفي معظم** الحالات يصيب الرئتين والعقد اللمفاوية لكنه يمكن أن يؤثر أيضًا في العينين والجلد والقلب والأعضاء **الأخرى** [12-15] .

نظرا للدور الهام الذي يقوم به ECA، قمنا بتنقية الأنزيم من رئة الفأر وذلك بهدف معرفة خواصه الفيزيوكيميائية والأنزيمية، فبعد سحق الأنسجة الرئوية والحصول على الهموجينات، تطبق المراحل الكروماتوغرافية بدءا بكروماتوغرافيا التبادل الأيوني على هلامة DEAE-SpherodexM، كروماتوغرافيا الألفة **على** Sepharose CL- 4B/MK -521 وأخيرا الترشيح الجزيئي على هلامة Superose 12 **في** كروماتوغرافيا السائل ذات الأداء العالي(HPLC).

I- المواد والطرق المستعملة

I- 1- أستخلاص الأنزيم

استعملت في هذا البحث فئران ذكور من سلالة Albino *Wistar* جلبت من معهد باستور بالجزائر العاصمة، فبعد نزع الرئتين تنظف من الدم والأنسجة الرابطة، وتغسل بكمية مناسبة من NaCl 0.5M ثم تجزأ إلى قطع صغيرة وتوضع في محلول منظم (KH_2PO_4 0.01 M (pH=7.5 **بنسبة** 5ml **لكل** 1g من الرئة. بعدها يطحن مزيج الأنسجة الرئوية في جهاز كهربائي (RHG. RHEMA- LABORTECHNIK). يعالج الخليط المتحصل عليه بمنظف غير أيوني Nonidet P-40 بتركيز 2% **تحت الرج الدائري لمدة** 2 سا ثم يترك المزيج **لمدة** 18**سا على درجة حرارة** 4°C+، يفصل الأنزيم المذاب على البقايا الأخرى عن طريق الترشيح على ورق سليلوزي ثم بواسطة عملية الطرد المركزي (6000 **دورة**/ دقيقة، 30د ، 4°C) . يؤخذ الرائق Surnageant وتجرى عملية التناضح dialyse ضد المحلول المنظم المستخدم في كروماتوغرافيا التبادل الأيوني KH_2PO_4 0.01M (pH=7.5)

I-2- تقدير كمية البروتين

قدرت كمية البروتين باستعمال طريقة (1978) ,.Markwell et al [16] المشتقة من طريقة (1951) ,.Lowry et al [17] والتي تسمح بإزالة التداخلات interferences الكيميائية التي يسببها المنظف Nonidet P- 40 (Merck) المستخدم في إذابة البروتينات الغشائية، يستخدم مصل ألبومين العجل BSA (sigma) في انجاز منحنى المعايرة.

I-3- تقدير نشاط الـECA

أ- طريقة اللمعان الحيوي

المقترحة من طرف (1976) , Friedland and Silverstein [18] حيث يقوم ECA بتحليل المركب HHL (-L- Hippurryl histidyl- L- Leucine) إلى ثنائي الببتيد L-histidyl- L- Leucine و حمض Hippurique الذي يتفاعل مع Orthophtadialdelyde مما يؤدي إلى ظهور اللون الأصفر اللامع (المتألق). تقاس شدة الكثافة الضوئية بإستعمال مصباح الأشعة فوق البنفسجية (طول موجة الحث Excitation 365 نانومتر) على جهاز Fluorimètre (Perkin Elmer).

ب- الطريقة السبكتروفوتومترية

وضعت هذه الطريقة من قبل Cushman and Cheng [19] . والتي تم تطويرها من طرف Baudin (1989) [20] ,حيث يعبر على النشاط الأنزيمي لـ ECA بـ (u/ml) وهي تقابل عدد النانومولات من حمض Hippurate المتكونة في الدقيقة على درجة حرارة 37°C و pH= 8.30 لكل 1ml من المحلول الأنزيمي. بعد تقدير النشاط الأنزيمي وكمية البروتين يتم حساب النشاط الأنزيمي النوعي لـECA والذي يعبر عنه بوحدة HHL لكل 1mg بروتين (u/mg).

II- مراحل التنقية: تم تنقية أزيم ECA بإستعمال طريقة (1985) ,.Bull et al [21] والتي تم تعديلها من قبل (1991) ,.Baudin et al [14] . وذلك بإتباع المراحل الآتية:

II-4-1 - كروماتوغرافيا التبادل أليوني على هلامة Spherodex M

استخدمت هلامة DEAE Spherodex M (IBF- Biotechnics) وهو مبادل أنيوني Anion exchange. فبعد موازنة العمود (3 30x cm) بمحلول الفوسفات المنظم 0.01M $KH_2 PO_4$ وبمعدل سريان مقداره 90ml/h، تمرر العينة على العمود وبعد تثبيتها على الهلامة يغسل العمود الكروماتوغرافي بنسبة 3 أضعاف حجمه بمحلول الفوسفات المنظم ، حتى تصل شدة الامتصاص الضوئي أقل من 0.05 على طول موجة ضوئية 280 نانومتر يشطف الأنزيم باستخدام مدرج خطي من 0 إلى 0.5M من NaCl وبزيادة في معدل السريان إلى 150ml/h ثم تجمع الأجزاء بحجم 25ml لكل جزء، حيث تقدر شدة الامتصاص الضوئي لجميع الأجزاء على طول موجة ضوئية 280 نانومتر. يسجل حجم الجزء الحاوي على أكبر نشاط أنزيمي وتقدر كمية البروتين والنشاط الأنزيمي. مع الملاحظة بأنه يجب الاحتفاظ في كل مرحلة من مراحل التنقية بجزء قليل لإجراء الهجرة الكهربائية.

II-4-2- كروماتوغرافيا الألفة على Sepharose CL- 4B /MK-521

استعملت هلامة Sepharose CL-4B (Pharmacia) وذلك بعد تثبيت مثبط نوعي لأنزيم ECA على الهلامة وهو(MK -521) Lisinopril. وذلك بإتباع المراحل الآتية:

II-4-2-1 - تحضير هلامة Sepharose CL-4B/MK-521

أ- تنشيط هلامة Sephrose CL-4B وتثبيت الذراع

- تغسل 50ml من هلامة Sephrose CL-4B بلتر من الماء المقطر و يضاف 50ml من محلول KBH_4 بتركيز 0.053 M المذاب في البوتاس 0.6 N.

- يضاف وبهدوء50ml من Butanediol diglycidyl ether ويوضع المزيج على درجة حرارة 23°C لمدة 8 سا.

ب- عملية ربط أو تثبيت المثبط النوعي للإنزيم (Lisinopril (MK-521

يغسل المزيج بواسطة 2ml من الماء المقطرأولا ثم باستعمال حجم يتراوح من 50 إلى 100ml من بيكربونات البوتاسيوم 0.3 N (pH=11). توضع الهلامة في حوجلة محكمة الإغلاق. ويضاف إليها 50ml من محلول MK-521 (22mM) المذاب في نفس المحلول المنظم. تترك الهلامة لمدة 72سا على درجة حرارة 37°C+ تحت الرج الهادئ.

جـ - تثبيط مجاميع الـ Epoxy الحرة وحفظ الهلامة

تغسل الهلامة بـ40ml من المحلول المنظم Gly 1 M (pH=10) ثم توضع في حوجلة محكمة الإغلاق، ويضاف إليها 50ml من محلول glycine المنظم. وتترك لمدة 18سا على درجة حرارة 37°C+. يغسل المزيج من جديد بـ450ml من المحلول المنظم (pH=8.0) NaCl 1M، $NaHCO_3$ 0.1 M . تحفظ هلامة الألفة التي تم تحضيرها في 50ml من محلول ملحي على درجة حرارة 4°C+.

II-4-2-2 - تنقية الـ ECA على هلامة Sephrasore Cl-4B/MK-521

يتم موازنة عمود الألفة الحاوي على Sepharose CL-4B/MK-521 بالمحلول المنظم (pH=7.5) Hepes 0,01 M, KCl 0.33, Zn Cl_2 1M في عمود حجمه 20ml (20 x 1.6 cm) وبمعدل سريان 20ml/h، فبعد إجراء عملية التناضح باستخدام محلول الألفة المذكور سابقا، تمرر الأجزاء الغنية بالنشاط الأنزيمي بعد مرحلة التبادل الأيوني على عمود الألفة وبنفس معدل سريان عملية الموازنة.

يغسل العمود بالمحلول المنظم حتى الحصول على شدة امتصاص اقل من 0.01 على طول موجة ضوئية 280 نانومتر، بعد أن يثبت الأنزيم بالمثبط المرتبط بهلامة الـ Sepharose CL-4B يشطف نوعيا وذلك بإضافة مثبط تنافسي لـECA في المحلول المنظم السابق وهو Enalaprilate 1mM وبزيادة معدل السريان إلى 30ml/h. ثم تقدر شدة الامتصاص الضوئي للأجزاء المتحصل عليها على طول موجة ضوئية 280 نانومتر، ثم تجرى عملية

التناضح في وجود المحلول المنظم المتكون من : Tris 0.002 M. NaCl 0.1M. EDTA 0.1mM (pH=8) لمدة 48 سا للأجزاء الغنية بالنشاط الأنزيمي لأجل إزالة المثبط المرتبط بالأنزيم، ثم التناضح باستعمال الماء المقطر لأجل التخلص من EDTA.

تجري عملية Lyophilisation للعينة المتحصل عليها وتطبق كروماتوغرافيا السائل عالي الأداء (HPLC) في المرحلة الموالية من مراحل التنقية.

II-4-3 - الترشيح الجزيئي في كروماتوغرافيا السائل عالي الأداء (HPLC) على هلامة Superose 12

تم تطبيق تقنية HPLC على جهاز من نوع Gilson الذي يتكون من: مضخة من نوع 302، كاشف للأشعة فوق البنفسجية (UV) من نوع 111LC ومجمع للأجزاء Collector من نوع 201، حيث استخدمت هلامة Superose12 (pharmacia) يذاب المسحوق الجاف من الأنزيم في اقل كمية ممكنة من المحلول المنظم المتكون من: Tris 0.05 M. NaCl 0.1 M (pH=7) ثم يطرد مركزيا (10.000 دورة /دقيقة ، 10د) و يرشح على مصفاة خاصة ذات ثقوب دقيقة (Millipore). بعد موازنة جهاز الـHPLC بالمحلول المنظم السابق وبمعدل سريان مقداره 0.25ml/h ، يتم حقن المحلول الأنزيمي بحجم مقداره 200µl.

II-4-4 - الهجرة الكهربائية على هلامة البولي أكريلاميد في وجود SDS (PAGE-SDS)

استخدمت طريقة Laemmli (1970) [22]، لأجل معرفة مدى نقاوة الأنزيم في كل مرحلة من مراحل التنقية، وكذلك في تعيين الوزن الجزيئي (MW) باستعمال مجموعة من البروتينات القياسية (Bio-rad): SDS – PAGE molecular weight standards-high، في جهاز الهجرة الكهربائية Hofer scientific instrements–San fransisco,CA) vertical Slab. Unit model SE 400) المتصل بمولد كهربائي (Rhema labortechnik desaga desatronic x) 5000v/100v.

III - النتائج والمناقشة

يبين الشكل (1) منحنى شطف الأنزيم ECA على هلامة Spherodex M بتطبيق مدروج خطي من NaCl (0-0.5 M). يلاحظ من خلال هذا المنحنى وجود جزء كبير في بداية عملية الشطف (320ml) والذي يضم أكبر قيمة للنشاط الأنزيمي النوعي والتي بلغت 0.543 u/mg. أدى تطبيق تقنية كروماتوغرافيا التبادل الأيوني إلى زيادة النشاط الأنزيمي النوعي بحوالي 10 مرات مقارنة بالنشاط الأنزيمي لمستخلص الأنزيم الخام Homogenate حيث ازداد النشاط النوعي من 0.056 u/mg إلى 0.5 u/mg يقابله زيادة في عامل التقنية Facteur de purification من 0.638 إلى 9.70 مرة. مع نقص في المردودية Rendement de purification كما هو مبين في الجدول (1) ، مما يدل على فعالية هده المرحلة في تنقية الأنزيم. يلاحظ توافق في النتائج التي تحصلنا عليها مع نتائج بعض الباحثين، حيث تحصل كل من (1991) ,.Baudin et al [14] على نشاط نوعي لـ ECA رئة الخنزير مقداره 0.22 u/mg وزيادة في عامل التنقية وصل 4.8 مرة بتطبيقهم لنفس مراحل التقنية بينما تحصل كل من (1977) Nishimura et al., [23] على نشاط نوعي مقداره 0.684 u/mg . ودلك من خلال تنقية ECA من رئة الإنسان على هلامة Cellulose M - DEAE وهي قيمة تقارب تلك التي وجدها كل من (1975) ,Das and Soffer [24] التي بلغت 0.42 u/mg بتطبيق نفس المبادل الأيوني. يعود الاختلاف في النتائج التي تحصلنا عليها مع نتائج الأبحاث الأخرى إلى نوع المصدر الأنزيمي المستخدم في تنقية الـECA بالدرجة الأولى وإلى نوع المبادل الأيوني المستخدم.

