Greener Journal of Biomedical and Health Sciences
Vol. 5(1), pp. 1-8, 2021
ISSN: 2672-4529

http://gjournals.org/GJBHS

Comparing day 0 and day 28, Hematological and Biochemical parameter changes, on Male white Rattus *norvegicus* exposed to *Urtica dioica* leaves & stem Ethanolic Extracts; a herb used in the management of Diabetes mellitus by the Tugen Community-Kenya

Kipbichii Chebor[1*], Osano Odipo[3], Isaboke Job[3], and Ng'wena Magak[2]

1. Department of Community Health and Extension (C.H.E.), School of Nursing, Midwifery and Paramedical Sciences (SONMAPS), Masinde Muliro University of Science and Technology (MMUST), P.O. Box 190-50100 Kakamega.

2. Department of Medical Physiology, School of Medicine (S.O.M.), Maseno University (M.U.), P.O. Box 333, Maseno.

3. Department of Environmental Biology and Health (E.B.H.), School of Environmental Studies (S.E.S.), University of Eldoret (UoE), P.O. Box 1125-30100.

ARTICLE INFO

Article No.: *092621093*
***Type*: Research**
Full Text: *HTML, EPUB*

Submitted: *26/09/2021*
Accepted: *01/10/2021*
Published: *06/11/2021*

***Corresponding Author**
Isaboke Job
E-mail: *senvebhm00119@ uoeld.ac.ke; fillmanjob@ gmail. com*
Phone: *+254718774783*

Keywords: *Urtica dioica leaves and stems extract;, ethanolic extracts; hematological biochemical parameters; Rattus norvegicus*

ABSTRACT

Introduction: With an increase in the use of herbal medications in the world today, so is the increasing concern about their safety/toxicity. Toxicity tests are essential for the development of new drugs and for extending the therapeutic potential of existing drugs. One such test is the examination of hematological and biochemical components of animals exposed to such herbs. One such herb is Urtica *dioica*, an herb commonly prescribed by herbalists in managing diabetes, hypertension nephritis, and hematuria, around the world, exposed to Rattus *norvegicus* for 28 days.

Objective: To compare day 0 and day 28 hematological and biochemical parameters changes on male white *Rattus norvegicus* **,** exposed to Urtica *dioica*, stems & leaves mixtures, ethanolic extracts.

Materials and methods: Experimental design was employed. About 2kg of fresh Urtica *dioica* (U.D.) mixture of leaves &stems, obtained from Baringo County, Eldama-Ravine forest, Kenya, were cleaned with distilled water at the site to remove debris and transported to the University of Eldoret, where its identity was taxonomically ascertained by the University taxonomists. Later, the herb was dried at room temperature and crushed into a powder form. Five hundred (500g) grams was used in [ethanolic] the extraction [duration was 72 hours], after which the percentage yield, phytochemicals, quantity (done using HPLC), and later the dosages were determined, after exposing six rats, to different concentrations of the herb. The rats used, were obtained from the University of Eldoret department of biological sciences. Rats' acclimatization was done for 7 days to make them adapt to the lab environment. Cage 1, which had six (6) rats were used for dosage optimization; cage 2, which had five rats, was used in the actual experiment (duration- 28 days). Cage 3 were the controls -given distilled water (controls). Blood samples for hematological; (*hemoglobin [Hb.), red blood cells [R.B.C.], and white blood cells [WBC]*) and biochemical (*Urea, creatinine, Alanine Transaminase [A.L.T.s], Aspartate Aminotransferase [A.S.T.s]*) tests, were drawn from the rats' tail end, at day 0 and day 28. Day 0 lab tests results were compared to day 28 lab test results. Analysis was done using *ADVIA 220i* for hematology and *Chemistry analyzer Cobas C311* for biochemicals. The outcomes were descriptively analyzed and presented in tabular form and figures. All the tests were 2-tailed, where applicable, and were considered significant at a p=0.05. Ethical approval to conduct this study was sought from, Animal Research and Ethics Committee (HAREC) of the University of Eastern Africa Baraton (UEAB)- Kenya (R.E.C.: UEAB/3/1/2018).

