Journal of Applied Biological Sciences
E-ISSN: 2146-0108 17(3): 451-459, 2023
DOI: https://doi.org/10.71336/jabs.1241
Research Article

INVESTIGATION OF CHOLESTEROL AND BLOOD GLUCOSE-REDUCING PROPERTIES AND ACUTE TOXICITIES OF THE TEA PREPARED BY *VERNONIA CINEREA* L. USING WISTAR RAT ANIMAL MODEL AND QUANTIFICATION OF FLAVONOID AND PHENOLIC CONTENTS

Abeywardena Kavindu Kusal[1] , Thammitiyagodage Mayuri Geethanjalie[2*],
Warahakgoda Gedara Sarath Sisira Kumara[2], Alosius Thushari Nelka Munasinghe[1],
Liyanage Dona Ashanthi Menuka Arawwawala[3]

[1]*Harishchandra National School, Temple Road, Negombo, Sri Lanka*
[2]*Medical Research Institute, Dr. Danister de Silva Mawatha, Colombo 8, Sri Lanka*
[3] *Industrial Technology Institute, Bauddhaloka Mawatha, Colombo 7, Sri Lanka*

**Corresponding Author:*
E-mail: drmayuri.geetha@gmail.com

(Received 12th June 2023; accepted 09th October 2023)

ABSTRACT. According to Ayurvedic and folkloric medicine of Sri Lanka, tea prepared using dried leaf powder of *Vernonia cinerea* L. (S. Monarakudummbiya**)** has many therapeutic effects including blood glucose and cholesterol-reducing properties. The study investigate cholesterol and blood glucose-reducing properties and acute toxicities exerted by *V. cinerea L* tea and quantify the total phenolic and flavonoid content. Three months Wistar (20) rats were recruited for the study and divided randomly (n=10) into two groups. One group of rats was daily given tea prepared using the leaf powder of *V. cinerea* while the other group was given an equal volume of distilled water. Their baseline biochemical parameters, such as blood glucose, total cholesterol, high-density lipoprotein (HDL-cholesterol), triglycerides, creatinine levels, alanine transaminase (ALT), aspartate transaminase (AST) activities were measured. This experiment was carried out for 28 continuous days and repeated the same biochemical parameters on the 29th day. In addition, total phenolic and flavonoid contents were evaluated in the *V. cinerea* tea. There was a significant increment of HDL cholesterol levels whilst total cholesterol, triglycerides and blood glucose remained unaffected. There was no significant increase in ALT, AST and creatinine, indicating no acute toxicities in the prepared tea. Total phenolic and total flavonoid content in *V. cinerea* tea were GAE/g of extract at 28.62±1.06 and 19.20±2.7 respectively. *V. cinerea* tea was rich in therapeutically important phytochemicals such as phenols and flavonoids that can significantly increase HDL cholesterol levels in Wistar rats. *V. cinerea* can be further improved as a green tea.

Keywords: *V. cinerea, C. sinensis, phytochemicals, wistar rats, health benefits.*

INTRODUCTION

Vernonia cinerea (L.) Less plant belongs to the Asteraceae family, commonly seen in Asian countries. This plant is widespread in many other parts of the world including

China, Bangladesh and Sri Lanka. It is a terrestrial annual erect herb and its height is approximately 80 cm. Leaves are alternate spirals, and elliptic while flowers are bisexually grouped together in a terminal head. Stems are rounded solid and hairy [1,2, 3]. It contains a number of alkaloids and it is claimed to have many therapeutic effects [2]. In addition, steroids, glycosides, triterpenoids and esters were present in the methanolic extract of stem bark and leaves of *V. cinerea*. Structure elucidation studies also confirmed the presence of lupeol, 12-oleanen-3-ol-3ß-acetate, stigmasterol and ß-sitosterol in the plant [3]. Some therapeutic activities such as anti-hyperglycemic activity on alloxan-induced mice, anti-oxidant, antitumor, anti-ameliorative, sedative, anti-diarrhoeal, antibacterial, analgesic, antipyretic, anti-inflammatory and anti-helminthic activities were investigated using animal models and other biochemical methods [2, 9]. According to Ayurvedic and folkloric practitioners in Sri Lanka, *V. cinerea* leaf powder has cholesterol-lowering properties. However, the lipid-lowering properties of *V. cinerea* and its hypoglycemic effect on normoglycemic rats are yet to be scientifically validated. Though plant-based medicines have widely been used as therapeutic agents by believing herbal remedies are safe [10] scientific evidence is needed to justify the safety of herbal medicines. Therefore, an attempt was made to investigate (a) glucose and lipid-lowering ability along with possible acute toxic effects in terms of liver and kidney toxicities and (b) quantification of total phenolic and total flavonoid contents of *V. cinerea*.