يوضع الشكل(2) منحنى شطف الأنزيم على هلامة الألفة Sepharose CL- 4B/MK-521. والذي يبين على شطف الأنزيم في جزء واحد فقط، حيث تحصلنا على زيادة كبيرة في قيمة النشاط الأيزيمي والتي بلغت 11.9u/mg مقارنة مع المرحلة السابقة يقابله ارتفاع هام في عامل التنقية الذي بلغ 214,643 مرة وبمردودية 29.971 % (الجدول 1) وهي نتيجة تتوافق نسبيا مع تلك التي توصل إليها كل من (1986) ,.Ehlers et al [25] حيث تحصلوا على نشاط نوعي مقداره 104 u/mg وبعامل تنقية يساوي 347 مرة لأنزيم ECA المنقى من رئة الإنسان،

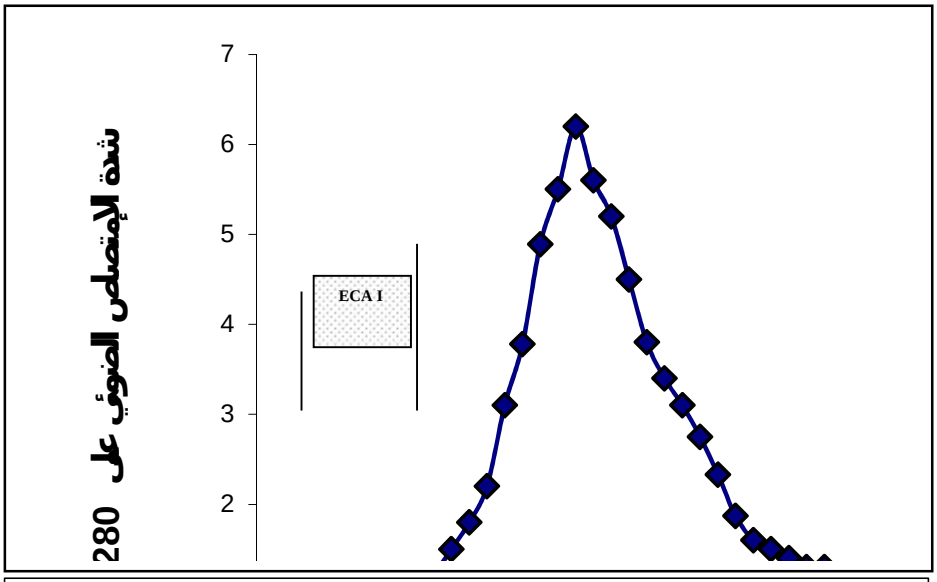
بينما تمكن (1984) ,.Pantoliano et al [26] من التوصل إلى نشاط نوعي مقداره 82u/mg مقابل عامل تنقية يساوي 356 مرة لـ ECA المنقى من رئة الأرنب. غير أن (Sakharov et al., 1987) [27-28] تحصلوا على قيمة قليلة للنشاط النوعي والتي وصلت إلى 38 u/mg بالنسبة لـ ECA قلب الإنسان وعلى 40 u/mg لأنزيم ECA الكبدي البشري

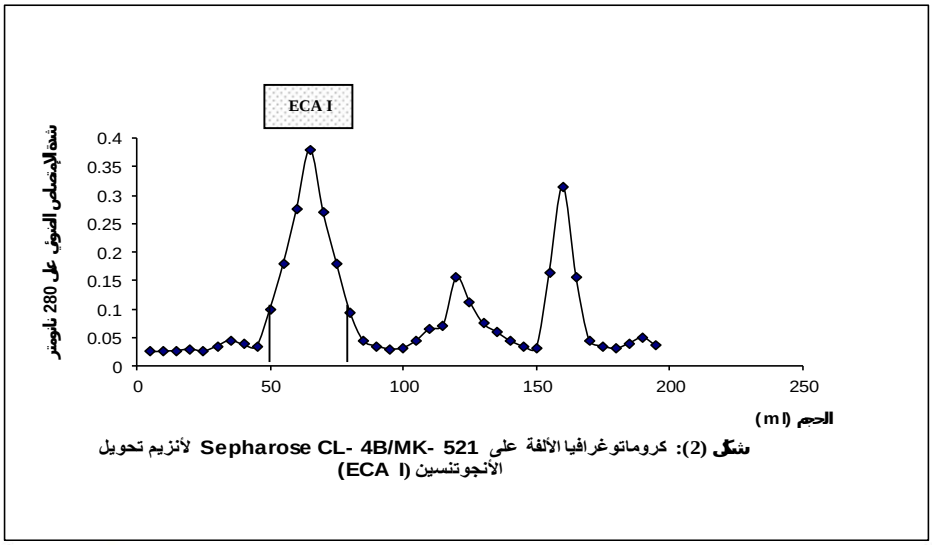
الشكل(3) يوضح منحى كروماتوغرافيا السائل عالي الأداء(HPLC) على هلامة الترشيح الجزئي Superose 12 لأنزيم ECA. والدي يبين على شطف الأنزيم في جزء (Pic) واحد دقيق ومتناظر بأقصى شدة امتصاص ضوئي له تساوي 0.335 على طول موجة ضوئية 280 نانومتر، حيث بلغ نشاطه النوعي 33.43 u/mg (الجدول 1). وبالتالي حصول زيادة كبيرة في النشاط النوعي مقارنة بالمرحلة السابقة.

يبين **الشكل(4)** الهجرة الكهربائية لأنزيم ECA على هلامة الأكريلاميد في وجود SDS للأجزاء الأنزيمية: يمثل كل جزء مرحلة من مراحل التنقية الثلاثة (مستخلص الأنزيم الخام، التبادل الأيوني، الألفة).

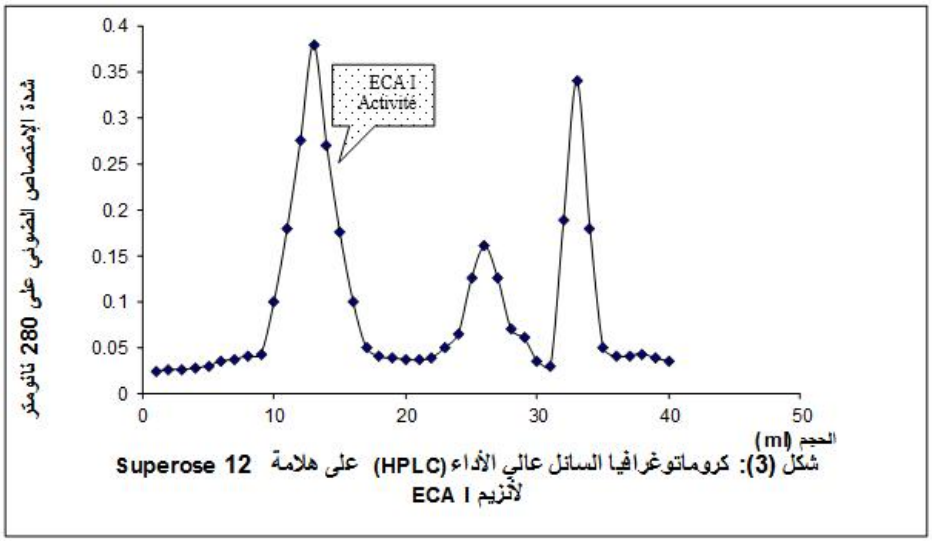
إن النتائج المتحصل عليها مشابهة لنتائج بعض الأبحاث الخاصة بتنقية ECA من الأعضاء الحيوانية المختلفة مع وجود اختلاف طفيف في نتائج عامل التنقية والنشاط النوعي وهذا يعود إلى نوع وكمية المصدر الأنزيمي أولا وإلى المراحل المتبعة في تنقية الأنزيم ثانيا والتي تعتمد أساسا على خواص الأنزيم البيوكيميائية.

يلاحظ ظهور شريط وحيد للأنزيم بعد مرحلة الألفة مع وجود بعض الشوائب الأخرى ، لاسيما البروتينات ذات الوزن الجزيئي المنخفض والتي تم التخلص منها بتطبيق تقنية HPLC حيث يبين الشكل(5) على ظهور شريط واحد فقط للأنزيم خالي من جميع الشوائب الأخرى. مما يبين نقاوة الأنزيم (تجانس الأنزيم).





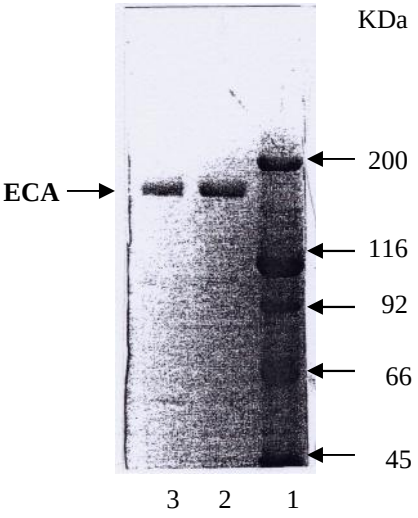
شكل (2): كروماتوغرافيا الألفة على Sepharose CL- 4B/MK- 521 لأنزيم تحويل الأنجوتنسين (ECA I)



شكل (3): كروماتوغرافيا السائل عالي الأداء (HPLC) على هلامة Superose 12 لأنزيم ECA I

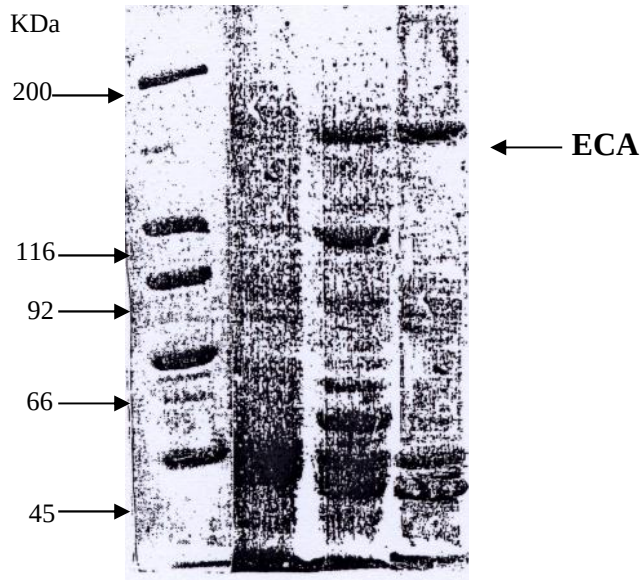
جدول (1) : الصفات البيوكيميائية لأنزيم الأنجيوتنسين I (ECAI) المقى من رئة الفأر (110g)

مراحل التنقيــة	الحجم (ml)	النشاط الكلي (بالوحدات U)	كمية البروتين الكلية (mg)	النشاط الأنزيمي النوعي (U/mg)	عامل التنقية (مرات التنقية)	المردودية (%)
مستخلص الأنزيم الخام	650	62.143	1095.90	0.056	1	-
مستخلص الأنزيم + Nonidet P-40	600	207.70	4513.48	0.036	0,638	100
كروماتوغرافيا التبادل الأيوني على DEAE - Sphérodex M	320	79.26	145.85	0.543	9,70	38.16
كروماتوغرافيا الألفة على Sépharose CL-4B/MK-521	44	62.250	5.20	11.97	214,643	29.971
HPLC على هلامة الترشيح Superose12	-	28.08	0.84	33.43	596,96	13.519



1- بروتينات قياسية (HMW. Bio-rad).
2- مستخلص الأنزيم الخام.
3- مرحلة التبادل الأيوني على DEAE-Sphérodex M
4- مرحلة الألفة على Sépharose CL-4B/MK-521

شكل (4): الهجرة الكهربائية على هلامة البولي أكريلاميد في وجود SDS للأجزاء الغنية بأنزيم ECA I (بعد التلوين بأزرق الكوماسي)

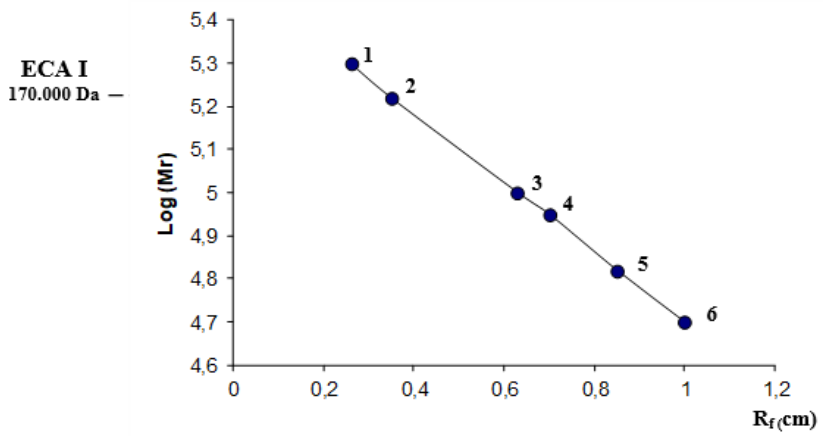


1- بروتينات قياسية (HMW. Bio-rad).
2 - 60µg من ECA.
3 - 50µg من ECA.

شكل (5) : أختبار نقاوة ECA بعد مرحلة الترشيح على هلامة Superose 12 **في** الـHPLC وتعيين الوزن الجزيئي (Mr) بإستخدام تقنية (PAGE-SDS)**.**

III – 1 - تعيين الوزن الجزيئي لأنزيم ECA

تبين نتائج الهجرة الكهربائية الشكل على الهلامة البولي أكريلاميد (بتركيز 6.5 %) **في وجود الـ** SDS **على** ظهور الأنزيم في شريط واحد فقط حتى في غياب SDS مما يدل بأن الأنزيم يتكون من سلسلة بولي ببتيدية واحدة. إضافة إلى ذلك يتضح بأن عملية النفسخ **أو فقدان** الأنزيم لبنيته الطبيعية Denaturation غير حساسة لاختزال الجسور الكبريتية. ومن خلال نتائج الشكل (5) **و للشكل** (6) يتبين بأن الوزن **الجزيئي** للأنزيم وصل 170000 دالتون وهي نتيجة تتوافق مع تلك التي تحصل عليها Lanzillo and Fanburg (1977) [29] والتي بلغ فيها الوزن الجزيئي للأنزيم رئة **الفأر** 185000 **دالتون**.

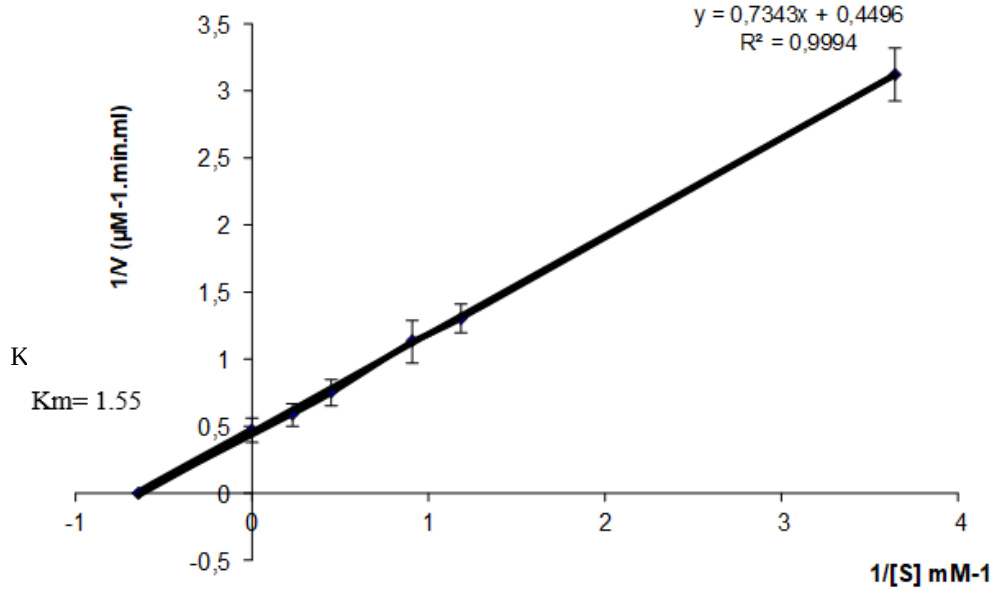


شكل (6) : تعيين الوزن الجزيئي للأنزيم عن طريق PAGE-SDS باستعمال البروتينات القياسية التالية :
1- Myosin, 200000; 2- ECA, 170000; 3 - β-Galactosidase, 116000; 4 - Phosphorylase B, 92000; 5- Serumalbumine, 66000; 6- Ovalbumine, 45000

III- 2- الدراسة الأنزيمية

III– 2- 1 - الدراسة الحركية

يبين الشكل(7) الدراسة الحركية لـECA والذي سمح بتحديد الثوابت الحركية للأنزيم يلاحظ من خلال هدا الشكل بأن ECA يقوم بتحليل الجوهر النوعي المتمثل في HHL بثابت ميخائليس Km و Vmax مقدارهما : 1.55mM و 1.382 µmole $Min^{-1}mg^{-1}$ على الترتيب. **فما تمكن كل من** (1988) ,.Rohrbach et al [30] من تحديد Km و Vmax لـ ECA رئة البقر حيث بلغت 1.3mM وU^{-1}. min^{-1}.1,2mol **على التوالي**. **مع بعض** [25] Das and Soffer, (1975) **وكذلك** [24] Ehlers et al., (1986) يمكن القول بأن النتيجة المتحصل عليها تتوافق مع نتائج كل من **الاختلافات** في قيمة Km والتي ترجع إلى العديد من العوامل من بينها مصدر الأنزيم، نوع الجوهر (مادة التفاعل) وكذلك نوع وحساسية الطريقة المستعملة في تقدير النشاط الأنزيمي [31].