Results: With regards to hematological parameters, all the changes were within the normal ranges. WBC especially recorded a decrease in all the parameters on the 28 days. Although biochemical parameters recorded some changes (Urea recorded a significant increase from day 0 to day 28, p-value of 0.001), these changes were also within normal ranges.

Conclusions and Recommendations: Urtica *dioica* leaves and stems ethanolic extracts showed some elevation in urea levels, though they were within the normal ranges and a decrease in WBC, which may signify that U.D. possesses, some anti-microbial or anti-inflammatory activities. Nevertheless, U.D. is generally safe (*all changes were within the normal ranges*); however, more studies are needed.

BACKGROUND

With an increase in the use of herbal medications used today, so to the increasing concerns about their safety/toxicity. These concerns may be either general or herb-specific, hence the need to study them. A toxicity test is essential for developing new drugs and for the extension of the therapeutic potential of existing drugs. This toxicity/safety of medicinal plants may be related to the mixtures of active compounds that they contain; their interactions with other herbs and drugs, contaminants, adulterants; or their inherent toxicity (Rodriguez-Fragoso *et al.*, 2008).

More than 20,000 medicinal plants are used to manage numerous pathologies in Africa today, but less than 1% of them have been scientifically investigated for safety (Taika *et al.*, 2018). An herb might be effective but not safe. Therefore, it's imperative to study its safety/toxicity. Animals have been used since time immemorial as surrogates when examining the safety and efficacy of medicinal herbs. One such animal is the use of white *Rattus norvegicus*. One among many outcomes, tested/ studied parameters is hematological (the red blood cell [R.B.C.], white blood cells [WBC], hemoglobin [H.b.]content), and biochemical (Urea, creatinine, electrolytes, enzymes such as A.S.T.s and A.L.T.s) changes. Deviation from the regular usually signifies something (Arika *et al.*, 2016).

Therefore, this study wanted to compare day 0 and day 28 hematological and biochemical parameter on white *Rattus norvegicus*, exposed to ethanolic extracts of *Urtica dioica* (*stinging nettle*) leaves & stems, a herb that comes from the *Urticaceae* family(Patel *et al.*, 2018). Traditionally, used as a blood purifier, diuretic, a treatment for rheumatism, eczema,

anemia, nephritis, hematuria, jaundice, menorrhagia, diabetes mellitus, hypertension, and diarrhea. It is widely distributed throughout the temperate and tropical areas around Africa, Asia, and the world (Chebor K, 2020; Joshi *et al.*, 2014).

MATERIALS AND METHODS

Study area: The study was carried out in the University of Eldoret, Biotechnology Centre laboratory.

Study design: Experimental

Collection of the herbs, preparation, and extraction:

Collection, identification, and extraction

2 Kgs. of Urtica *dioica* plant materials were collected from Baringo County-Kenya and taxonomically verified based on their morphological characteristics (Beattie *et al.*, 2005) by the University of Eldoret's-Kenya, Department of Botany, taxonomists. They were cleaned at the place of origin (Eldama-ravine forest) with distilled water to remove external debris attached to them, then transported to the University of Eldoret (Kenya), Biotechnology Laboratory, where they were air-dried at room temperature to complete dryness before being crushed with a grinder- OHMS OCG-200, into a powder form in readiness for extraction.

Ethanol extraction
[98% concentration-analytical reagent purchased from Sigma-Aldrich-Kenya]

The herb was extracted using the maceration extraction process. Five hundred grams (500 g) of the herbs were soaked in two liters of ethanol for 72 hours (Azwanida, 2015)at room temperature, after which the resultant mixtures were filtered using Whatman filter paper (No.1) and the filtrate concentrated to dryness using a vacuum-rotary evaporator machine- BUCHI Rotavapor R-3000 at a temperature range of between 40^0C -50^0C. Fifty milliliters (50mls) of distilled water were then added to the container containing the concentrated dried substance, and then, using a stirring rod, the contents of the container were stirred to dissolve the dried substance much as possible.

Determination of extraction yield

After extraction, U.D., percentage (%) yield was determined based on the formula described by (Qaid, 2020). Lyophilization was done with the help of '*Harvest Right freeze drier (U.S.A)'* for 24 hours (ethanol extract).

The resultant yield powder was dissolved in 200mls of distilled water.