MATERIALS AND METHODS

Ethical clearance for the present study was obtained by the ethics review committee of the Medical Research Institute, Colombo 8, Sri Lanka (ethical clearance No:51/2018). All the animal experiments were conducted according to the guidelines provided by the International Council for Laboratory Animal Science (ICLAS) and in the local settings in the non-infectious animal experimental area at the Medical Research Institute of Sri Lanka.

Study design

Plant material

V. cinerea plants were collected from the different areas of the Western province (longitude: 80.008775 and latitude: 6.901609) of Sri Lanka. Sri Lanka has no seasonal variation and this plant was distributed throughout the year in the country. Voucher specimens were collected in May 2019. The authentication of this plant was carried out by a Botanist at the Botany Department, University of Kelaniya, Sri Lanka. Subsequently, the leaf parts of *V. cinerea* were cleaned and air dried in low temperatures (28 ^{o}C - 30 ^{o}C) under moderate sunlight for three days. Finally, dried leaves were crushed and kept in a sealed container for further use.

Preparation of Vernonia cinerea tea

Crushed *V. cinerea* leaves (2.5 g) were added to a cup containing boiling water (240 mL) and kept for 5 min. Then filtered, the filtrate was subjected to quantification of total phenols and flavonoids. The same filtrate was used to feed the rats in the experimental group.

Animals

Wistar male rats (160g-180g) bred and maintained for research purposes at the Medical Research Institute, Sri Lanka were included in the present study, and female Wistar rats were excluded from the study. All the rats were taken to the noninfectious animal experimental area at the Animal Centre, MRI, and allowed to acclimatize for one week prior to the experimentation. They were maintained in an air-conditioned (22 °C-24 °C) and light-controlled room (12 hrs. light-dark cycle). Rats were group housed in standard polypropylene rat cages and 2-4 rats were assigned to each experimental unit. Autoclaved wood shavings were used as their bedding materials. They were fed with standard laboratory animal feed prepared at the Medical Research Institute using locally available feed ingredients and feed and water were given in *ad libitum* quantities.

Sample Size calculation:

Ten rats were assigned per group by following the below calculation [11].
Sample size per group = 2 SD2 (Zα/2 + Zβ)2/d2
Were,
SD - Standard deviation
Zα/2 = Z 0.05/2 = Z 0.025 = 1.96 (From Z table) at type 1 error of 5%
Zβ = Z 0.20 = 0.842 (From Z table) at 80% power
d = effect size = difference between mean value
Hence,
Sample size = 2 SD2 (1.96 + 0.842)2/d2
= 2(8)2 (1.96 + 0.842)2/10.52
= 9
Expected attrition or death of animals: 10%
Corrected sample size = Sample size/ (1− [% attrition/100])
Corrected sample size = 9/0.9 = 10

Feeding of Wistar rats with tea prepared from Vernonia cinerea

Rats were divided randomly into two groups by assigning ten rats to each experimental group. One group of rats (n=10) was fed daily with the tea prepared using the leaf powder of *V. cinerea* while the other group (n=10) was fed daily with an equal volume of distilled water. Dosage calculation was done according to OECD guidelines based on 2mL aqueous solution for 100 g body weight per rat and according to the body weight of the animal individual dosage was calculated. Their baseline blood glucose, creatinine, total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides levels and ALT, AST activities were measured. This experiment was carried out for 28 continuous days and repeated the same parameters on the 29th day.

Blood collection

Animals were mildly sedated using Isoflurane anaesthesia. Their tail was dipped in a lukewarm water bath (40 °C) as described by Wariyapperuma and co-workers [12] and gently placed on a rat holder. Then 1mL of blood was drawn from their lateral tail vein using a 23-gauge needle attached to a 1mL syringe. Blood samples were collected at baseline levels and after 28 days from rats treated with either *V. cinerea* tea or distilled water. Blood samples were collected by a qualified veterinarian according to the

guidelines given for humane care and the use of laboratory animals for research according to ICLAS International. Blood samples were collected by one investigator and serum separation and analysis were done by another investigator. The serum was separated by centrifugation at, 3000 rpm for 10 minutes. In the serum separation and analysis process, the second investigator was blinded to prevent bias.

