Asian Journal of Biochemistry

ISSN 1815-9923

Academic
Journals Inc.

www.academicjournals.com

Asian Journal of Biochemistry 10 (6): 306-311, 2015
ISSN 1815-9923 / DOI: 10.3923/ajb.2015.306.311

Sub-Chronic Esculetin (6,7-Dihydroxy-Coumarin)-Induced Alteration in Some Haematological and Serum Parameters in Normal Male Wistar Rats

[1]Anthony Cemaluk C. Egbuonu, [2]Ambrose E. Ogbu, [1]Ifeoma I. Ijeh and [2]Lawrence U.S. Ezeanyika

[1]Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Nigeria

[2]Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka, Nigeria

Corresponding Author: Anthony Cemaluk C. Egbuonu, Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Nigeria

ABSTRACT

The sub chronic esculetin (6,7-dihydroxy-coumarin)-induced response on malondialdehyde concentration and some haematological and lipid metabolism parameters was studied in normal male Wistar rats to further understand the underlying esculetin metabolism and effects. Five groups (n = 6) of rats, A-E were respectively exposed to 6.0, 12.0 and 24.0 mg kg^{-1} b.wt. of esculetin, 0.2 mL of the vehicle control (10% dimethylsulfoxide (DMSO)) and 0.2 mL of the normal control (Distilled Water (DW)) per orally and daily for 28 days. Compared with the controls, the haematological parameters change in the esculetin-exposed rats was not significant ($p>0.05$) but decreased numerically with increasing dose viz: hemoglobin (99.83±5.04 g L^{-1}-91±7.04 g L^{-1}), packed cell volume (30±1.41%-27.33±2.16%), white blood cell count (9.63±0.91×10^9 L^{-1}-7.95±0.58×10^9 L^{-1}), neutrophil (1.93±0.51×10^9 L^{-1}-1.72±0.34×10^9 L^{-1}) and lymphocyte count (7.64±1.04×10^9 L^{-1}-6.18±0.63×10^9 L^{-1}). The reduction in the serum cholesterol (3.39±0.23 mmol L^{-1}-3.01±0.78 mmol L^{-1}) and triacylglyceride (0.49±0.45 mmol L^{-1}-0.15±0.05 mmol L^{-1}) concentrations in the esculetin-dosed rats was significant ($p<0.05$) and followed the numerical decreasing trend. The serum MDA concentration (1.90±0.53 mmol L^{-1}-2.82±1.50 mmol L^{-1}) decreased ($p<0.05$) but increased numerically with increasing dose. Thus, the sub-chronic esculetin-induced alteration was inversely related to esculetin dose and may not have resulted to significant pathologies or dysfunctional states in the rats.

Key words: Lipid peroxidation products, packed cell volume, neutrophil, hemoglobin, lymphocytes

INTRODUCTION

Esculetin (6,7-dihydroxycoumarin) is a coumarin (group of non-nutrient phenolic phytochemicals) derivative that is present in several plants, including *Citrus limonia*, *Aesculus hippocastanum*, *Euphobia lathyris*, *Fraxinus rhynchophylla* and *Artemisia capillaris* (Masamoto *et al*., 2003; Tien *et al*., 2011). Esculetin, like other coumarins, possesses diverse biological and pharmaceutical properties (Lacy and O'Kennedy, 2004) and is useful in drug synthesis (Kostova, 2005). It is common in foodstuffs, including vegetables, fruits, nuts, coffee, tea

and wine and it is widely used as flavour-enhancing agents for variety of foods (Leung *et al*., 2005). The average western diet contains approximately 1.0 g day^{-1} of mixed coumarins, including esculetin (Lacy and O'Kennedy, 2004) and in the sub Saharan African, including Nigeria, plants generally serve as source for foods and herbs. Thus, the dietary and therapeutic exposure of animals to esculetin may be quite significant. Recent reports of beneficial effects of esculetin in experimentally induced diseased state in animals (Kadakol *et al*., 2015; Kim and Lee, 2015; Prabakaran and Ashokkumar, 2012, 2013) may increase its dietary and pharmacologic use with possible adverse physiologic influence, warranting this study aimed at ascertaining the sub chronic esculetin (6,7-dihydroxy-coumarin)-induced response on malondialdehyde concentration and some haematological and lipid metabolism parameters in normal male Wistar rats. Agent-induced influence in animals has been reported (Egbuonu *et al*., 2010; Egbuonu and Ezeanyika, 2013; Egbuonu and Osakwe, 2011) even at a low dose (Egbuonu *et al*., 2009).

MATERIALS AND METHODS

Thirty male Wistar rats (10-12 weeks old and an average body weight of 114.8 g) were used in this study. The rats were purchased from the animal house of the Faculty of Biological Sciences, University of Nigeria, Nsukka, Nigeria.

Chemicals: Chemicals used in this research were products of reputable companies, including May and Baker, England; Merck, Germany; BDH Chemicals, UK and Sigma Chemical Compound, USA. Reagents used were commercial kits and are products of Randox Laboratories Ltd., UK. The effective study was conducted between June, 2007 and June, 2008.