*5mls was used in phytochemical screening (*screening done as per the description of *(Muralidharan, 2015)*

1ml used in HPLC (determining the quantities)
The screening was done using Shimadzu HPLC SYSTEM Machine from shimadzu cooperation Kyoto-japan. The column was: Silica 250 x 4 nm, ten µm. Injection.

50mls was used in optimization
144mls used in the actual experiment

N/B for the sake of optimization, the 50mls formed was classified as 100%. Further dilutions were made using distilled water—75%, 50%, 25% and 12.5%, and 0% and given to 6 rats via oral gavage at 9 am daily for seven days. First rat (1) rat given 100%, 1 rat 75%...and the last (1) rat given 0% (pure-distilled water). The aim was to determine the optimal concentration to be given to rats during the actual experiment. Physical and physiological parameters, e.g., temperature, behavior, etc. checked at seven days as described by (Hawkins *et al.*, 2011). Findings analyzed and optimal dose with no or minimal effect, picked.

During the actual experiment, the rats were given the determined dose via oral gavage every morning for the duration of the experiment (28 days).

The feeding protocol was done in reference to the Piero et al. study of 2011 (Piero *et al.*, 2011).

Collection, preparation, and protocol of feeding the rats (cases and controls)

Collection

Sixteen (16) white males *Rattus norvegicus,* between the ages of 4 and 6 months, weighing in the ranges of 200-230g, were recruited. The animals were obtained from the University of Eldoret-Kenya department of biological sciences. The sample size was selected based on systematic published peer-reviewed studies (Ranasinghe *et al.*, 2012; Yeh *et al.*, 2003).

Preparation (Acclimatization of the rats to the lab environment)

The rats were cared for under the laboratory procedure, and feeding was done using pellets from UNGA limited-Eldoret Kenya, morning and evening, and given water *ad-libitum*.
N/B Before the experiment commenced, the rats had to be acclimatized in the lab environment for seven days.

Experimental protocol

Before the experiment, blood samples were taken for baseline hematological (*Hemoglobin, R.B.C., WBC*) and biochemical tests (*Urea, creatinine and A.L.T.s, and ASTS*). The same was also done at the end of the experiment. Blood samples were collected through the tail end and put on heparinized sample bottles**,** where applicable, in readiness for the tests (the collection was done on days 0 and 28). Analysis was done using *ADVIA 220i* for hematology and *Chemistry analyzer Cobas C311* for biochemicals.

Cage 1 was used for optimization, cage 2 (cases) rats were given ethanolic extracts of U.D. via

oral gavage. In cage 3, the controls were fed with only distilled water and pellets from Unga Kenya *ad*-libitum.

The feeding of the cases was done once daily at 0.5mls U.D./100mg of a rat, orally at 0900 hours while continuing with the other regular feeding (i.e., the pellets and water, every morning and evening for the 28 days).

N/B the handling of the animals was as described by National Academies(Council, 2010).

Ethical approval

Approval of the research was done by the Human and Animal Research and Ethics Committee (HAREC) of the University of Eastern Africa Baraton (UEAB)-Kenya (R.E.C.: UEAB/3/1/2018)

Data analysis

The resultant data were entered into excel sheet office 19 and analyzed using SPSS software version 21. Descriptive statistics were used to describe the data and summarized in tabular form. All the tests were 2-tailed, where applicable, and were considered significant at a p=0.05.

RESULTS

Introduction

After collecting the Urtica *dioica* plant samples from Baringo County-where they are commonly used (Chebor K, 2020), the plants were extracted, and the extraction yield was determined as shown in *table 1*. Different quantities were then subjected to qualitative phytochemical screening, HPLC Analysis Hematological and biochemical responses using male white Rattus *norvegicus* rats. The hematological and biochemical responses were monitored for 28 days. The status at 0 day was compared to the status at 28 day.

N/B, Day 0 status with reference to the normal ranges was either low, normal, or high, at day 28th, and was described as either; increase-within normal ranges, increase above the normal ranges, decrease within the normal ranges, or a decrease below the normal ranges. [Normal ranges was based on the works of *(Giknis & Clifford, 2008; Sharp & La Regina, 1998)*]

The results are as shown below;

The percentage yield of Urtica dioica after extraction.