Analysis of Biochemical parameters

Serum glucose, triglyceride, total cholesterol, high-density lipoprotein (HDL) cholesterol, creatinine levels and ALT, AST activities were determined using a semiautomated biochemistry analyzer (Start Fax 3330, India) using the reagent kits provided by Human Diagnostic (Human Diagnostic, Germany).

Total Phenolic and flavonoid content of Vernonia cinerea herbal tea

Total phenolic and flavonoid contents were determined using colorimetric methods as follows.

Total Phenolic Content (TPC)

The total polyphenol content of *V. cinerea* herbal tea was determined by the Folin-Ciocalteu spectrophotometric method [15] using gallic acid as the standard phenolic compound. Twenty microliters of three different concentrations of *V. cinerea* herbal tea were added to 110.00 µL of ten times diluted freshly prepared Folin-Ciocalteu reagent and incubated with 70.00 µL of 10 % sodium carbonate solution at room temperature (25±2 ℃) for 30 minutes. Absorbance was recorded at 765 nm. Five different concentrations of gallic acid (mg/mL) were used to construct the standard curve. Total polyphenol content was expressed as mg gallic acid equivalents (GAF)/g of extract. The test was triplicated. The same procedure was followed for low-grown Sri Lankan green tea as prepared similarly to *V. cinerea* herbal tea mentioned previously.

Total Flavonoid Content (TFC)

Total flavonoid content was determined according to the methods described by Meda and co-workers [16] with slight modifications. One hundred microliters of 2 % aluminum chloride in a methanol solution were incubated with three different concentrations of *V. cinerea* herbal tea (100 µL) at room temperature (25±2 ℃) for 10 minutes and absorbance was recorded at 415 nm. Quercetin was used to construct the standard curve and total flavonoid content was expressed as mg quercetin equivalents (QE)/g of extract. The test was triplicated. The same procedure was followed for low-grown Sri Lankan green tea as prepared similarly to *V. cinerea* herbal tea mentioned previously. Total phenolic and Flavonoid contents of plant materials were analyzed at the Industrial Technology Institute in Colombo.

Data analysis

Statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS, version 16). Biochemical parameters obtained from the test group and the control group of rats after 28 days of the experimental period were compared using the student t-test (mean values were compared).

RESULTS AND DISCUSSION

Rats used in the control and the test arms in this intervention were fed with a normal laboratory animal rat diet prepared based on the WHO formula prescribed by Saboudry [17] using locally available feed ingredients. The moisture, fat, protein, fiber, ash, carbohydrates, and caloric content in 100g of rat feed were 8.61±0.02%, 7.27g±1.15, 18.02g±0.01, 3.76g±0.42, 5.10g±0.04, 57.69g and 368.27 kcal respectively when determined using the method prescribed by Samaranayake and co-workers [18]. The main fat constituent of this diet was soya oil. There are studies demonstrating when dietary saturated fatty acids are replaced with soybean oil, blood cholesterol levels are lowered [19]. In the present study, *V. cinerea* tea significantly ($p \leq 0.05$) increased high-density lipoprotein in Wistar rats while total cholesterol and triglyceride levels remained unaffected (Table 1). Similar results were observed with hot water and cold ethanolic extracts of *Trichosanthes cucumerina* Linn in normolipidemic Wistar rats [20]. In addition, there was no significant ($p \geq 0.05$) change in fasting blood glucose levels in normoglycemic Wistar rats administered with *V. cinerea* tea for 28 consecutive days compared to the control group (Table 1). In the present study test and control group of rats were given the same rat formula prepared with soya oil as the main fat constituent. That may be the reason for unaffected total cholesterol and triglyceride levels observed in the test as well as in the control group of rats in the study. Thus, in the present study, it is scientifically proven that the capability of *V. cinerea* tea increases HDL-cholesterol levels while not altering the levels of total cholesterol, triglycerides, and fasting blood glucose levels (Table 1). The higher intake of polyphenols had a direct association with increased levels of HDL-cholesterol [21].