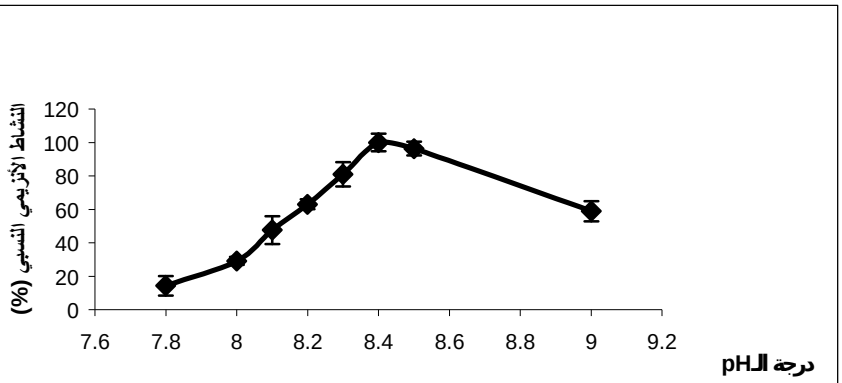


شكل (7): تعيين الثوابت الأنزيمية (Km و Vmax) بإستخدام تمثيل Lineweaver -Burk .

III– 2 – 2 - الظروف المثلى للإماهة الأنزيمية

أ - تأثير درجة الـ PH على الفعالية الأنزيمية

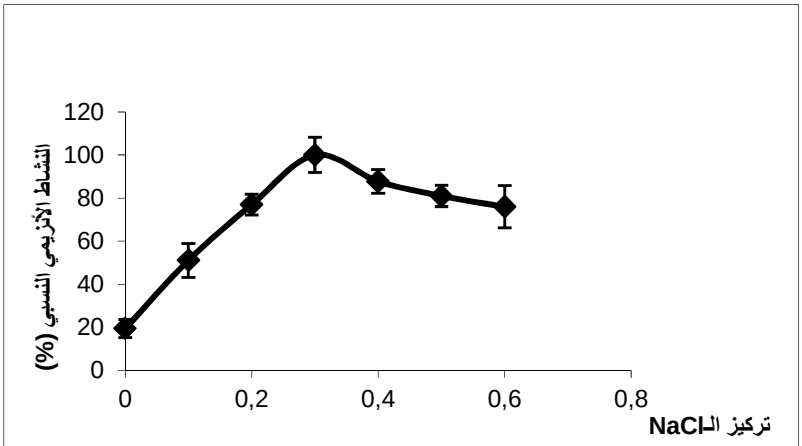
يمثل الشكل (7) فعالية ECA في درجات الـpH المختلفة حيث نلاحظ أنه عند كل درجة pH يبدي الأنزيم قيمة نشاط نسبي متميز وتبلغ أقصى قيمة للنشاط الأنزيمي النسبي (100 %) عند درجة pH تقابل 8.4 وهي درجة الـpH المثلى للأنزيم مما يوافق النتائج المتحصل عليها من قبل العديد من الباحثين لنفس الأنزيم [33,23] .



شكل (8): تأثير درجة الـpH على النشاط الأنزيمي النسبي لأنزيم ECA I

ب – تأثيرتركيز أيونات الكلور على الفعالية الأنزيمية

يزداد النشاط الأنزيمي بزيادة أيونات الكلور حتى يصل إلى أقصى قيمة له (100%) ، والذي يقابل التركيز المثالي لأيونات الكلور 0.3M (الشكل 8). يلاحظ بأن الأنزيم فقد حوالي 80% من نشاطه في غياب هذه الأيونات ، ثم يسترجع نشاطه تدريجيا بزيادة تركيز أيونات الكلور، حيث يعادل النشاط النسبي في وجود 0.2 M من NaCl قيمة مقدارها 77% من النشاط الأنزيمي مقارنة بالتركيز المثالي لأيونات الكلور. مما يدل على أهمية هذه الأيونات في النشاط التحفيزي لأنزيم الـECA [33,32] .



شكل (9): تأثير أيونات الكلور على النشاط الأنزيمي النسبي لأنزيم ECA I

ج – تأثير بعض المثبطات على الفعالية الأنزيمية

يضم الجدول (2) قيم IC-50 و الذي يحسب من العلاقة التالية : ($K_{i\ (\text{العسابي})}$) (1 + [S]/ K_m) = IC-50. إضافة لقيم كل من K_i الظاهري و K_i الحسابي للمثبطات المدروسة حيث يتضح من خلال النتائج المبينة في الجدول (2) بأن Enalaprilate يمثل المثبط الأكثر فعالية في تثبيط الأنزيم ، لأنه يمتلك أصغر قيمة لـIC-50 و K_i التي بلغت 1.475 nM و 0.92 nM على الترتيب ، ثم يليه Lisinopril بقيمة IC-50 و K_i التي وصلت 5.623 nM و 2.20 nM على الترتيب. مما يتوافق مع نتائج العديد من الأبحاث [34-36] . غير أن لـ EDTA قدرة تثبيط ضعيفة للأنزيم بإعتباره مثبط غير نوعي (مخلب Chélateur للأيونات ثنائية التكافؤ) للأنزيم [37].

جدول (2) : تثبيط نشاط ECA I بستخدام Enalaprilate
تعيين: IC-50 و Ki . EDTA, Lisinopril,

Ki (الظاهري) nM	(الحسابي) Ki nM	IC-50 nM	المثبط
0.92	0.74	1.475	**Enalaprilate**
2.200	2.811	5.623	**Lisinopril**
^{3}10 x300	^{3}10 x340.4	^{5}10x 680.77	**EDTA**

خاتمة

من النتائج الأولية التي تحصلنا عليها فيما يخص تنقية ECA من رئة الفأر باستخدام عدة مراحل كروماتوغرافية متتالية لأجل دراسة بعض الخواص البيوكيميائية، يمكننا أن نستنتج ما يلي:

- إمكانية تنقية كمية معتبرة من إنزيم تحويل الأنجيوتنسين I وذلك بتطبيق المراحل الكروماتوغرافية السابقة: التبادل الأيوني على هلامة DEAE-Spherodex M ، الألفة على Sepharose CL-4B/MK-521 . HPLC على Superose 12.
- بلغت قيمة Km و Vmax قيمة مقدارها 1.55 mM و $1.382\mu mole\ Min^{-1}\ mg^{-1}$ على الترتيب.
- وصل الوزن الجزيئي للإنزيم حوالي 170000 دالتون باستخدام تقنية PAGE-SDS.
- تركيز أيونات الكلور المثلى لـ ECA: 0.3M ودرجة الـ PH المثلى له تساوي 8.40.
- يمتلك المثبط Enalaprilate قدرة تثبيط لنشاط ECA تفوق بحوالي 4 مرات Lisinopril. أما EDTA فيمتلك قدرة تثبيط ضعيفة باعتباره مثبط غير نوعي مقارنة بالمثبطين السابقين.

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Study on the alleviating effect of *Punica granatum* against toluene-induced renal and oxidative stress damage in rats

Fatma Zohra Arkoub[1,2]*, Rahma Boukarine[1,2], Leila Hamdi[1], Labiba Kahalerras[3], Kamel Khelili[1], and Cherif Abdennour[1]

[1]Laboratory of Animal Ecophysiology, Department of Biology, Faculty of Sciences, University Badji Mokhtar-Annaba, Annaba 23000, Algeria.

[2]Laboratory of Environmental Sciences and Agroecology, Department of biology, Faculty of Life and Natural Sciences, University Chadli Bendjedid- El Tarf, El Tarf 36000, Algeria.

[3]Department of Agronomy, Institute of Veterinary Sciences and Agronomic Sciences, University El Hadj Lakhdar-Batna-1, Batna 05000, Algeria.

Article Info

Article history:

Received 02/02/2025
Revised 08/04/2025
Accepted 08/04/2025

Key words:

Antioxidant, kidney, oxidative stress, *Punica granatum,* toluene, rats.

Abstract

Organic solvents exposure has been shown to affect the functional integrity of various organs. The current study was carried out to evaluate the renal dysfunction induced by toluene (Tol) toxicity, and the potential protective role of *Punica granatum* fresh peel aqueous extract (FPAE) in male rats. The orally treated rats were divided as follows: Control (C), positive controls (corn oil: 1.25 mL/kg BW; and FPAE: 400 mg/kg BW), Tol1: 275 mg/kg BW, Tol2: 550 mg/kg BW and a mixture each of FPAE-Tol1 and FPAE-Tol2. After 6-week study period, urea, creatinine, and uric acid were estimated, as well as renal histology and oxidative stress markers. Results showed that Tol group has significant increase of serum urea and creatinine levels, with a significant elevation in renal malondialdehyde (MDA) levels, and a significant decrease of glutathione content and glutathione peroxidase activity. FPAE co-administration partially retrieved the changes in almost all studied parameters compared with the Tol group. Tol induced histopathological kidney damage, which was minimized as a result of *P. granatum* treatment. In conclusion, this study provides evidence that FPAE attenuates renal oxidative injury induced by Tol, supporting the traditional claims of its beneficial effects, possibly due to its antioxidant potential.

Corresponding Author : *f.arkoub@univ-eltarf.dz*

1. INTRODUCTION

Nowadays, renal diseases represent a major global public health issue, significantly contributing to a high incidence of morbidity and mortality **[1].** Kidneys are vital organs composed of thousands of functional units called nephrons that continuously filter the blood to excrete waste matters **[2]**. They act like a chief player in eliminating toxic metabolites produced from catabolism, making them frequent targets of various xenobiotics as medications, industrial toxins, which are primarily excreted through those organs **[2]**.

Humans' constant exposure to hazardous pollutants is largely driven by lifestyle behaviors. Despite being less widely recognized than illicit drugs, inhalants abuse represents a common problem in the adolescents around the world**.** The ease of their availability and low cost, have made them easier for consumption **[3].** Toluene is an aromatic hydrocarbon commonly used for abuse in different ways**.** It is also present in gasoline and some cleaning products. As a lipophilic substance, it has a strong affinity for lipids and can easily diffuse into fatty tissues **[4].** Toluene abuse is associated with harmful effects on health, including euphoria followed by depression0. Prolonged or high-level

exposure can damage the central nervous system, heart as well as reproductive changes, hepatic and renal failure **[5,6].** These effects are primarily attributed to suppression of free radical scavenging function and the enhancement of ROS by toluene induced, leading to oxidative stress and lipid peroxidation **[7].** Toluene toxicity can be mitigated through antioxidant defense mechanisms, which help reduce ROS, neutralize free radicals, and enhance the elimination of toxic compounds. This toxicity can be mitigated through dietary supplementation with antioxidant-rich products, which reduce or eliminate reactive oxygen species and free radicals **[8]**.

Currently, natural antioxidants are used as potential health benefits that are abundant in various spices, herbs, vegetables, and fruits **[9,10].** The natural antioxidant defense system plays a crucial role in neutralizing many toxic effects in the body **[10,11].** The *Punica granatum,* commonly called pomegranate, is a seasonal crop of the Punicaceae family grown in the Mediterranean region, as well as Pakistan, India, and Iran**.** Pomegranate is a rich source of bioactive molecules such as hydrolyzable tannins (ellagitannin, punicalagin, punicalin and pedunculagin), flavonoids, anthocyanins etc…. These components place pomegranate in a higher grade compared to other fruits **[12].**

P. granatum have attracted a great deal of attention because of its potential health benefits, which include antioxidant properties and high potency in the elimination of free oxygen radicals, as well as anti-cancer, anti-inflammatory, anti-lipoperoxidation, and DNA repair activities; in which its consumption has been linked to a possible risk reduction of some cancers, heart diseases, diabetes, and obesity **[12]**, and to male fertility improvement **[13].**

Despite the studies carried out demonstrating its antioxidant properties and medicinal benefits of *Punica granatum*, however, none has dealt with the association between this fruit and toluene consumption. Therefore, the present study was conducted to shed some light on the likely attenuating potentials of *P. granatum* against toluene renal damage of Wistar rats.

2. MATERIAL AND METHODS

Chemicals

Toluene ($C_6H_5CH_3$, purity 99.5%), manufactured by Sigma-Aldrich Chemicals Co. (St. Louis, MO, USA), was purchased from a chemical shop in Annaba, Algeria. All other chemicals used in this experiment were analytical grade and supplied by Animal Ecophysiology Laboratory.

Fruit collection and extract preparation

Mature *Punica granatum* were obtained from a farmland (Besbes-El-Taref). Following collection, fresh fruits were cleaned under running tap water and the fresh peels were removed, crushed into fine pieces using a blender (Sinbo, Turkey). The pieces were extracted using distilled water (via cold maceration) for 72 h at room temperature with continuous shaking. The mixture was decanted and filtered using cotton gauze.

Animals and experimental design

Adult male Wistar rats weighing 230±30 g, were obtained from the Pasteur Institute of Algiers and housed at the breeding house of Badji Mokhtar Annaba University. They were kept in standard polypropylene cages with access to water and standard rat food purchased from the agro-food complex (El-Kseur, Bejaia). The animals were maintained at an ambient temperature of 22±2°C, humidity, and natural photoperiod.

After acclimatization period, rats were divided into seven groups (each containing ten rats) and were maintained for the experimental period of 45 days, as follows:

C: It is served as a normal control group. Animals of this group given distilled water.
CO: Animals of this group given 1.25 mL/kg BW of corn oil.
FPAE: Animals of this group received 400 mg/kg BW of *P. granatum* peel fresh aqueous extract.
Tol1: Rats received 275 mg/kg BW of toluene dissolved in corn oil as vehicle.
Tol2: Rats received 550 mg/kg BW of toluene dissolved in corn oil as vehicle.
FPAE-Tol1: Animals received Tol (275 mg/kg BW), followed after 2 hours by FPAE (400 mg/kg BW).
FPAE-Tol2: Rats received Tol (550 mg/kg BW), followed after 2 hours by FPAE (400 mg/kg BW).

Samples collection

Animals were sacrificed by decapitation and their kidneys were removed; one kidney was quickly frozen at -20°C for the assessment of oxidative stress parameters, while the second was fixed in formalin 10% for histological examination. Blood samples were collected in dry tubes, and serum was obtained by centrifugation at 3000× g for 10 min to measure biochemical parameters.