Table 1: Yield outcomes of extraction yields

	Urtica *dioica* ***Ethanolic extract***
A. Dry quantity before extraction in mg of the herb	500,000mg
B. Extracted dry quantity after lyophilization in mg	1,376
% yield extracted $=^{B}/_{A}*100$	0.28%

The rate of extraction using the maceration method yielded 1,376 mg of the herb for 72 hours. This is approximately 0.28% extract obtained from the plants. After extraction, the extracts were subjected to qualitative phytochemical screening.

Phytochemical screening of ethanol extract

Table 2: Qualitative analysis of phytochemicals of Urtica *dioica ethanolic extract*

No	*Phytochemicals compounds*	*Qualitative presences*
1	*Alkaloids*	++
2	*Flavonoids*	++
3	*Phenols*	_
4	*Saponnins*	+++
5	*Tannins*	_
6	*Quinones*	+
7	*Oxalates*	_
8	*Terpenoids*	_
9	*Glycosides*	+
10	*Steroids*	_
11	*Coumarins*	+
12	*Sterols and Triterpenes*	++
13	*Xanthones*	_
14	*Catechins*	_
% presence of phytochemicals in reference to the total number of phytochemicals tested		*7/14* ***50%***

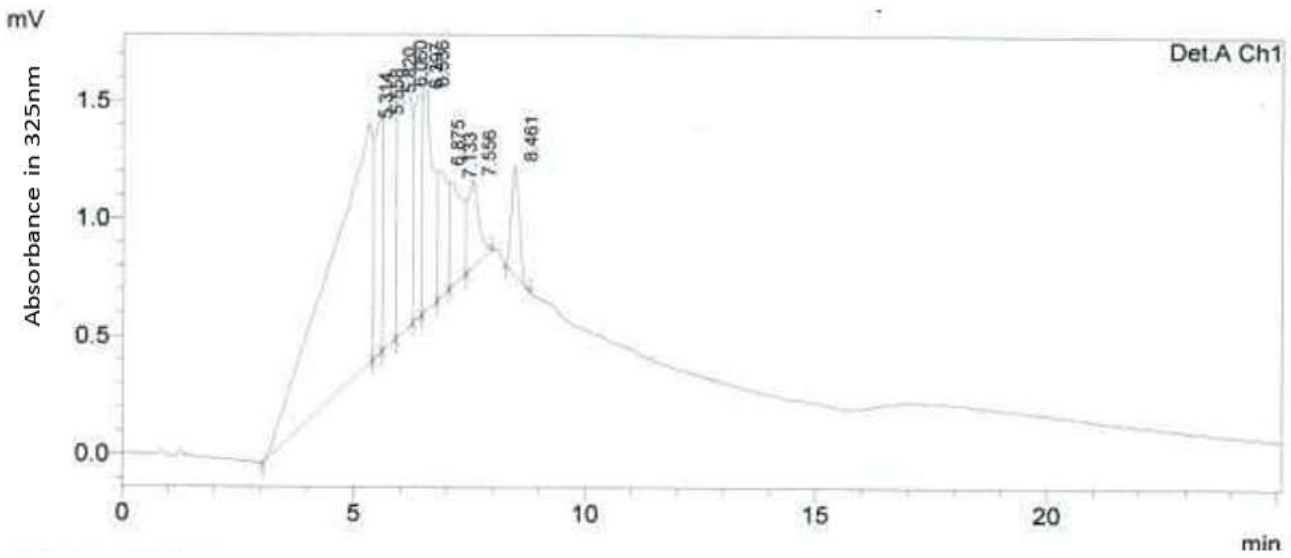

Fig 1: HPLC Chromatogram Analysis of Urtica dioica ethanol extract

Fourteen phytochemicals were tested qualitatively, and 50% of the tested compounds were present. Saponins were found to be of high quantity with the domination of "+++" while alkaloids, Flavonoids, and sterols were moderately present. Quinones, Glycosides, and coumarins were seen in trace amounts "+." After a qualitative phytochemical screen of the extract, the outcome was further tested in HPLC to confirm the presence of the phytochemicals.

The HPLC analysis chromatogram of the plant produced ten peaks with different retention times and different areas. This confirmed more than seven (7) different compounds that can be extracted by ethanolic solvents. The extract was then tested for a hematological and biochemical response on the white Rattus *norvegicus* male rats.