Table 1. *Effect of Vernonia cinerea tea on Blood Glucose Level and lipid profile in Healthy Wistar rats*

Biochemical tests	Group 1 (Control Group)	Group 2 (Test Group)
Fasting blood glucose		
Day 0	98.70±3.27	100.21±3.22
Day 28	109.55±3.78	110.18±3.00
Total Cholesterol		
Day 0	34.31±2.39	35.36±2.89
Day 28	51.23±2.55	50.95±1.53
HDL Cholesterol		
Day 0	23.30±0.80	20.02±1.39
Day 28	21.19±1.37	28.13±2.6*
Triglyceride		
Day 0	116.51±10.28	103.55±7.76
Day 28	168.05±13.55	168.05±13.55

*Values are expressed as mean ± S.E.M., (n = 10). All the units are given in mg/dL, *Significant when compared to the control; $p \leq 0.05$*

Atherosclerosis is a major cardiovascular disease caused by the accumulation of lipids in the endothelial wall, thickening the endothelial wall and reducing the lumen. The major risk factor in the development of atherosclerosis is an imbalance of blood lipids, known as dyslipidemia. Atherogenic dyslipidemia, defined as a triad of increased low-density lipoprotein (LDL) particles, decreased HDL-cholesterol, and increased triglycerides, was found to be an important independent risk factor for cardiovascular diseases [15, 16].

The phenolic and flavonoid content of *V. cinerea* tea were 28.62±1.06 GAE/g of extract and 19.20±2.7 QE/g of extract respectively. Moreover, the total phenolic content and total flavonoid content of low-grown Sri Lankan green tea were 30.20±2.10 GAE/g of extract and 21.34±1.30 QE/g of extract respectively [22]. Results revealed that the total phenolic and total flavonoid content of *V. cinerea* tea was similar to those of low-grown Sri Lankan green tea ($p<0.05$) [22]. Secondary metabolites such as flavonoids and phenols have been reported to play very important roles in lipid metabolism and protective functions against the incidence of lipid peroxidation and cardiovascular diseases. The favorable effects of flavonoids are mainly due to their antioxidant properties, which result from their ability to decrease the oxidation of low-density lipoproteins, thereby improving the lipid profiles. The other main function of flavonoids on the cardiovascular system is the ability to produce vasodilation and regulate the apoptotic processes in the endothelium. Further, flavonoids can prevent platelet aggregation based on their involvement in arachidonic acid metabolism [23].

Some phenolic compounds were reported to work together with nonsteroidal anti-inflammatory drugs (NSAIDs) to inhibit pro-inflammatory mediators or gene expression. Phenolic compounds reported the ability to inhibit the angiotensin-converting enzyme (ACE) and have been used to treat hypertension. The inhibition of carbohydrate hydrolyzing enzyme represents a type 2 diabetes mellitus therapy [24].

V. cinerea tea was found to be non-toxic in terms of liver and kidney functions after administration to Wistar rats for 28 consecutive days. As indicated by the effects of *V. cinerea* tea on serum levels of AST and ALT activities any adverse effects were not found even after 28 days of continuous treatment (Table 2). Creatinine is formed by the nonenzymatic breakdown of creatine, and changes in its serum concentration are the result of alterations in renal blood flow, renal function, or urine outflow [25]. If kidney function falls, the creatinine level will rise. In the present study, the creatinine levels of rats administered with *V. cinerea* tea were similar to that of the control group (Table 2). ALT and AST activities are considered reliable markers for liver disease and good indicators of overall health. However unaffected ALT and AST activities were observed in the current study after ingesting tea prepared by *V. cinerea* indicating that it has no adverse effects on liver function. Tea prepared from *C.sinensis* is the most widely used non-alcoholic beverage in the world [26]. Green tea is popular in Asian countries, mainly in Japan and China and tea prepared using *V. cinerea* showed equivalent concentrations of Phenols are Flavonoid compounds compared to the low-grown Sri Lankan green tea [22].

Table 2. *Effect of Vernonia cinerea tea on liver and kidney functions in healthy Wistar rats*

Biochemical tests	Group 1 (Control Group)	Group 2 (Test Group)
AST(U/L)		
Day 0	86.87±4.0	86.62±3.58
Day 28	68. 48±2.62	71.98±3.69
ALT(U/L)		
Day 0	36.01±1.90	39.90±3.84
Day 28	30.99±1.73	26.43±1.61
Creatinine (mg/dL)		
Day 0	0.52±0.02	0.53±0.37
Day 28	0.55±0.022	0.54±0.163

Values are expressed as mean ± S.E.M., (n = 10).
Not significant when compared to the respective control groups; $p \geq 0.05$
AST: Aspartate transaminase, ALT: Alanine transaminase

CONCLUSION

The present investigation revealed the capability of increasing HDL-cholesterol and nontoxicity in terms of renal and hepatic toxicity in *V. cinerea* tea in Wistar rats. *V. cinerea* tea can be further improved as a green tea after performing further clinical investigations.