Determination of biochemical parameters

Serum urea, creatinine, and uric acid levels were determined using an automated chemistry analyzer (ERBA XL-600, ERBA Diagnostics Mannheim GmbH, Mannheim, Germany).

Determination of oxidative stress parameters

The reduced glutathione level (GSH) was measured spectrophotometrically at 412 nm, using the method of **Wekbeker and Cory [14].** Glutathione peroxidase (GSH-Px) activity was assessed according to the method of **Flohe and Gunzler [15].** Lipid peroxidation was measured as MDA determined by thiobarbituric acid (TBA) assay according to **Ohkawa et al. [16].** Total protein concentration was determined according to the method of **Bradford [17]** by using bovine serum albumin as a standard.

Histopathological examination

Formaldehyde-fixed tissues were dehydrated in gradual ethanol (70–100%) and were embedded in paraffin wax. Sections of 5 µm thicknesses were then prepared using a rotary microtome following staining by using hematoxylin and eosin (H&E) dye for microscopic observation under alight microscope (BioLab, Mumbai, India) **[18]**.

Statistical analysis

Statistical analysis of the data was performed using GraphPad Prism 7 (GraphPad Software, San Diego, CA, USA) and Statistical Package for the Social Sciences SPSS version 26.0 for Windows (IBM Corporation, Armonk, NY, USA). Results are expressed as mean ± SEM (Standard Error of the Mean). All measured parameters were analyzed using one-way ANOVA, and all statistical analyses were followed by a post hoc test (Tuckey's test), with significance set at $p<0.05$.

3. RESULTS

Kidney weight

The kidney absolute weight is shown in figure 1. Exposure to toluene (Tol2) significantly increased kidney weight compared to the control and positive control groups. In contrast, the combined group (FPAE-Tol2) showed an insignificant decrease in kidney weight compared to the Tol group.

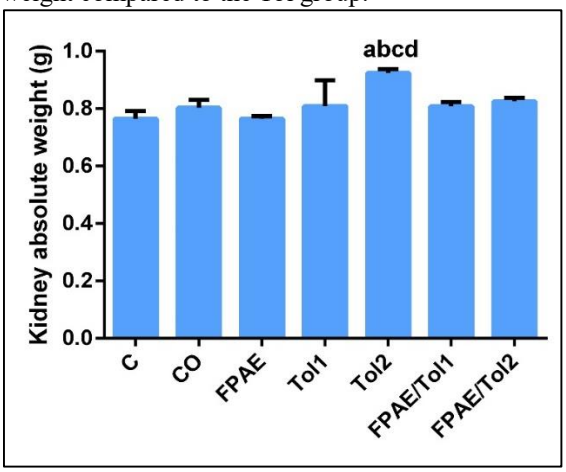

Figure 1. Kidney absolute weight of rats in different experimental groups (mean±SEM).
a: statistically different Vs control. **b**: statistically different Vs CO group. **c**: statistically different Vs PJ group. **d**: statistically different Vs PJ group.**e**: statistically different Vs Tol group.
C: Control, **CO**: Corn Oil, **FPAE**: Fresh Peel Aqueous Extract, **Tol1**: Toluene dose 1; **Tol2**: Toluene dose 2, **FPAE-Tol1**: Toluene dose 1 + Fresh Peel Aqueous Extract, **FPAE-Tol2**: Toluene + Fresh Peel Aqueous Extract).

Serum biochemical parameters

As shown in figures 2 and 3, oral administration of toluene resulted in a significant increase in serum urea and creatinine concentrations compared to the control and positive control groups. However, supplementation with pomegranate peel aqueous extract FPAE-Tol significantly reduced the concertation compared to the Tol2 group.
The serum uric acid of Tol2 group showed a significant decrease compared with the control and positive control groups (Figure 4). No significant differences were noted in the Tol1 group compared to the control animals.

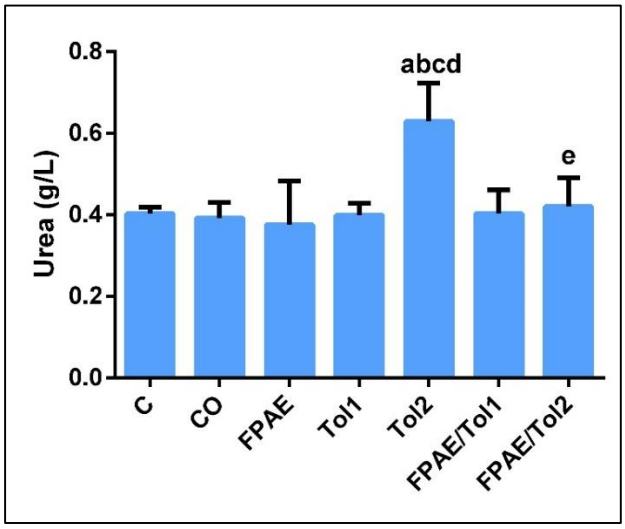

Figure 2. Urea levels in different experimental groups (mean±SEM).
a: statistically different Vs control. **b**: statistically different Vs CO group. **c**: statistically different Vs PJ group. **d**: statistically different Vs PJ group.**e**: statistically different Vs Tol group.
C: Control, **CO**: Corn Oil, **FPAE**: Fresh Peel Aqueous Extract, **Tol1**: Toluene dose 1; **Tol2**: Toluene dose 2, **FPAE-Tol1**: Toluene dose 1 + Fresh Peel Aqueous Extract, **FPAE-Tol2**: Toluene + Fresh Peel Aqueous Extract).

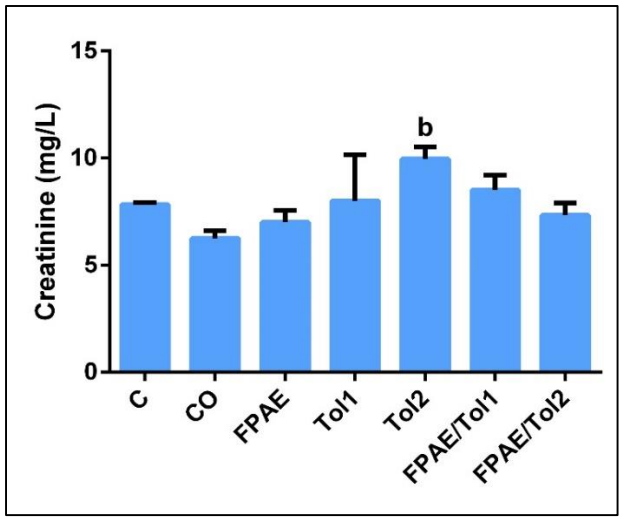

Figure 3. Creatinine levels in different experimental groups (mean±SEM).

a: statistically different Vs control. **b**: statistically different Vs CO group. **c**: statistically different Vs PJ group. **d**: statistically different Vs PJ group.**e**: statistically different Vs Tol group.
C: Control, **CO**: Corn Oil, **FPAE**: Fresh Peel Aqueous Extract, **Tol1**: Toluene dose 1; **Tol2**: Toluene dose 2, **FPAE-Tol1**: Toluene dose 1 + Fresh Peel Aqueous Extract, **FPAE-Tol2**: Toluene + Fresh Peel Aqueous Extract).

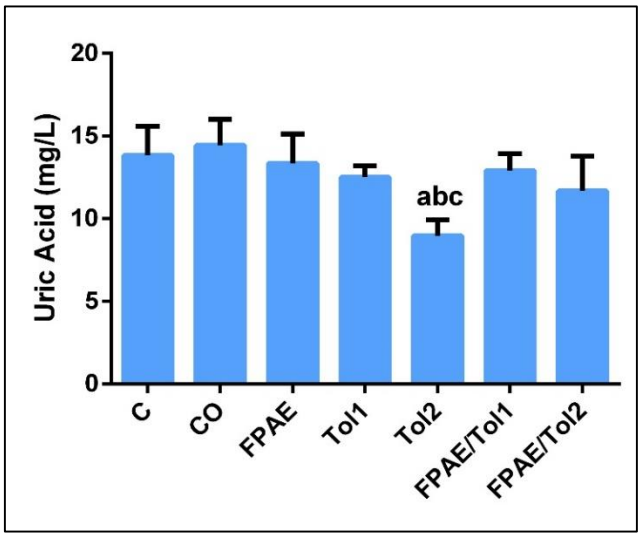

Figure 4. Uric Acid levels in different experimental groups (mean±SEM).

a: statistically different Vs control. **b**: statistically different Vs CO group. **c**: statistically different Vs PJ group. **d**: statistically different Vs PJ group.**e**: statistically different Vs Tol group.
C: Control, **CO**: Corn Oil, **FPAE**: Fresh Peel Aqueous Extract, **Tol1**: Toluene dose 1; **Tol2**: Toluene dose 2, **FPAE-Tol1**: Toluene dose 1 + Fresh Peel Aqueous Extract, **FPAE-Tol2**: Toluene + Fresh Peel Aqueous Extract).

Oxidative stress parameters

As indicated in table 1, oxidative stress results revealed a significant reduction in the renal GSH level and GPx activity, whereas the renal MDA concentration was significantly increased in rats of Tol2 group compared to the control and positive control groups (CO and FPAE). On the other hand, the group Tol1 did not show significant difference compared controls.

Conversely, the administration of *P. granatum* FPAE significantly increased the level of GSH and the activity of GPx compared to Tol2 group. Results also showed a significant decrease in MDA levels of FPAE supplemented rats compared to the group treated with Tol2.

Table 1. Oxidative stress parameters of rats in different experimental groups (mean±SEM).
C: Control, **CO**: Corn Oil, **FPAE**: Fresh Peel Aqueous Extract, **Tol1**: Toluene dose 1; **Tol2**: Toluene dose 2, **FPAE-Tol1**: Toluene dose 1 + Fresh Peel Aqueous Extract, **FPAE-Tol2**: Toluene + Fresh Peel Aqueous Extract).

Groups	GSH Kidney (nmoL/mg Prts)	GPx Kidney (nmoL GSH/mg Prts)	MDA Kidney (nmoL/mg Prts)
C	56.11±3.75	0.44±0.007	0.32±0.01
CO	54.83±2.71	0.43±0.02	0.34±0.01
FPAE	57.03±2.59	0.45±0.004	0.29±0.007
Tol1	54.81±3.13	0.42±0.02	0.36±0.008
Tol2	38.76±5.27^{abcd}	0.31± 0.02^{abcd}	0.53±0.02^{abcd}
FPAE-Tol1	53.88±0.78	0.42±0.009	0.34±0.02
FPAE-Tol2	51.27±0.81	0.40±0.01^{e}	0.43±0.01^{de}

Histological examination

As shown in Figure 5, Renal microscopic observation in the control rats (C, H, PJ, FPAE) revealed a normal renal parenchyma with normal architecture, consisting of the glomerulus (GL) surrounded by a narrow and clear space. Tol2 exposure (E) resulted in renal parenchyma changes and disorganization, characterized by extensive vacuolization, dilatation of Bowman's space, degeneration of the renal tubular epithelium, and glomerulus atrophy. Whereas renal tissues of rats in FPAE-Tol2 (G) groups were less affected compared with the Tol2 group.

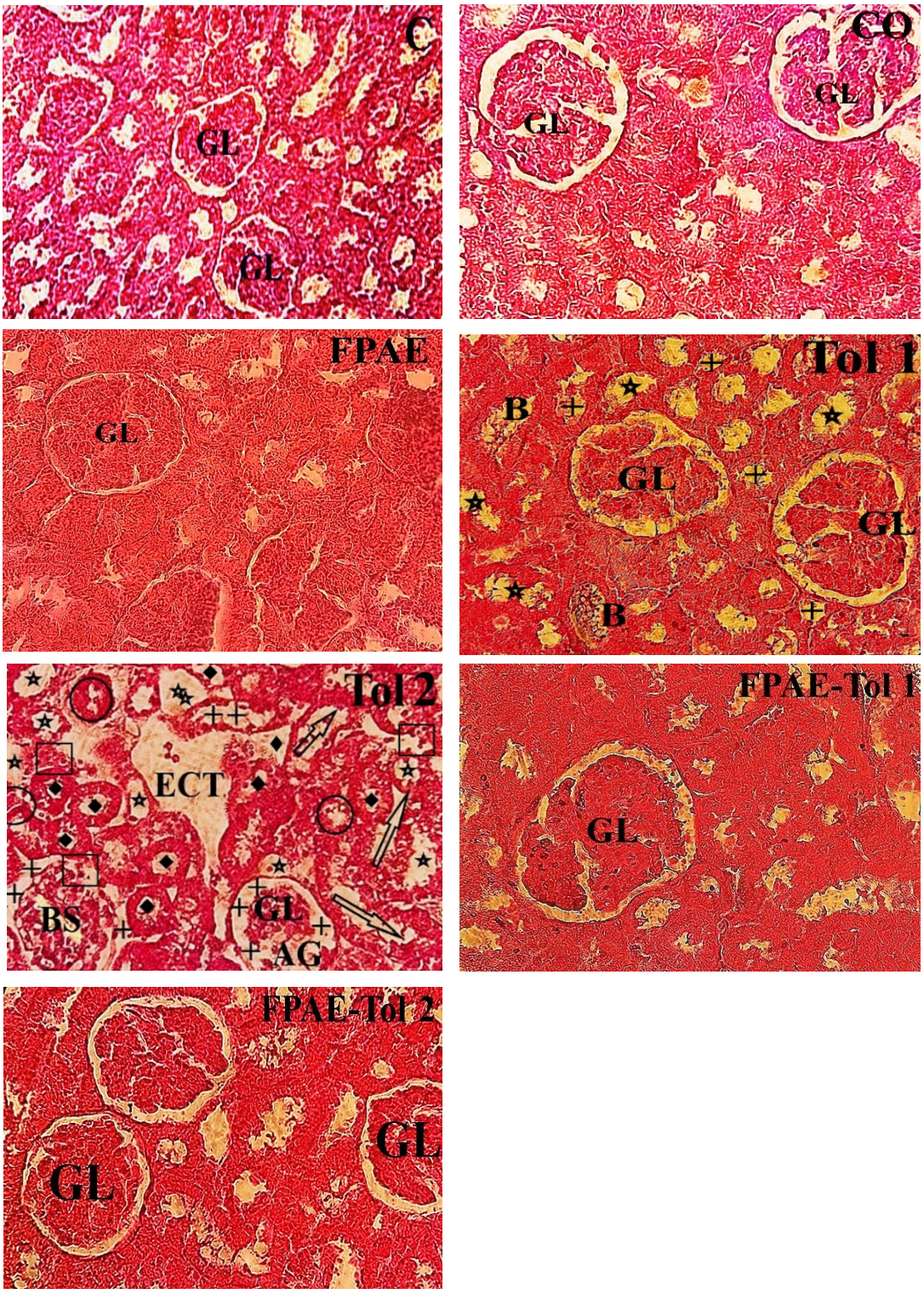

Figure 5. Light microphotographs of kidney tissues from different experimental groups (×40).