Weight averages of the rats

Table 3: day 0 and 28 average weights

EXTRACTS	DAY 0, Average weight in grams	DAY 28 Average weight in grams
Controls	209	213
Cases Ethanol	204	215

Dosages given to the rats

Based on the results of optimization, there was no notifiable effect on the six rats after exposing to various concentrations of Urtica *dioica;* hence the dosages were given using the 100% concentration. The dosages given to rats was, therefore, 0.5ml/100g of rat, which is equivalent to ***3.44mg/100g of a rat once daily for 28 days.***

<u>Hematological parameters changes</u>

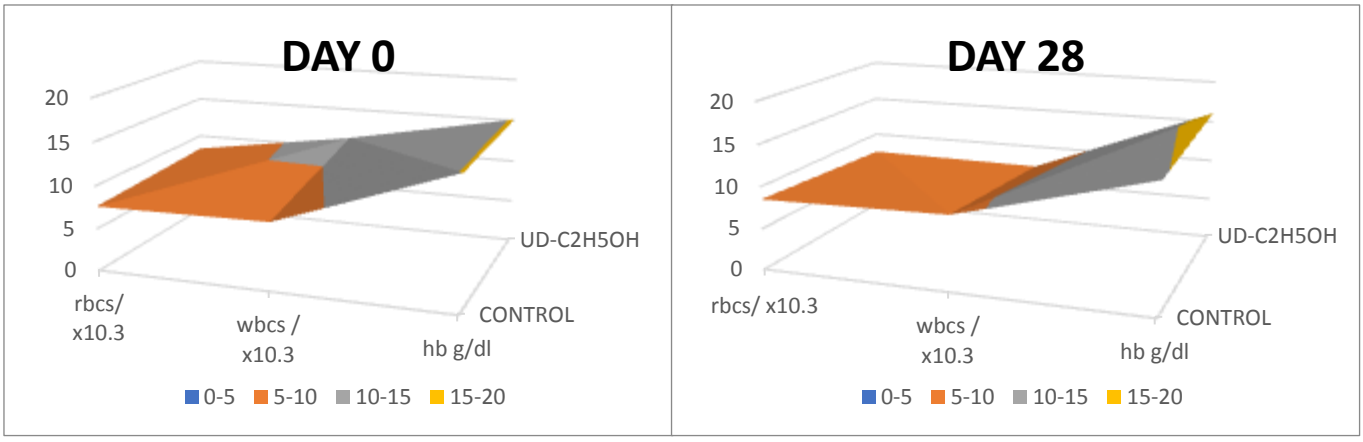

Fig 2: Surface plots for Hematological responses of male* white Rattus *norvegicusrats exposed to Urtica dioica ethanol extracts: the test of red blood cells, white blood cells, and hemoglobin.

There was a significant increase of both R.B.C. and WBC in the controls but a decrease in hemoglobin (P<0.05). The hemoglobin of the rats administered with the extract (cases) significantly increased by 12.1% (p<0.05) from day 0 to day 28, while that of control decreased by 0.7%. WBC in cases generally decreased.

Biochemical responses

Different biochemical parameters were also tested on the responses from exposures of Urtica dioica, namely Urea, creatinine, ALTS, and ASTL.