Acknowledgment: The authors like to acknowledge Prof. R.A. Seetha Priyangani from the Department of Botany, University of Kelaniya for helping with the authentication of the plant and Dr (Mrs.) W.A. N. M. Wariyapperuma, Assistant Librarian, University of Jayewardenapura for contributing to the analytical part of this experiment.

Conflict of Interest. The authors declared that there is no conflict of interest.

Authorship Contributions. Concept: K.K. Abeywardena, A.T.M. Munasinghe, Design: K.K. Abeywardena, M.G. Thammitiyagodage Data Collection: M.G. Thammitiyagodage, W.G.S.S Kumara, K.K. Abeywardena, Analysis or Interpretation: K.K. Abeywardena, M.G. Thammitiyagodage, L.D.A.M. Arawwawala Literature Search and Writing: M.G. Thammitiyagodage, L.D.A.M. Arawwawala

Financial Disclosure. This research received no grant from any funding agency.

REFERENCES

[1] Digwal, M., Tarekar, D., Surji, A., Amravati, D. (2017): Pharmacognostic studies of *Vernonia cinerea* (L.) Leaves - an antismoking plant. Pharmaceut Res 6 (1), pp. 875-882.
[2] Lakshmi Prabha, J. (2015): Therapeutic uses of *Vernonia cinerea*. A short review. International Journal of Pharmaceutical and Clinical Research 7(4): 323-325.
[3] Haque, M.A., Hassan, M.M., Das, A., Begum, B., Ali, M.Y., Horshed, H. (2012): Phytochemical investigations of *Vernonia cinerea* (Family: Asteraceae). Journal of Applied Pharmaceutical Science 2(6): 78-83. https://doi.org/10.7324/JAPS.2012.2617.

[4] Iwalewa, E.O., Iwalewa, O.J., Adeboye, J.O. (2003): Analgesic, antipyretic, anti-inflammatory effects of methanol, chloroform and ether extracts of *Vernonia cinerea* Less leaf. Journal of Ethnopharmacology 86(2-3): 229-234. https://doi.org/10.1016/S0378-8741(03)00081-3.

[5] Gupta, M., Mazumder, U.K., Manikandan, L., Haldar, P.K., Bhattacharya, S., Kandar, C.C. (2003): Antibacterial activity of *Vernonia cinerea*. Fitoterapia 74(1-2):148-150. https://doi.org/10.1016/s0367-326x(02)00291-5.

[6] Kumar, P.P., Kuttan, G. (2009): *Vernonia cinerea* L. scavenges free radicals and regulates nitric oxide and proinflammatory cytokines profile in carrageenan-induced paw edema model. Immunopharmacology and Immunotoxicology 31(1):94-102. https://doi.org/10.1080/08923970802438391.

[7] Daffodil, E.D., Lincy, P., Mohan, V.R. (2014): Study of the whole plant of *Vernonia cinerea* Less for in vitro antioxidant activity. International Journal of Pharmacy 4(2): 172-178.

[8] Sonibare, M.A., Aremu, O.T., Okorie, P.N. (2016): Antioxidant and antimicrobial activities of solvent fractions of *Vernonia cinerea* (L.) Less leaf extract. African Health Sciences 16(2):629-39. https://doi.org/10.4314/ahs.v16i2.34.

[9] Pakpisutkul, J., Suwapraphan, J., Sripayak, N., Sitkhuntod, N., Loyrat, S., Yahayo, W., Supabphol, R. (2022): The effects of *Vernonia cinerea* Less extracts on antioxidant gene expression in colorectal cancer cells. Asian Pacific Journal of Cancer Prevention 23(11), 3923 – 3930. https://doi.org/10.31557/APJCP.2022.23. 11.3923.

[10] Marcus, D.M., Grollman, A.P. (2016): Toxicity of Botanical Medicines: An Overlooked Global Health Problem. American Journal of Public Health 106(1):16-7. https://doi.org/ 10.2105/AJPH.2015.302937.

[11] Charan, J., Biswas, T. (2013): How to calculate sample size for different study designs in medical research. Indian Journal of Psychological Medicine 35(2):121-126. https://doi.org/10.4103/0253-7176.116232.