Control (A), CO (B), FPAE (C), and Tol1 (D) revealed a normal renal glomerulus (GL) and tubular epithelium (×10). Tol2 group (E) showed a deleterious morphological change versus those of the control groups, which was illustrated by glomerular atrophy (GA) with dilatation of Bowman's space (BS) and degeneration of the renal tubular epithelium (stars, circles, and arrows). Yet, FPAE-Tol1 (F) and FPAE-Tol2 (G) groups displayed a better-preserved epithelium and morphology with a normal architecture nearly like those of controls.

(GL: glomerulus, BS: Bowman's Space, AG: atrophy of the glomerulus with widening of Bowman's capsule, stars: dilatation of the convoluted tubule, (+): dilatation of Bowman's space, arrow: tubular dilatation and degeneration of the renal tubular, ECT: empty convoluted tubule). C: Control, CO: Corn Oil, PJ: Pomegranate Juice, PAE: Peel Aqueous Extract, Tol: Toluene; PJ-Tol: Toluene + Pomegranate Juice, PAE-Tol: Toluene + Peel Aqueous Extract).

4. DISCUSSION AND CONCLUSION

Humans are exposed to numerous environmental agents that can impair their physiologic systems capacity. Renal function is known to be highly sensitive to many chemical and physical agents generated by industrial and agricultural activities **[2,10,11]** The kidney is the target organ for many occupational and environmental chemicals and acts as chief player in their elimination **[19]**. As the primary excretory organ, it becomes a major target for various xenobiotics, such as toluene, which can be eliminated in the urine after 12 hours following exposure, mainly as metabolites.

Exposure to toluene showed a significant increase in rats' kidney absolute weight. This result agrees with that obtained when Wistar rats were exposed to this organic solvent. Indeed, following exposure to toluene at 4.5 ml/kg on male rats for 7 days, a drastic increase in absolute kidney weight was observed **[20]**, which could be attributed to an inflammation of renal tissues. It has been shown that exposure to toluene in provoked an elevation of pro-inflammatory cytokines levels (IL-4, IL-13, TNF-α, and IFN-γ) **[21]**. In people smelling toluene chronically from adhesives, accumulation of immune complexes in the kidneys has been observed **[22]**. This increase may also be the result of tubular cells swelling, their disorganization and congestion **[8].**

Toluene induced an increase in the blood urea and creatinine levels, which was concur with the results of previously reported work **[8, 20].** As markers, uric acid, urea and creatinine was stated to be essential elements to estimate renal function and glomerular filtration **[23].** Numerous mechanisms might explain the high blood levels of these metabolites, including dehydration **[24],** which has been observed in our study**.** According to the study of **Taros et al. [22]** exposure to toluene brings irreversible renal insufficiency due to distal tubular cell damage. Additionally, this increase may be attributed to the elevated proteins catabolism into amino acids to form urea and creatinine **[25]** Since toluene is mostly eliminated through the kidneys in the form of a hippuric acid, it is evident that it can damage renal glomerular cells; hence, the excretion of urea and creatinine could be disrupted **[6].** The significant decrease in serum uric acid may be interpreted by its strong free radical-scavenging molecule and its protective response against ROS production. Some major epidemiological studies have identified low uric acid levels as a translation to the greater oxidative stress generation **[26].**

The metabolic perturbation induced by toluene has been found to be related to its biotransformation, resulting in particular production of toluene epoxides, which are high reactivity species that can induce damage to various body systems **[7].** Therefore, it appears that it can disturb antioxidant defence system by decreasing GSH levels and GPx activity, along with a rise in MDA levels, which is also seen in our study. Our results seem to be conceivable with those obtained by other researchers **[27]**. It has been mentioned that NO production and its reaction with superoxide radicals generates highly cytotoxic ROS, which can significantly increase the progression of renal failure. This, in turn, resulting in a reduction of intracellular glutathione activity. Several scholars have highlighted the relationship between VOCs, including toluene, and elevated levels of H2O2, COX-2 and NO. These compounds affect enzymatic activity, DNA oxidation, thiol oxidation, nitrosylation, and lipid peroxidation **[7].** Lipid peroxidation can be assessed by measuring MDA, the end product of polyunsaturated fatty acids oxidation causing structural impairment and loss of function of cell membranes. Overproduction of ROS is a key pathogenic process contributing to the disruption of intracellular redox homeostasis or indirectly activate signal transduction pathways **[28]**, which is known to cause tissues pathological changes. Regarding the drop observed in GSH concentration the reason perhaps was associated not only to the excessive production of ROS but could also result from impaired regeneration by GSH reductase. Decreased regeneration of GSH may occur due to a deficiency in the reducing equivalent NADPH, which can result from the alterations in mitochondrial membrane permeability by oxidative stress **[29].**

In line with our previous findings in this study, several structural changes were observed renal in rats treated with Tol. Thus, the main renal histopathological features included abnormal nephrotic changes varied from degenerative to necrotic in some tubular epithelium besides to atrophy of glomerular part. renal tubular cells were swollen, had loss of staining capacity, and nuclei appeared to be dilated, presence of blood clot was noted in toluene-treated rats. Renal tubules swelling and distortion lining with congestion of interstitium was mentioned after exposure of rats to 900 mg/kg of toluene **[6]**. The critical reason of the cellular damage caused by toluene is a caspase dependent process, which plays an important role in the production of inflammatory mediators and apoptosis. It was suggested that Bax/Bcl2 ratio was directly associated with renal apoptosis and progressive diseases in other organs **[6].**

Pomegranate has a protective effect as it contains high content of phenolic compounds, phenolic acids, ellagic tannins (punicalin, punicalagin, gallagic, and ellagic acid), flavonoids (anthocyanins, catechins, rutin, epigallocatechin-3- gallate), and anthocyanins (delphinidin, cyaniding, and pelargonidin), which possess many prominent capacities **[9,30,31].** In terms of the investigation of *Punica granatum*'s role, our study revealed that treatment with PAE and PJ generally improved the impairment induced by toluene in kidney. Our results are in harmony with the findings other findings **[32],** who suggested that the administration of pomegranate peel extract significantly attenuated the damaging

impact of phenylhydrazin on the kidney in rat models. It has been affirmed that phytoconstituents such as gallic acid was proved to be very effective against oxidation by restoring damaged tissues in rats, which might be explained by their ability to donate a hydrogen atom from their phenolic hydroxyl groups **[30].** Other **a**uthors also established that flavonoids and ellagic acid can elevate the biosynthesis of glutathione and/or prevent its degradation by increasing the vital enzyme in GSH synthesis (C-glutamyl cysteine synthetase) and serum paraoxonase activity, potentially protecting against lipid peroxidation **[33].** Furthermore, punicalagin exhibits the capacity to inhibit inflammation and apoptosis by modulating the production of NO, COX, Bax and Bcl-2, therefore protecting cells from death **[12,13].**

In conclusion, the current study affirmed that the sub-chronic exposure to a moderate dose of toluene induces kidney injuries mediated via oxidative stress, confirmed by lipid peroxidation, and down-regulated antioxidant parameters. But further studies are needed to ascertain the precise mechanisms of its action on mitochondrial function. Supplementation of *Punica granatum* fresh peel aqueous extract has been proved to own excellent antioxidant activities that prevented the effects of this toxic solvent by the restoration of renal tissue structure and function.

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Mancozeb has inhibited the reproduction mechanism in male domestic pigeons (*Columba livia domestica*) and altered hepatic function

Souheila Slimani[1]*, Badis Aouzal[1], Cherif Abdennou[2], Mohamed Salah Boulakoud[2]

[1] *Laboratory of Interaction Research, Biodiversity, Ecosystems and Biotechnology, Faculty of Sciences, University 20th August 1955 Skikda, Algeria.*
[2]*Laboratory of Animal Ecophysiology, Department of Biology, University Badji Mokhtar-Annaba, Annaba 23000, Algeria*

Info. Article

Historique de l'article

Reçu le 06/12/2024
Révisé le 26/01/2025
Accépté le 13/04/2025

Keywords:

Mancozeb, pigeon, saisonal reproduction, photoperiod, biochemical markers.

ABSTRACT

Pesticides are toxic chemicals, very much used in agriculture in order to increase the agricultural outputs. These molecules can affect reproductive function of animals. This study aimed to the toxicity of the fungicide mancozèb on certain blood biochemical markers and seasonal reproduction of male domestic pigeons (Columba livia domestica) under long photoperiod (20L: 04D). The fungicide was orally administered at 2 and 5 g/l (doses used in agriculture). The obtained results revealed that under a long photoperiod, the sexual activity lasted only 04 weeks. In addition, mancozeb administration induced gonadic regression, delayed the refractory phase, provoked a hyperglycemia, a hyperlipidemia, an elevation in the activity of alkaline phosphatase and aminotransferase, and a reduction in creatinine level. The toxic effects of mancozeb was apparent in higher doses. To conclude, mancozeb has inhibited the reproduction mechanism in male pigeons and altered hepatic function.

* ***Corresponding author***: shsouheila@yahoo.fr

1. INTRODUCTION

Mancozeb is a fungicide widely applied in the fields in order to eradicate plant pests. It is a famous product due to its low toxicity on non-target organisms, with a weak acute toxicity [1]. Furthermore, it is known to be lethal to nematodes at agricultural concentrations, while the sub lethal doses had affected nervous system, heat shock reactions and larval growth [2]. Mancozeb has also been shown to induce significant increases in reactive oxygen species (ROS) and cause mitochondrial inhibition in *in vitro* studies. It has been repported that Mancozeb have heamatotological and biochemical effects and thyroid toxicity [3].

As for reproductive toxicity in general, it is difficult to ascertain whether the chemicals are harmful to such physiological function. Mancozeb impairs female reproductive performance with blastomeric apoptosis in embryos at very low concentrations [4]. Mancozeb was demonstrated to alter implantation in mice [4]. Sexual organs were shown to be reduced in rats exposed to mancozeb, while long period treatment by mancozeb has disrupted spermatogenesis, it raised testicular lipid levels, degeneration in somniferous and epididymal tubules with loss of sperms. Biochemical changes of gonads were observed in male rats after chronic exposure to mancozeb [5].

In view of paucity of information on reproductive system, the present study has been undertaken to know the possible effects of mancozeb on reproductive cycle and some biochemical markers of domestic male pigeon (*Columba livia domestica*).

2. MATERIALS AND METHODS

2.1. Chemicals

Mancozeb or Zn, Mn-ethylene bisdithiocarbamate, a carbamate fungicide (bayer France) was diluted with drinking water of pigeons to obtain the required concentrations, which then given to birds.

2.2. Animals

Male pigeon (*Columba livia domesica*), which had been captured locally, were kept in light-controlled rooms in metal cages measuring (100 x100x100) cm, with six birds per cage. They had been under a natural photoperiod, ambient temperature and humidity of 20±2 °C and 55% ± 4, respectively for 15 days. Food (chick crumbs) and water were provided *ad libitium*. Eighteen pigeons were divided equally to three groups, in which the first group was used as a control, but the second one has received 5 g/l of mancoeb (tech 75% purity); a concentration used in agriculture. However, the third group was given 2 g/l of mancozeb. All groups were held under artificial photoperiod of (20L: 4D) using electric horologe of an intensity of 72 watts. Hence, water containing mancozeb was renewed every 48h.

2.3. Laparotomy and blood sampling

Gonadal development was assessed by laparotomy at intervals of approximately 15 days. The gonads were examined through a small incision in the body wall between the last two ribs, after anesthetizing the incision with viscous lidocain. And the dimensions of the left testis measured to the nearest 0.5 mm. Testicular volume was calculated $V= 4/3\ \pi\ a2\ b$; where a is half the width and b is half the length (long axis).

Blood samples were obtained by pricking a superficial wing vein and collecting approximately 1 ml blood into heparinised tubes.

2.4. Analytical procedures

The plasma enzyme activities of alanine aminotransferase (ALT), plasma alkaline phosphatase (ALP), creatinine were measured by using commercially diagnostic kits obtained from Randox Laboratories (Ardmore, Northern Ireland, UK) using an automate CX9 (BECKMAN, Brea, CA, USA) as well as for glucose (kits supplied by Diamond Diagnostic).

The total cholesterol (TC) and triglycerides (TG) were analyzed by the enzymatic colorimetric methods (Randox reagent). TC was measured according to the enzymatic endpoint cholesterol oxidase-phenol aminophenazone method. TG were measured according to the glucose oxidase-phenol-aminophenazone method after enzymatic hydrolysis by lipases.

2.5. Statistical treatment

Data were presented as mean values ± SEM by using ANOVA, followed by Student's t test to assess significant differences among treated groups. All statistical analyses were performed using Minitab version 16.The significance was defined as $p\leq 0.01$.

3. RESULTS

Changes in testicular size

Testicular volumes were presented in fig 1. At the beginning of the experiment, all birds had mean testicular size of 188.3±36 mm^3. Control birds that were kept on 20L: 4D; long photoperiod throughout the experiment, all maintained fully reproductive cycle, characterized by significant ($p\leq 0.01$) increase in the volume of their testes at week 4, followed by spontaneous gonadal regression, with testes reaching a minimal size of 43.21 ± 8.4 mm^3 ($p\leq 0.05$) by week 8 of the experiment. No significant changes were observed testicular volume during the period of study. Though, treated pigeons have higher testicular volume compared to the control at the last week.

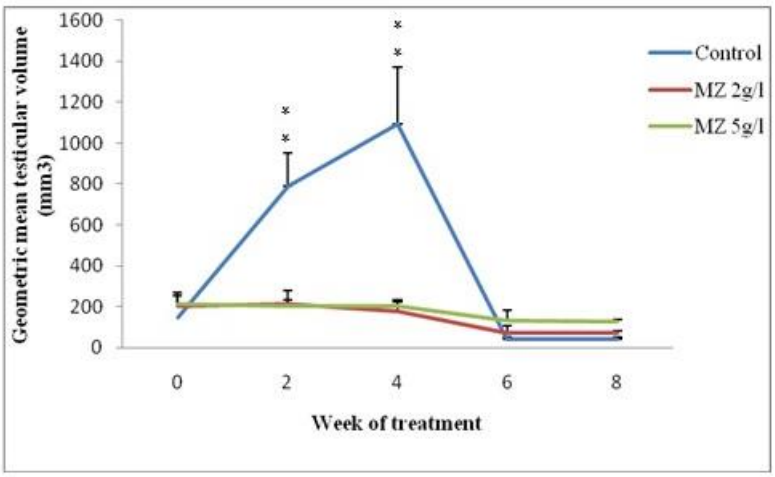

Fig 1: Change in testicular volume (mm3) in male pigeons treated at two different doses of mancozeb (2 and 5g/l) and placed under 20L: 4D. Data points correspond to geometric mean±SEM (n=6). Significant differences at $p \leq 0.05$ and $p\leq 0.01$ (ANOVA followed by Student's t test).