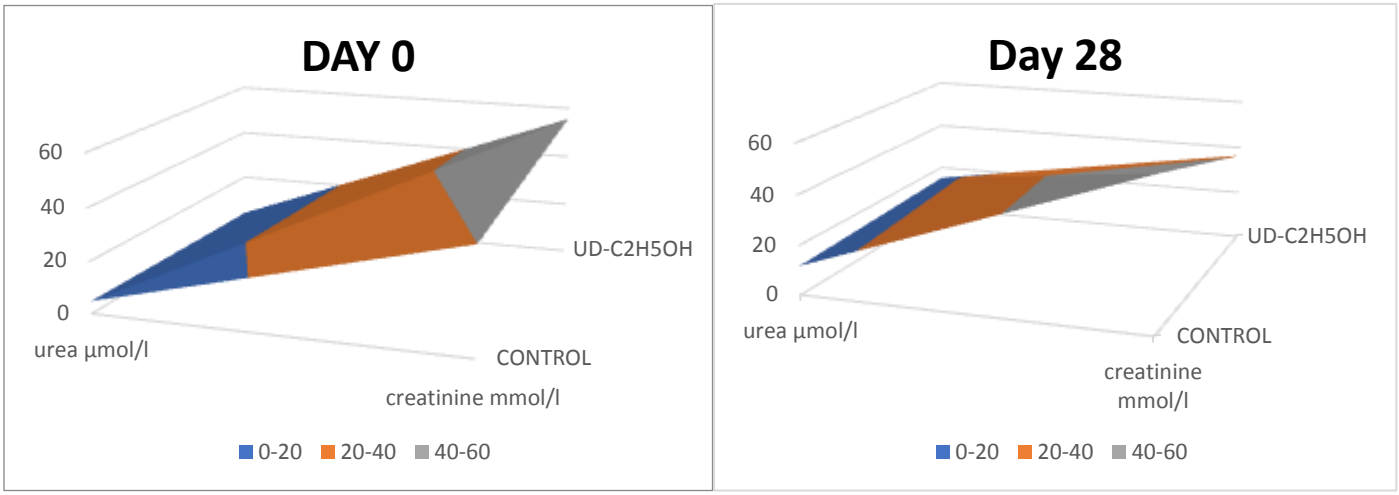

Fig 3: Surface plots for Biochemical responses of* white male Rattus *norvegicus exposed to Urtica dioica ethanol extracts: the test of Urea and Creatinine.

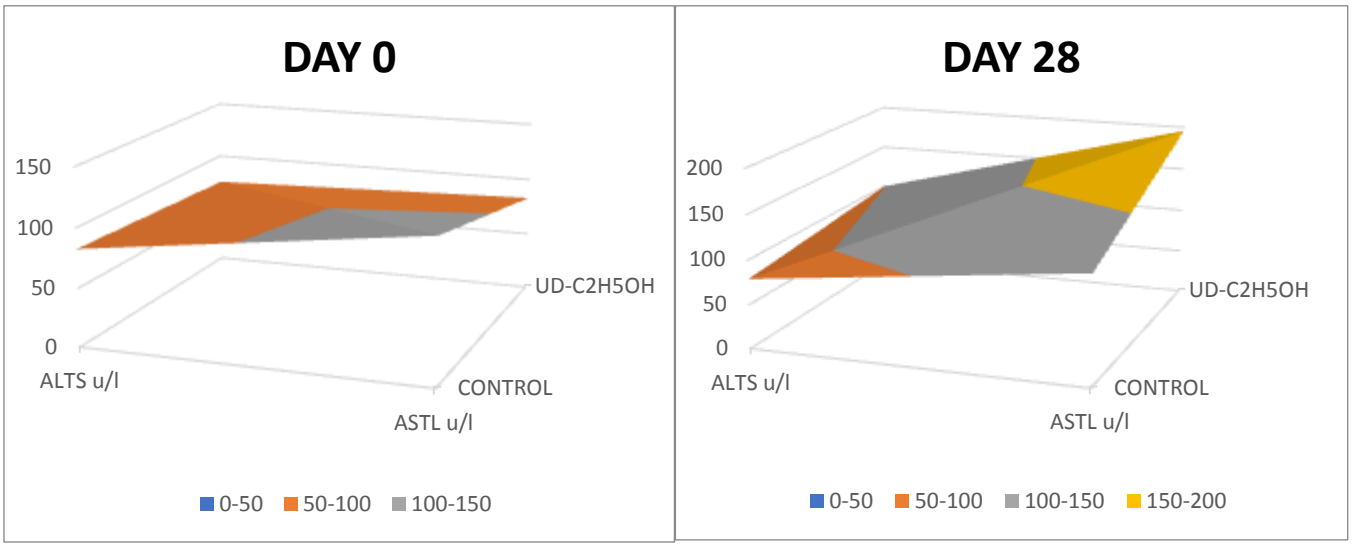

Fig 4: Surface plots for Biochemical responses of* white male Rattus *norvegicus rats exposed to Urtica dioica ethanol extracts: the test of ALTS and ASTL.

There was a significant increase in Urea from day 0 to day 28, p-value of 0.001 for both the control and the cases. With regards to creatinine, there was an increase in the control but a decrease in the cases.