[12] Wariyapperuma, W.A.N.M., Jayawardena, B.M., Kannangara, S.D.P., Wijayasinghe, Y.S., Subramaniam, S.S., Karunakaran, R., Kumara, W.G.S.S., Thammitiyagodage, M.G. (2019): Blood sample collection procedure and biochemical parameters of 6-8 weeks old male and female Wistar rats under Isoflurane anesthesia. Proceeding of Annual Scientific Sessions of the Sri Lanka Association for Laboratory Animal Science.

[13] Singleton, V. L., Orthofer, R., Lamuela-Raventós, R.M. (1999): Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods in Enzymology 299: 152-178. http://dx.doi.org/10.1016/S0076-6879(99)99017-1.

[14] Meda, A., Lamien, C. E., Romito, M., Millogo, J., Nacoulma, O.G. (2005): Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chemistry 91(3): 571-577. https://doi.org/10.1016/j.foodchem.2004.10.006.

[15] Musunuru, K. (2010): Atherogenic dyslipidemia: Cardiovascular risk and dietary intervention. Lipids 45(10): 907-914. https://doi.org/10.1007/s11745-010-3408-1.

[16] Moore, K.J., Tabas, I. (2011): Macrophages in the pathogenesis of atherosclerosis. Cell 145(3):341– 355. https://doi.org/10.1016/j.cell.2011.04.005.

[17] Saboudry, M.A. (1988): Breeding and Care of Laboratory Animals. Volume 1, pp18-19.WHO. Health laboratory Technology Unit. Geneva. Switzerland.

[18] Samaranayake, H.A.E., Chackrewarthy, S., Wickremasinghe, A.R., Thammitiyagodage, M.G., Karunakaran, R. (2015): Determination of the proximate composition of rat feed used at the Medical Research Institute (MRI), Sri Lanka. Proceedings of the 2nd Scientific Session of the Sri Lanka Association for Laboratory Animal Science.

[19] Messina, M., Shearer, G., Petersen, K. (2021): Soybean oil lowers circulating cholesterol levels and coronary heart disease risk and has no effect on markers of inflammation and oxidation. Nutrition (89), 2021, 111343.

[20] Arawwawala, L.D.A.M., Thabrew, I., Arambewela, L.S.R. (2011): Evaluation of toxic potential of standardized aqueous and ethanolic extracts of *Trichosanthes cucumerina* in rats. Latin American and Caribbean Bulletin of Medicinal and Aromatic Plants (BLACPMA) 1, 11-22.

[21] Castro-Barquero, S., Tresserra-Rimbau, A., Vitelli-Storelli, F., Doménech, M., Salas-Salvadó, J., Martín-Sánchez, V., Rubín-García, M., Buil-Cosiales, P., Corella, D., Fitó, M… et al. (2020): Dietary Polyphenol Intake is Associated with HDL-Cholesterol and A Better Profile of other Components of the Metabolic Syndrome: A PREDIMED-Plus Sub-Study. *Nutrients*.; 12(3):689.

[22] Abeywardena, K.K., Thammitiyagodage, M.G., Kumara, W.G.S.S., Munasinghe, A.T.M., Arawwawala, L.D.A.M (2023): Phenol and Flavonoid Contents of *Vernonia cinerea* (L.) and Low-Grown Sri Lankan Green Tea *Camelia sinensis*. Proceeding of the 10th Annual Scientific Sessions and International Conference of the Sri Lanka Association for the Laboratory Animal Science, Colombo, Sri Lanka.

[23] Ciumărnean, L., Milaciu, M.V., Runcan, O., Vesa, Ș.C., Răchișan, A.L., Negrean, V., Perné, M.G., Donca, V.I., Alexescu, T.G., Para, I., Dogaru, G. (2020): The Effects of Flavonoids in Cardiovascular Diseases. Molecules 21;25(18):4320.

[24] Rahman, M. M., Rahaman, M.S., Islam, M.R., Rahman, F., Mithi, F.M., Alqahtani, T., Almikhlafi, M.A., Alghamdi, S.Q., Alruwaili, A.S., Hossain, M.S., Ahmed, M., Das, R., Emran, T.B, Uddin, M.S (2021): Role of Phenolic Compounds in Human Disease: Current Knowledge and Future Prospects. Molecules 30;27(1):233.

[25] Ferguson, M.A., Waikar, S.S (2012): Established and emerging markers of kidney function. Clin Chem. 58(4):680-689.

[26] Wang, C., Han, J., Pu, Y., Wang, X. (2022): Tea (*Camellia sinensis*): A Review of Nutritional Composition, Potential Applications, and Omics Research. Applied Sciences 12(12):5874. https://doi.org/10.3390/app12125874.