Changes in biochemical markers

Alanine amino transferase

The changes in alanine amino transferase (ALT), of males' pigeons (Columba livia domestica) held under 20 L: 4 D and treated with mancozeb are given in Fig 2. Treatment of domestic pigeon with mancozeb induced a significant increase ($p \leq 0.05$) in ALT (27.45, 30.73 and 34.54 U/l for the control, 2 and 5g/l mancozeb, respectively) at the 4th week of experiment.

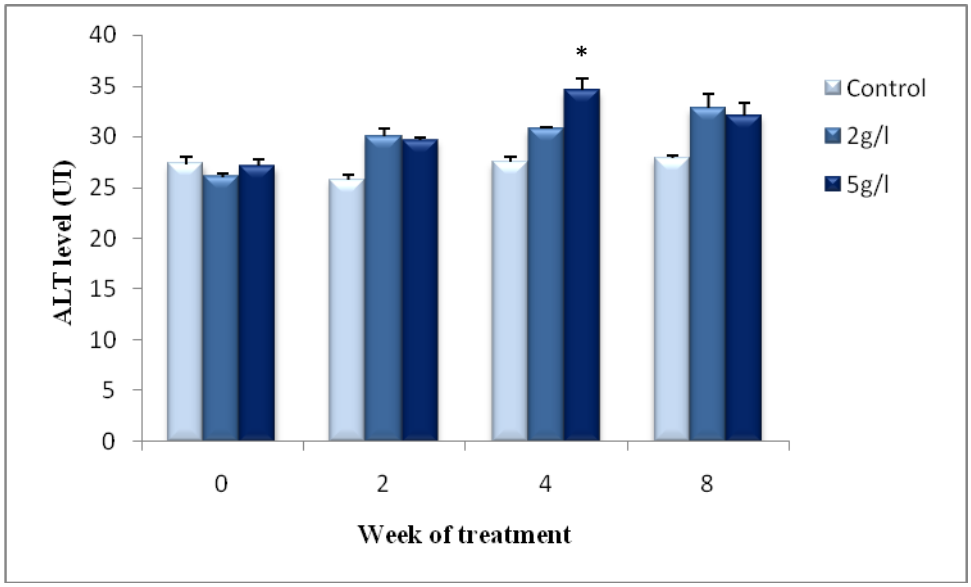

Fig 2: Change in (ALT) level (UI/L) in male pigeons treated at two different doses of mancozeb (2 and 5g/l) and placed under 20L: 4D. Data points correspond to geometric mean±SEM (n=6). Significant differences at $p \leq 0.05$ (ANOVA followed by Student's t test).

Alkaline phosphatase

Results relative to changes in alkaline phosphatase (ALP) are summarized in Fig 3. Obtained results revealed that treatment by mancozeb had produce a significant increase ($p \leq 0.01$) in enzymatic activity of alkaline phosphatase (ALP) until the 2nd week of experience, where we have recorded a mean level of 147.22, 599.75 and 703 UI/L for controls 2 and 5g/l mancozeb, respectively. It is important to note that control pigeons hadn't shown any changes in (ALP) through the experiment.

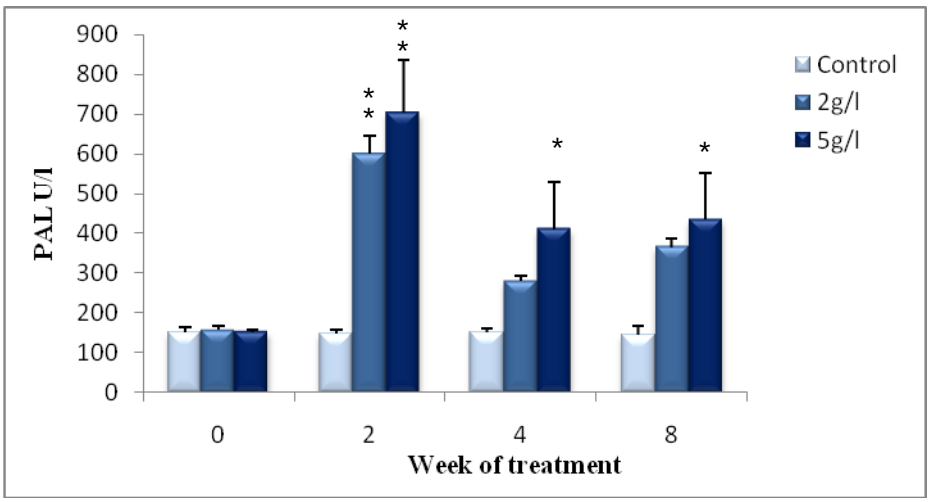

Fig 3: Change in (PAL) level (UI/L) in male pigeons treated at two different doses of mancozeb (2 and 5g/l) and placed under 20L: 4D. Data points correspond to geometric mean±SEM (n=6). Significant differences at $p \leq 0.05$ and $p \leq 0.01$ (ANOVA followed by Student's t test).

Glucose

The glucose concentration in blood of male pigeons (*Columba livia domestica*) placed under 20L: 4D and treated with mancozeb was significantly increased ($p \leq 0.05$) through the study from 3.28 g/l to 3.78 g/l in pigeon received 2g/l of mancozeb and from 3.34 to 4.86 g/l of blood glucose of pigeon treated with 5g/l of mancozeb. However, control pigeons have revealed a slight decrease in plasma glucose (Fig 4).

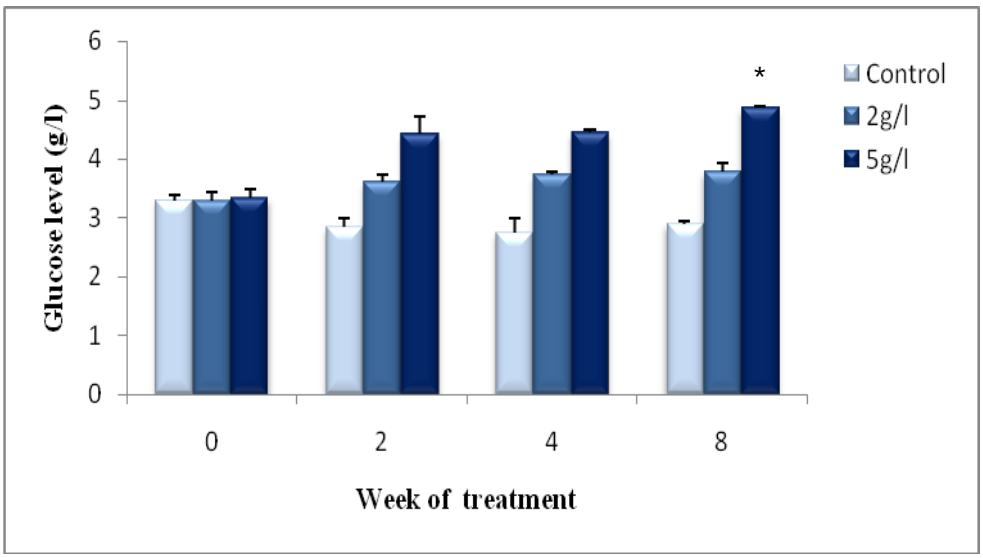

Fig 4: Change in plasma glucose (g/l) in male pigeons treated at two different doses of mancozeb (2 and 5g/l) and placed under 20L: 4D. Data points correspond to geometric mean ± SEM (n=6). Significant differences at $p \leq 0.05$ (ANOVA followed by Student's t test).

Creatinine

The results obtained revealed a significant decrease ($p \leq 0.05$) in plasma creatinin. Whose we are recorded a value of 7.6 g/l of creatinin at the beginning of the study and 2.01 g/l at the end in 5 g/l of mancozeb treated. Control subject have reserved the same concentration of creatinin through the study (Fig 5).

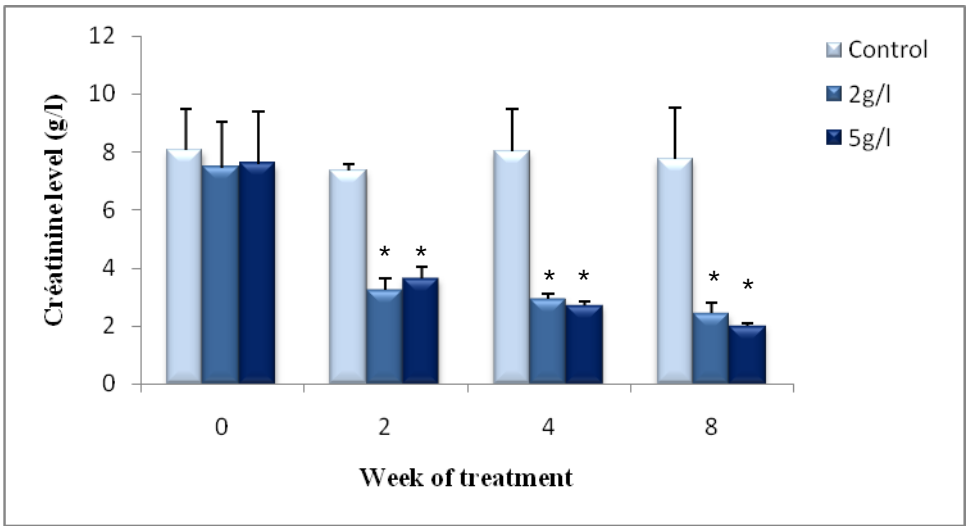

Fig 5: Change in plasma creatinine (g/l) in male pigeons treated at two different doses of mancozeb (2 and 5g/l) and placed under 20L: 4D. Data points correspond to geometric mean ± SEM (n=6). Significant differences at $p \leq 0.05$ (ANOVA followed by Student's t test).

Cholesterol

The mean plasma cholesterol of control and treated male pigeons (Columba livia domestica) placed under long daily photoperiod 20L: 8D and orally treated with 2 and 5 g/ were shown in Fig 6. The treatment with mancozeb caused a significant ($p \leq 0.05$) elevation in plasma cholesterol till the 4th week of experiment in treated groups. While, controls had shown a non significant decrease in plasma cholesterol.

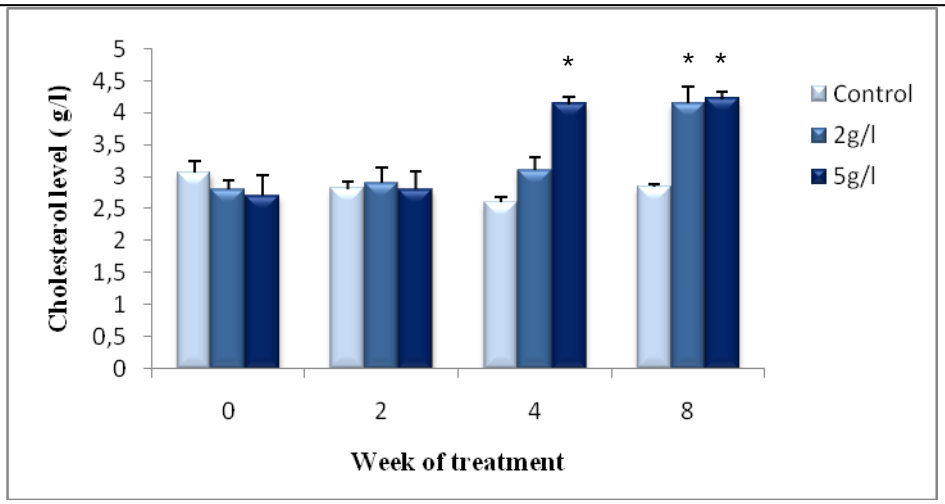

Fig 6: Change in plasma cholesterol (g/l) in male pigeons treated at two different doses of mancozeb (2 and 5g/l) and placed under 20L: 4D. Data points correspond to geometric mean ± SEM (n=6). Significant differences at $p \leq 0.05$ (ANOVA followed by Student's t test).

Triglycerides

The results related to triglycerides recorded in this study are summarized in Fig 7. Data show a significant ($p \leq 0.05$) decrease in circulating triglycerides in mancozeb treated groups through the experiment. From 1.6 g/l to 4.3 g/l in 2g/l treated pigeons, and from 1.52 to 4.55 g/l among pigeons treated by the highest dose of mancozeb. There were no notable changes in triglycerides concentration in control subjects.

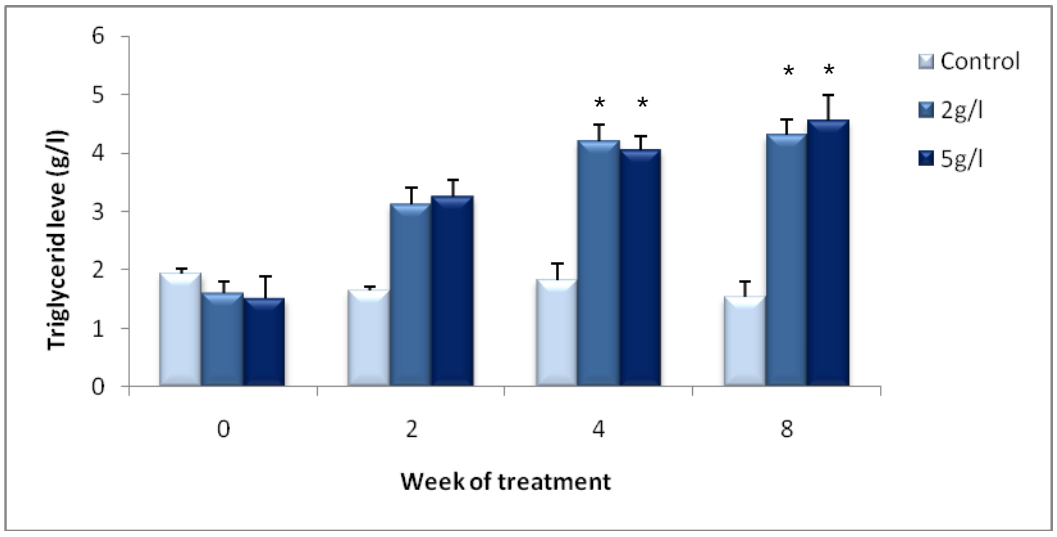

Fig 7: Change in plasma triglycerides (g/l) in male pigeons treated at two different doses of mancozeb (2 and 5g/l) and placed under 20L: 4D. Data points correspond to geometric mean ± SEM (n=6). Significant differences at $p \leq 0.05$ (ANOVA followed by Student's t test).

4. DISCUSSION

In birds, the gonadal growth and regression are highly seasonal and relate to environmental factors such as food availability and the photoperiod length. Therefore, the day length has been well defined as the regulator of different metabolic and reproductive activities in many avian species. It is known that birds use their extra-retinal photoreceptors, in combination with a circadian clock, to measure photoperiod [6]. Findings from this work indicate that under artificial photoperiod (20 L: 4D), birds maintained a fully reproductive cycle characterized by full mature testes at the 4th week, followed by spontaneous gonadal regression, as an increase in photoperiod rises the secretion rate of gonadotrophin-releasing hormone (GnRH) leads to increased LH and FSH, and then to steroid hormone synthesis [6]. However, the administration of mancozeb at a rate of 2 g/l and 5 g/l to male pigeons under long days, has inhibited the development of testes, and disturb their reproductive cycle.