Experimental design: Approval for this animal study was sought and obtained from the Ethical Committee, Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka, Nigeria. The rats were housed in separate cages, acclimatized for 7 days and then randomly assigned to five groups (A, B, C, D and E) of 6 rats each. The rats in group A, B and C were exposed to esculetin solution at a dose of 6, 12 and 24 mg kg^{-1} b.wt., respectively.

The rats in group D, the vehicle control were exposed to 0.2 mL of 10% dimethylsulfoxide (DMSO) whereas those in group E, the normal control were exposed to 0.2 mL of Distilled Water (DW). The administration was per oral for 28 consecutive days. All the animals were handled humanely and fed *ad libitum* with pelletised growers feed manufactured by Grand Cereals and Oil Mills Limited, Jos, Nigeria. The rats were scarificed on day 29 to obtain the samples (whole blood which shared to two and one part processed to obtain the serum).

Methods: Hemoglobin was determined by the method described by Cheesbrough (2000) based on the principle that haemoglobin converts to stable haemiglobincyanide (HiCN) following haemolysis of whole blood cells on dilution with modified Drabkin's solution (potassium ferricyanide and potassium cyanide). The absorbance of the coloured HiCN solution was read with a spectrophotometer at 540 nm and compared with that of a reference HiCN standard solution. The intensity of the colour produced is directly proportional to the amount of haemoglobin present. The differential white blood cell count was determined by counting the different white cell types in a Leishman stain using a microscope as described in Cheesbrough (2000).

The Packed Cell Volume (PVC) was measured by the method of Cheesbrough (2000) as the proportion of whole blood occupied by red cells, expressed as a ratio while the WBC count was determined by diluting whole blood with a fluid that cause haemolysis of the erythrocytes but had no effect on leukocytes, which can then be counted microscopically using a haemocytometer as the number of white blood cells per liter of blood calculated (Cheesbrough, 2000).

The serum MDA concentration was determined by the method of Wallin *et al.* (1993) based on the principle as in Egbuonu and Ezeanyika (2012). The serum concentration of total cholesterol and triacylglyceride were determined with Randox enzyme kit based on the method of Trinder (1969) and on the principle of colorimetric estimation and quantification of quinoneimine formed after the enzymatic hydrolysis and oxidation of either cholesterol or triacylglyceride.

Statistical analysis: The data were analyzed by the Least Significant Difference (LSD) and significant difference in means accepted at $p<0.05$, using one way analysis of variance (ANOVA). The results were expressed as mean value±standard deviation of the measured variables in each group.

RESULTS

On comparison with the controls (D and E), the esculetin-induced alteration on the haematological parameters was not significant ($p>0.05$) but decreased numerically from the lowest to highest esculetin-exposed group viz: hemoglobin (99.83±5.04 g L^{-1}-91±7.04 g L^{-1}), packed cell volume (30±1.41%-27.33±2.16%), white blood cell count (9.63±0.91×10^9 L^{-1}-7.95±0.58×10^9 L^{-1}), neutrophil (1.93±0.51×10^9 L^{-1}-1.72±0.34×10^9 L^{-1}) and lymphocyte count (7.64±1.04×10^9 L^{-1}-6.18±0.63×10^9 L^{-1}) (Table 1).

The reduction in the serum concentration of cholesterol and triacylglyceride in the esculetin-dosed rats was significant ($p<0.05$) and followed the numerical decreasing trend in the concentration of cholesterol (3.39±0.23 mmol L^{-1}-3.01±0.78 mmol L^{-1}) and triacylglyceride (0.49±0.45 mmol L^{-1}-0.15±0.05 mmol L^{-1}). Lipid peroxidation products (MDA) concentration decreased significantly ($p<0.05$) but the numerical decreasing trend was reversed (1.90±0.53 mmol L^{-1}-2.82±1.50 mmol L^{-1}) (Table 2).

Table 1: Haematological indices in controls and esculetin-exposed rats

Treatment groups	Hemoglobin (g L^{-1})	WBC count (×10^9 L^{-1})	Packed cell volume (%)	Neutrophil (×10^9 L^{-1})	Lymphocyte (×10^9 L^{-1})
A (6.0 mg kg^{-1})	99.83±5.04	9.63±0.91	30.00±1.41	1.93±0.51	7.64±1.04
B (12.0 mg kg^{-1})	98.00±11.52	9.30±2.84	29.50±3.39	1.92±0.47	6.83±2.27
C (24.0 mg kg^{-1})	91.00±7.04	7.95±0.58	27.33±2.16	1.72±0.34	6.18±0.63
D (Vehicle control)	99.00±3.41	7.93±1.01	29.67±1.03	1.83±0.60	6.08±0.84
E (Normal control)	95.67±20.32	7.50±1.87	28.50±6.69	1.40±0.29	6.07±1.66

*Statistically significant difference ($p<0.05$) between the control and esculetin-exposed groups. Results were expressed as Mean±SD for n = 6 rats, WBC: White blood cells

Table 2: Serum malondialdehyde, triacylglyceride and cholesterol concentration in controls and esculetin-exposed rats