Both ALTS and ASTL increased significantly on the rats exposed orally to the herb extract while there was no significant increase (A.L.T.s) and decrease (A.S.T.) in controls (p>0.05)

DISCUSSION

This study aimed to determine the phytochemical's constituents of Urtica dioica and their hematological and biochemical effects on exposure to white male Rattus *norvegicus* rats after 28 days.

Regarding phytochemicals, out of the fourteen tested, seven were present: alkaloids, flavonoids, saponins, quinones, glycosides, coumarins, sterols, and terpenoids. Based on the confirmation of phytochemicals by the HPLC analysis, there were more than seven (7) different peaks, with variations in retention times and area. This variation confirms different phytochemicals as confirmed in literature (Bourgeois *et al.*, 2016; Dar *et al.*, 2013; Grauso *et al.*, 2020).

Concerning hematological changes, Hb, in cases increased significantly (p<0.05), indicating that this herb might be hemo-protective and hepato-

protective, a finding that is in congruence with the findings (Juma *et al.*, 2015), while a decrease was seen in WBC, signifying that this herb might be possessing an anti-microbial or autoinflammatory tendency. A finding that concurs with the studies of (Dar *et al.*, 2012). R.B.C. parameter, also reduced as compared to the controls, nevertheless the levels were within the normal range

Regarding biochemical parameters, all urea levels recorded a higher level on day 28 than day 0, a finding suggesting that this herb might affect the Kidneys. The same increasing scenario was also seen with regards to creatinine levels. ALTS and A.S.T.s, though there were some changes, all the changes were within normal ranges—a finding concurrent with the findings of (Mukundi *et al.*, 2017).

CONCLUSIONS AND RECOMMENDATIONS

With regards to hematological parameters, all the changes were within the normal ranges. WBC especially recorded a more of a decrease in all the parameters on the 28 days. Concerning biochemical parameters, though all the others recorded a change within normal ranges, Urea recorded some changes, higher than the normal. Nevertheless, U.D. is generally safe. However, much more studies are needed.

REFERENCES

Arika, W., Nyamai, D., Musila, M., Ngugi, M., & Njagi, E. (2016). Hematological markers of in vivo toxicity. *Journal of Hematology & Thromboembolic Diseases*.

Azwanida, N. (2015). A review on the extraction methods use in medicinal plants, principle, strength and limitation. *Med Aromat Plants, 4*(196), 2167-0412.1000196.

Beattie, A. J., Barthlott, W., Elisabetsky, E., Farrel, R., Kheng, C. T., Prance, I., . . . ten Kate, K. (2005). Chapter 10 New Products and Industries from Biodiversity. In R. Hassan, R. Scholes & N. Ash (Eds.), *Ecosystems and Human Well-being: Current State and Trends, Volume 1* (Vol. 1, pp. 273-295). Washington, U.S.A.: Island Press, Washington, DC.

Bourgeois, C., Leclerc, É. A., Corbin, C., Doussot, J., Serrano, V., Vanier, J.-R., . . . Lainé, É. J. C. R. C. (2016). Nettle (Urtica dioica L.) as a source of antioxidant and anti-aging phytochemicals for cosmetic applications. *19*(9), 1090-1100.

Chebor K, O. O., Isaboke J and Ng'wena M. (2020). Identity, Preparation, Dosages and Conservation Knowledge of the Antidiabetic Herbs Used by The Tugen Living in Baringo County-Kenya. *African journal of health sciences- KEMRI, 33*(3), 1-17.

Council, N. R. (2010). *Guide for the care and use of laboratory animals*: National Academies Press.

Dar, S. A., Ganai, F. A., Yousuf, A. R., Balkhi, M.-u.-H., Bhat, T. M., & Sharma, P. J. P. B. (2013). Pharmacological and toxicological evaluation of Urtica dioica. *51*(2), 170-180.

Dar, S. A., Yousuf, A., Ganai, F. A., Sharma, P., Kumar, N., & Singh, R. (2012). Bioassay guided isolation and identification of anti-inflammatory and anti-microbial compounds from Urtica dioica L.(Urticaceae) leaves. *African Journal of Biotechnology, 11*(65), 12910-12920.