It has been recorded a lower means in testes sizes during the experiment in treated birds. Physiologically, it is possible that is attributed to the mechanism of photoperiod measurement. Therefore, they hadn't estimated the true photoperiod, and consequently all photoperiod would be regarded as being short [7].

On the other hand, it is possible that mancozeb have interfered directly on the testis as number of pesticides has showed testicular toxicity [7]. Study demonstrated that mice treated with mancozeb had a decreased testes weight and an inhibited spermatogenesis [5]. Other studies revealed a testicular atrophy with damaged germinal epithelium, accompanied with reduced sperm motility and viability in male adult pigeons exposed to maneb [8]. The toxic effect of pesticides on reproductive cycle of domestic pigeons (*Columba livia*) associated with a decreased testes size, reduced tubule size, and the reduced number of germ cells have been reported [9], [10].

Few studies have been carried out on the mechanisms of organometallic fungicide action on target organisms. However, many studies have been reported on the effects of heavy metals alone in a variety of organisms [11]. Exposure to most metals result in metal accumulation in certain tissues and organs of the exposed organisms. Chronic inhalation of high levels of Mn has been associated with a neurodegenerative disorder characterized by both central nervous system abnormalities and neuropsychiatric disturbances [12]. Zn is well known to accumulate in two particular organs, namely liver and kidney, where they may cause biochemical and histopathological changes [7].

In the present study, exposure to mancozeb resulted in biochemical disorders. Alanine amino transferase (ALT) and alkaline phosphatase (ALP) are primarily used to evaluate hepatic damage in clinical findings [13]. The results of this study demonstrated a notable elevation of plasma ALT and ALP activities of pigeons intoxicated by mancozeb fungicide. These findings agreed with that recorded in serum of thiram treated pigeons [13]. A higher activity of ALT in delthametrin, abamectin alone and combination treated pigeons [14]. This can be attributed to the tissue damage, especially hepatocytes [14]. Such findings are in-line with that reported earlier on the hepatocytes necrosis and aminotransferase activities elevation [13] and confirming that it may be attributed to liver injury [14].

Receiving mancozeb at dose of 2 and 5g/l led to an increase in plasma glucose in *Columba livia*., as a result of increasing catecholamines and corticosteroid hormones levels through the activation of gluconeogenesis pathway. Increased plasma glucose levels were recorded after exposing pigeons to thiram [13]. In this study plasma creatinine values were gradually decreasing with increasing dose of mancozeb. This decrease might be resulted from kidney functional impairments, which have been confirmed by tubular histopathological studies. Plasma cholesterol and triglycerides were significantly elevated, is possibly an indication of hepatocytes permeability [13]. Thus, the obstruction of bile duct can contribute in the decrease of cholesterol secretion through the bile, leading to its raise in the plasma [14].

5. CONCLUSION

In conclusion, our results indicate that under a long daily photoperiod of (20L: 4D), male domestic pigeon (*Columba livia domestica*) maintained a fully reproductive cycle characterized by full testicular maturity after one month, followed by spontaneous gonadal regression. However, the administration of mancozeb at a rate of 2 g/l and 5 g/l to male pigeons under long days, has inhibited the development of testes, and disturbed their reproductive cycle. Biochemical findings of this study suggest that mancozeb induced hepatotoxicity via an increase of alanine amino transferase and alkaline phosphatase activity, accompanied with hyperglycemia, hyperlipidemia and an elevation in creatinine level.

Acknowledgements

Authors are grateful to the PNR projects, Algiers for the financial support which was carried out within animal ecophysiology laboratory.

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Olea europaea leaf extract can modulate the reproductive state of cadmium-poisoned rats

Rym Chaker[1], Ouarda Mansouri[2], Farida Loudjani[1], Cherif Abdennour[1]

[1] Laboratory of Animal Ecophysiology, Department of Biology, Faculty of Sciences, Badji Mokhtar University, Annaba 23000, Algeria
[2] Laboratory of Animal Ecophysiology, Faculty of Medicine, Badji Mokhtar University, Annaba 23000, Algeria

Article Info.

Article history:

Received: 02/02/2025
Revised: 17/04/2025
Accepted: 17/04/2025

Keywords:

Cadmium,
Fertility,
GSH,
MDA,
Rat.

ABSTRACT

Cadmium is a widely used heavy metal and is considered a powerful endocrine disruptor, with adverse effects on fertility and the body's physiological activities. The aim of the study is to investigate the benefits of the aqueous extract of fresh olive leaves (*Olea europaea* L.) in reducing cadmium toxicity. In this context, 80 male *Wistar* rats were divided into eight groups and treated daily for one month by gavage. The negative control received a standard diet; three positive control groups (LEO1, LEO2, and LEO3) respectively received different extracts (0.25, 0.5, and 1 g/kg rat), one group received fresh cadmium chloride solution (40 mg/kg rat), and the last three groups received a combination of LE + $CdCl_2$ with the same dosages. The results demonstrated cadmium led to a decrease in fertility parameters (concentration, mobility, LH, and testosterone), with a significant decrease in GSH and an increase in MDA levels. Supplementation of olive leaf extract significantly improved all parameters of the three combined treatment groups with different degrees. In conclusion, olive leaf extract was able to mitigate cadmium-intoxicated rats and preserved reproductive parameters.

***Auteur correspondant**: manouriouarda22@gmail.com*

1. INTRODUCTION

Increased environmental pollution due to industrialization has become a major health concern worldwide [1; 2]. Heavy metals used in many applications continue to have very harmful effects on the environment and human health [3]. Cadmium is present in water, soil, and air, and it is toxic even at low concentrations. It also tends to accumulate in living organisms [4]. Due to its high toxicity and prolonged persistence in the environment, Cd, which is not essential, requires special attention [5]. Cadmium is a widespread environmental contaminant from industrial and agricultural activities, causing unavoidable exposure [6]. It is easy for it to enter the food chain and endangering human health [7]. Long-term exposure to cadmium in the environment and occupational activities has been associated with several pathophysiological disorders affecting vital organs [8; 9].

Mechanisms that induce testicular toxicity due to this metal include lipid peroxidation (LPO) and over production of reactive oxygen species (ROS). Cadmium has negative effects on various organs of the body, but its reproductive toxicity in men is of particular concern due to its potential for infertility [10]. The presence of cadmium impairs spermatogenesis, reduces sperm quantity and quality, and causes structural and functional abnormalities of the testis [11].

Metal toxicity neutralizers are commonly found in medicinal plants. They are considered a valuable source of antioxidant compounds due to their free radical scavenging ability and potential health benefits associated with their consumption [12]. The olive tree, known as *Olea europaea* L., is an iconic tree of very ancient origin. The main use of

the olive tree has traditionally been based on the oil extracted from the olive fruit due to its economic and nutritional values. However, in recent decades, interest has gradually shifted to the olive leaf due to its important pharmaceutical values [13; 14; 15]. The leaves have been used in human nutrition in the form of extracts, herbal teas, and powders [16].

In this context, this study focuses on assessing the toxicity of cadmium by evaluating reproductive parameters (spermogram, sex hormones) and oxidative stress (testes) in Wistar rats and on the mitigating effect of olive leaf *Olea europaea* at different concentrations on this toxicity.

2. EQUIPEMENT AND METHODS

2.1 Biological material

This work was carried out on 80 young male Wistar rats from the Pasteur Institute of Algiers, weighing on average 250 grams. The animals were raised in plastic cages (42.5 x 26.5 x 15 cm. Animals were exposed to laboratory conditions.

2.2 Preparation of olive leaf extract

Fresh leaves of the olive tree (*Olea europaea L.*) were sampled and the plant part was ground by adding 10 ml of distilled water using a blender. After filtering the mixture, the obtained aqueous crude extract was administered to rats daily by gavage.

2.3 Preparation of cadmium chloride

Cadmium chloride $CdCl_2$ was dissolved in a volume of distilled water. The obtained solution was administered by gavage.

2.4 Animal treatments

Male Wistar rats were divided into 8 groups: The control group received standard diet (in the form of pellets); the LEO1, LEO2 and LEO3 groups were treated with olive leaf extract at doses of 0.25, 0.5, and 1 g/kg body weight of rats, respectively. The cadmium group was treated with cadmium chloride solution (40 mg/kg body weight). The other 3 groups were treated with a combination of cadmium and leaf extract (Cd+LEO1, Cd+LEO2, Cd+LEO3) at the same dosage. The treatment was administered by gavage for one month.

2.5 Blood sampling

After 30 days of treatment, rats were fasted overnight and then sacrificed in the morning by decapitation. Once the dissection was completed, the testes were removed and weighed. In order to assess oxidative stress parameters, part of this organ was stored in a freezer at -20 °C.

2.6 Spermogramme study

Sperm was collected from a small opening made at the epididymis. The sperm was then diluted in physiological serum (NaCl 0.9%) to study the concentration and mobility of spermatozoa carried out by the machine (Sperm Class Analyzer).

2.7 Estimation of LH and testosterone

The enzyme immunoassay method was used for the determination of luteinizing hormone and testosterone according to the technical sheet of the Diametra ELISA kit.

2.8 Estimation of stress oxidative merkers

Glutathion (GSH)

The glutathione assay was performed according to the method of [17]. The principle of this assay is based on the measurement of the optical absorbance of 2-nitro-5-mercapturic acid.

The latter results from the reduction of 5,5'-dithiobis-2-nitrobenzoic acid (Ellman's reagent, DTNB) by the (-SH) groups of glutathione. For this, a deproteinization of the homogenate is essential in order to keep only the thiol groups specific to glutathione.

To obtain the homogenate, 200 mg of the testicle was placed in 8 mL of 0.02 M ethylene diamine tetra acetic acid (EDTA) solution, cold ground using an ultrasonic grinder at a temperature of 4 ° C.

Malondialdehyde (MDA)

MDA is one of the end products formed during the decomposition of polyunsaturated fatty acids by free radicals. The testicular MDA level was evaluated according to the method of [18].

500 mg of the testis was placed in 5 mL of phosphate buffer solution (0.1 M, pH 7.4), then cold ground (4 °C) using an ultrasonic grinder for 30 seconds to obtain a homogenate.

The protein concentration is determined according to the method of [19] which is based on the use of Coomassie blue (G 250) as a reagent. The latter reacts with the amine groups (–NH2) of the proteins to form a blue complex.

2.9 Statistical analysis

Statistical results are expressed as mean ± standard error. Data analysis was performed by ANOVA test comparing the means of each parameter analyzed two by two, using Graph Pad Prism software. Results are considered significant when P≤0.05 is represented by (*).

3. RESULTS

3.1 Testicular absolute weight

According to the results obtained in Figure 1, a significant decrease was recorded in the group treated with Cd alone compared to the different treatment groups. A significant increase in the combined groups (Cd+LEO) compared to the group treated with Cd alone was recorded.

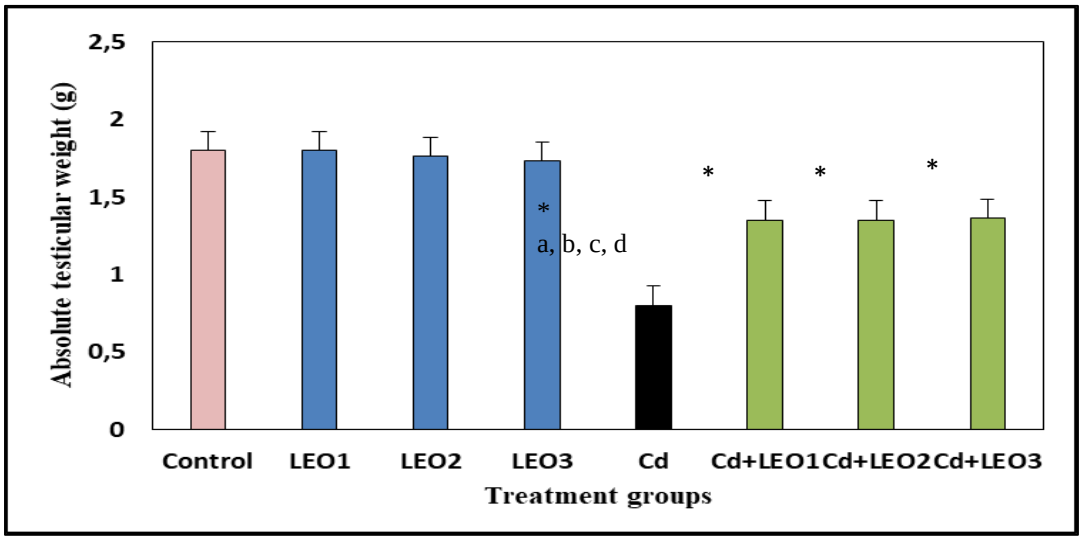

Figure 1: Change in absolute testicular weight (g) in different groups after 30 days of treatment.
a: Cd vs control; b: Cd vs LEO1; c: Cd vs LEO2; d: Cd vs LEO3; e: Cd vs LE

3.2 Spermatozoa concentration

According to the result presented in Figure 2, a significant decrease in sperm concentration in the Cd alone group compared to: (control, positive controls and the three groups combined).

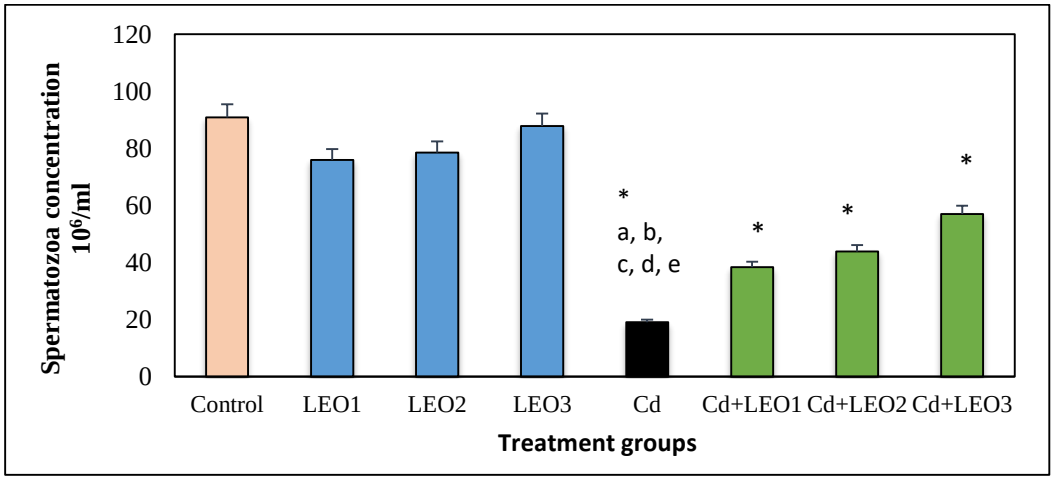

Figure 2: Sperm concentration (10^6/ml) in different treatment groups.
a: Cd vs control; b: Cd vs LEO1; c: Cd vs LEO2; d: Cd vs LEO3; e: Cd vs LE.