Giknis, M., & Clifford, C. B. (2008). Clinical laboratory parameters for Crl: W.I. (Han). *Charles River Laboratories*.

Grauso, L., de Falco, B., Lanzotti, V., & Motti, R. J. P. R. (2020). Stinging nettle, Urtica dioica L.: Botanical, phytochemical and pharmacological overview. *19*, 1341-1377.

Hawkins, P., Morton, D., Burman, O., Dennison, N., Honess, P., Jennings, M., . . . Wells, S. (2011). A guide to defining and implementing protocols for the welfare assessment of laboratory animals: eleventh report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement. *Laboratory animals, 45*(1), 1-13.

Joshi, B. C., Mukhija, M., & Kalia, A. N. (2014). Pharmacognostical review of Urtica dioica L. *International Journal of Green Pharmacy (IJGP), 8*(4).

Juma, K., Maina, S., Muriithi, J., Mwangi, B., Mworia, K., Mwonjoria, M., . . . Mburu, D. J. J. D. D. (2015). Protective Effects of Urtica dioica and cimetidine® on liver function following acetaminophen Induced Hepatotoxicity in Mice. *4*(130), 2.

Mukundi, M. J., Mwaniki, N. E., Piero, N. M., Murugi, N. J., Kelvin, J. K., Yusuf, A. A., . . . Gathumbi, K. (2017). Potential anti-diabetic effects and safety of aqueous extracts of Urtica dioica collected from Narok County, Kenya. *Pharm. Anal. Acta, 7*, 548.

Muralidharan, L. (2015). Hypoglycemic and biochemical remedies of Cathanthus roseus (Linn) on Alloxan–induced diabetic rat and its antioxidant status in rat lenses. *Int J Med Res Pharm Sci, 2*, 1-6.

Patel, S. S., Ray, R., Sharma, A., Mehta, V., Katyal, A., & Udayabanu, M. (2018). Antidepressant and anxiolytic like effects of Urtica dioica leaves in streptozotocin induced diabetic mice. *Metabolic brain disease, 33*(4), 1281-1292.

Piero, N. M., Joan, M. N., Kibiti, C. M., Ngeranwa, J., Njue, W. N., Maina, D. N., . . . Njagi, E. N. (2011). Hypoglycemic activity of some kenyan plants traditionally used to manage diabetes mellitus in Eastern Province.

Qaid, M. (2020). How to calculate extraction yield? Retrieved from: https://www.researchgate.net/post/How_to_calculate_extraction_yield/5f98e9695166084008151e0f

Ranasinghe, P., Jayawardana, R., Galappaththy, P., Constantine, G., de Vas Gunawardana, N., & Katulanda, P. (2012). Efficacy and safety of 'true'cinnamon (Cinnamomum zeylanicum) as a pharmaceutical agent in diabetes: a systematic review and meta-analysis. *Diabetic medicine, 29*(12), 1480-1492.

Rodriguez-Fragoso, L., Reyes-Esparza, J., Burchiel, S. W., Herrera-Ruiz, D., & Torres, E. (2008). Risks

and benefits of commonly used herbal medicines in Mexico. *Toxicology and applied pharmacology, 227*(1), 125-135.

Sharp, P., & La Regina, M. (1998). Experimental methodology. *Sharp P, La Regina M. The laboratory rat. Boca Raton: CRC Press LLC*, 140-144.

Taika, B. B., Bouckandou, M., Souza, A., Bourobou, H. B., MacKenzie, L., & Lione, L. (2018). An overview of anti-diabetic plants used in Gabon: Pharmacology and toxicology. *Journal of Ethnopharmacology, 216*, 203-228.

Yeh, G. Y., Eisenberg, D. M., Kaptchuk, T. J., & Phillips, R. S. (2003). Systematic review of herbs and dietary supplements for glycemic control in diabetes. *Diabetes care, 26*(4), 1277-1294.

Cite this Article: Kipbichii C; Osano O; Isaboke J; Ng'wena M (2021). Comparing day 0 and day 28, Hematological and Biochemical parameter changes, on Male white Rattus *norvegicus* exposed to *Urtica dioica* leaves & stem Ethanolic Extracts a herb used in the management of Diabetes mellitus by the Tugen Community-Kenya. Greener Journal of Biomedical and Health Sciences, 5(1), 1-8.