3.3 Spermatozoa motility

A significant decrease in sperm motility was recorded in the group treated with cadmium alone compared to the control and positive control groups. On the other hand, a significant increase was observed in the combined groups (Cd+LEO1, Cd+LEO2 and Cd+LEO3) compared to the group treated with Cd alone.

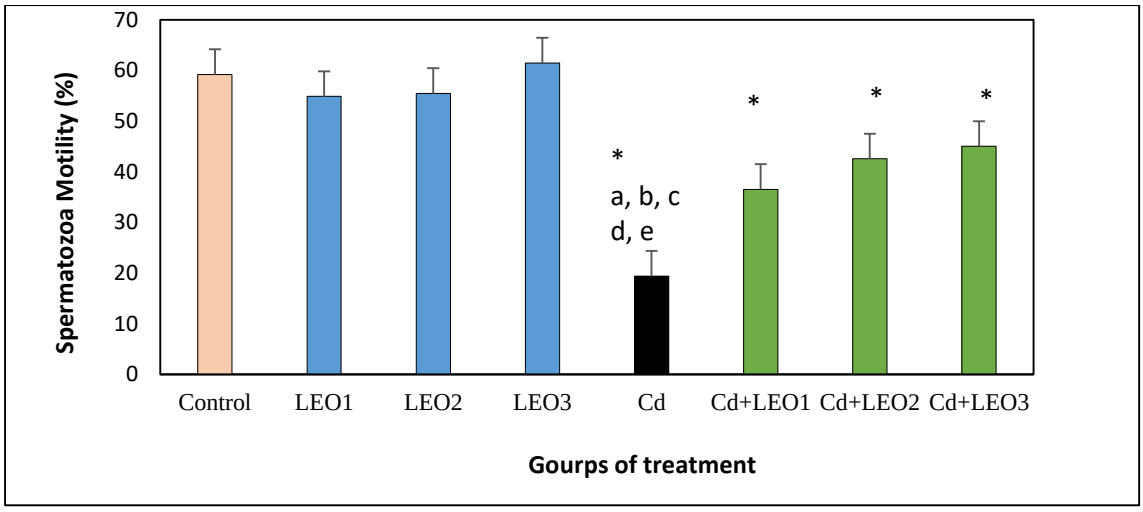

Figure 3: Sperm motility (%) in different treatment groups.

a: Cd vs control; b: Cd vs LEO1; c: Cd vs LEO2; d: Cd vs LEO3; e: Cd vs LE

3.4 Testosterone

Figure 4 showed a significant decrease in testosterone levels in the cadmium-exposed group compared to the control, positive controls, and groups treated with the combination of cadmium and olive leaves at different doses.

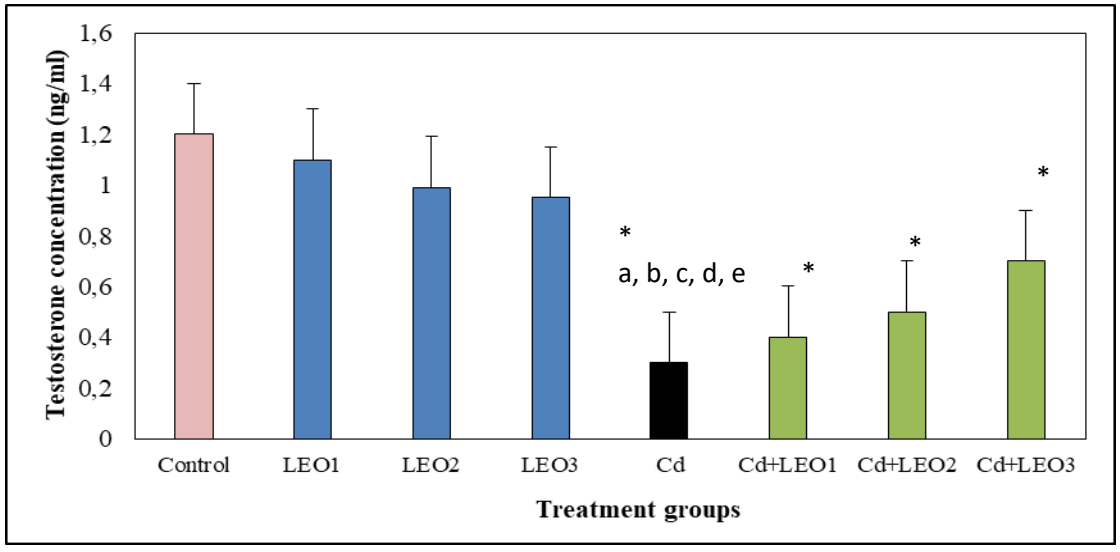

Figure 4: Variation in testosterone concentration (ng/ml) in different treatment groups.

a: Cd vs control; b: Cd vs LEO1; c: Cd vs LEO2; d: Cd vs LEO3; e: Cd vs LE.

3.5 Luteinizing hormone (LH)

A significant decrease was recorded in rats exposed to cadmium alone compared to control, positive control and combined groups.

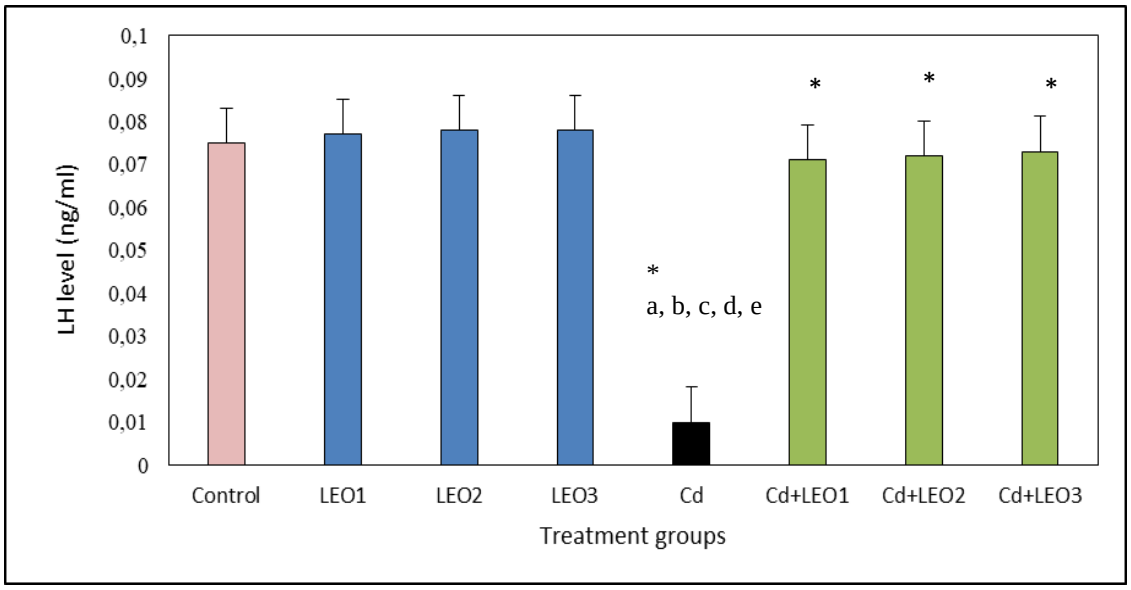

Figure 5: Variation in LH level (ng/ml) in different treatment groups.

a: Cd vs control; b: Cd vs LEO1; c: Cd vs LEO2; d: Cd vs LEO3; e: Cd vs LE.

3.6 Testicular glutathione

According to the results obtained in Figure 6, a significant decrease in glutathione level was recorded in the group treated with Cd alone compared to the control groups, LEO1, LEO2, LEO3 and Cd+LE groups.

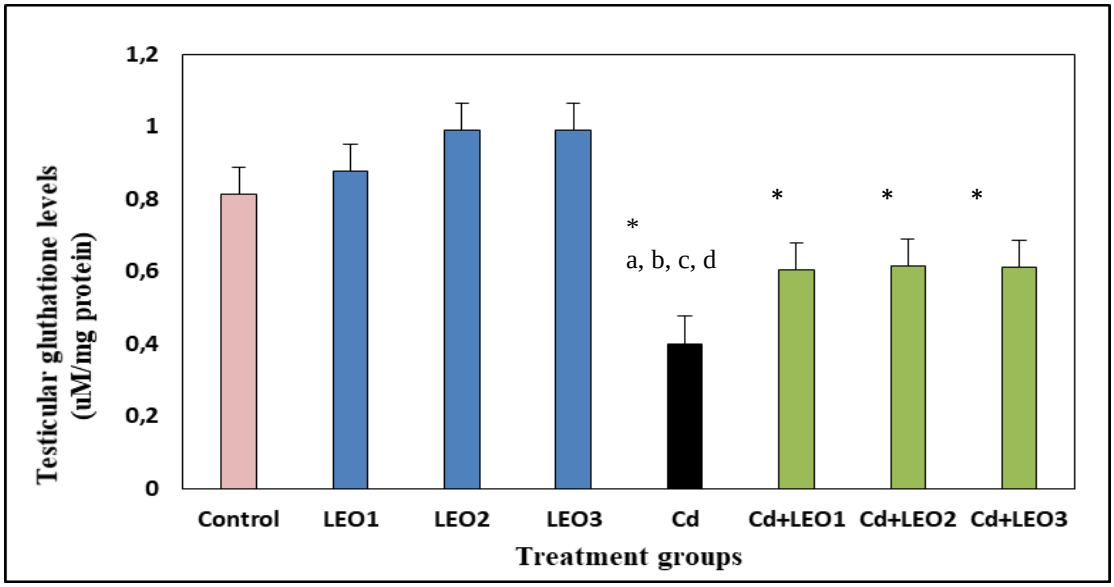

Figure 6: Variation in testicular glutathione levels (µM/mg protein) in rats exposed to cadmium and olive leaf extract after 30 days of treatment.

a: Cd vs control; b: Cd vs LEO1; c: Cd vs LEO2; d: Cd vs LEO3; e: Cd vs LE.

3.7 Testicular malondialdehyde

The results mentioned in Figure 7, indicated a significant increase in the MDA rate concerning the group exposed to cadmium alone compared to the control, positive control and Cd+LEO1, Cd+LEO2 and Cd+LEO3 groups, respectively.

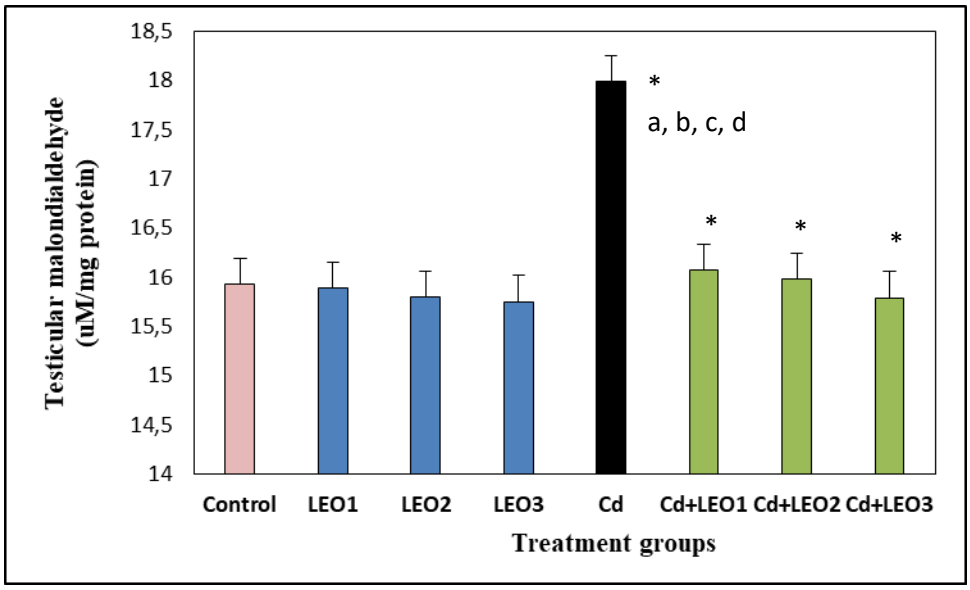

Figure 7: Variation in testicular MDA levels (µM/mg protein) of rats exposed to cadmium and olive leaf extract after 30 days of treatment.
a: Cd vs control; b: Cd vs LEO1; c: Cd vs LEO2; d: Cd vs LEO3; e: Cd vs LE

4 DISCUSSION

In recent years, the persistence of cadmium contamination has become a major concern due to its adverse effects on the reproductive organs. These properties make them vulnerable to free radical attack, which can lead to alterations in various membrane enzymes, as well as affect the structural integrity and fluidity of the membrane [20].

In our study, sperm motility and concentration were significantly reduced in rats exposed to cadmium alone compared to the control, positive control, and combined groups. The results obtained are similar to those of other studies [21]. However, studies conducted on the effects of cadmium on human sperm motility, concentration, and viability have shown that this metal not only significantly reduces these parameters; changes in ATP (adenosine triphosphate) production, which provides the energy required for sperm movement, are also observed [22].

It has been suggested that cadmium-derived ROS can propagate through lipid membranes and cause disturbances in various reproductive mechanisms. Chronic exposure to cadmium has been shown to impair mammalian reproductive functions by causing spermatogenesis impairment, sperm motility impairment, sperm morphology alteration, and decreased acrosome reaction rate [11]. Germ cells containing high concentrations of polyunsaturated fatty acids exhibit low antioxidant capacity.

Cadmium has detrimental effects on the action of the hormones LH and testosterone, which play an important role in spermatogenesis, while not ignoring its effects on Leydig cells responsible for testosterone secretion. In addition, its effects on the composition and effect of the hormone testosterone acting on the hypothalamic-pituitary axis explain the reason for the decreased fertility in poisoned rats. Recent studies [23] have shown that cadmium chloride causes the degradation of stem cells (spermatogonia), alteration of Sertoli cells, and spermatogenesis disorders.

With regard to oxidative stress markers, the results showed a significant decrease in testicular glutathione levels in the metal-exposed group. Cadmium appears to bind to the SH group of GSH, affecting its antioxidant activity, and it also has the ability to alter the activity of antioxidant enzymes. Co-administration of leaf extract to rats resulted in an increase in testicular GSH concentration and a decrease in MDA concentration. Studies conducted by several authors [24; 25] have shown that chronic exposure to cadmium chloride induces metabolic changes in rats, including a significant decrease in glutathione (GSH) antioxidant parameters along with an increase in morphological and malonic acid peroxidation indices, including methanogen dialdehyde (MDA). Known functions of glutathione include its role in biosynthetic pathways, detoxification, and redox homeostasis. Glutathione can interact with proteins in a variety of ways through thiol-disulfide exchange and other processes to protect body tissues and organs.

5 CONCLUSION

This study revealed that the toxicity caused by cadmium chloride after 30 days of exposure resulted in various alterations in reproductive function (testosterone and LH), sperm quality (concentration and motility) by disruption of the hypothalamic-pituitary-gonadal axis in male Wistar rats. In addition, cadmium increased the testicular MDA level, with a decrease in the GSH concentration. However, co-administration of the aqueous extract of fresh olive leaves with cadmium preserved these parameters in the normal physiological state, which determines the benefits of the plant constituents.

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