Treatment groups	Triacylglyceride (mmol L^{-1})	Cholesterol (mmol L^{-1})	Malondialdehyde (mmol mL^{-1})
A (6.0 mg kg^{-1})	0.49±0.45*	3.39±0.23*	1.90±0.53*
B (12.0 mg kg^{-1})	0.31±0.26*	3.21±0.30*	1.95±0.48*
C (24.0 mg kg^{-1})	0.15±0.05*	3.01±0.78*	2.82±1.50*
D (Vehicle control)	0.81±0.75	5.22±0.15	3.52±0.71
E (Normal control)	0.89±0.55	5.27±0.09	3.52±1.44

*Statistically significant difference ($p<0.05$) between the control and esculetin-exposed groups. Results were expressed as Mean±SD for n = 6 rats

DISCUSSION

Esculetin (6,7-dihydroxycoumarin) is coumarin derivative found in various plants, plant products and average western diet, hence may be inadvertently consumed on daily basis, warranting this study to further understand the underlying esculetin metabolism and effects. On comparison with the controls (D and E), the esculetin-induced alteration on the haematological parameters was not significant ($p>0.05$), implying negligible esculetin-induced influence on blood cells production, circulation and functions in the rats. The observed numerical decrease in the haematological indices from the lowest to the highest esculetin-exposed group could be a pointer to apparent esculetin-induced inverse response with varying dose in the rats.

The change in the WBCs in the rats exposed to esculetin was not significant ($p>0.05$), suggesting that esculetin may not have induced significant toxic effect that could trigger an immune response in the rats. The WBCs are important in immune response (Guilhermino *et al*., 1998) and are generally released in response to toxic effect in animals. The packed cell volume range in esculetin-treated rats (30.00±1.41%-27.33±2.16%) was not significant ($p>0.05$) but within the normal range (25%-45%) as reported by Oleforuh-Okoleh *et al.* (2015), though in broiler chicks. The reduction in the serum concentration of cholesterol and triacylglyceride in the esculetin-dosed rats was significant ($p<0.05$) and dose dependent, indicating significant up-regulation in the utilization or down-regulation in the synthetic pathways of cholesterol and triglyceride catabolism which could occur regardless of esculetin dose.

The hydroxycoumarins, including esculetin are typical phenolic compounds with metal chelating free radical scavenging potentials (Lin *et al*., 2000; Nishiyama *et al*., 2001; Kaneko *et al*., 2003). As expected, the lipid peroxidation products (MDA) concentration decreased significantly ($p<0.05$) and dose dependently in the esculetin-exposed groups compared to the controls. Similar observations were reported earlier by Lin *et al.* (2000), Martin-Aragon *et al.* (1998) and Gilani *et al.* (1998), suggesting prevailing healthy antioxidant status, perhaps due to the absence of significant esculetin-induced pathologies or dysfunctional states and/or potential antioxidant activities of esculetin in the rats. Antioxidant properties of esculetin have been related with its radical scavenging activity (Kostova, 2005), inhibition of tyrosine kinases (Masamoto *et al*., 2003) and enhancement of glutathione status (Martin-Aragon *et al*., 1998). The observed decrease in the serum MDA concentration in the esculetin-exposed rats appears to support the observation on the other parameters in this study and in earlier studies which reported by Egbuonu *et al.* (2012) and Egbuonu *et al.* (2015) suggesting the absence of significant esculetin-induced adverse influence in the rats.

The numerical decreasing trend observed in the other studied parameters was however reversed in the serum MDA concentration (1.90±0.53 mmol L^{-1}-2.82±1.50 mmol L^{-1}), which could imply novel underlying mechanism in esculetin metabolism and actions. The esculetin-induced decreasing response with increasing dose for some parameters but an increasing response with increasing dose for MDA as observed in this study may be apparently pointing to esculetin potential to selectively elicit potentiated effect, either at low or high concentration, that could diminish and possibly vanish with increasing or decreasing concentration, respectively. This is a novel observation that could be indicating interplay of multiple catabolic routes in esculetin metabolism, hence underlying complex mechanisms in the biochemical and therapeutic actions of esculetin, warranting further studies.

CONCLUSION

The sub-chronic esculetin-induced alterations were inversely related to esculetin dose and may not have resulted to significant dysfunctional states in the rats. The noted inverse dose related

response in either decreasing or increasing direction could be pointing to significant underlying complex mechanisms in esculetin metabolism and actions, warranting further investigations.

REFERENCES

Cheesbrough, M., 2000. District Laboratory Practice in Tropical Countries, Part 2. Cambridge University Press, Cambridge, UK., Pages: 299-320.

Egbuonu, A.C.C., O. Obidoa, C.A. Ezeokonkwo, L.U. Ezeanyika and P.M. Ejikeme, 2009. Low dose oral administration of monosodium glutamate in male albino rats may be nephroprotective. Bio-Research, 7: 470-473.

Egbuonu, A.C.C., P.M. Ejikeme and L.N. Obasi, 2010. Monosodium glutamate: Potentials at inducing prostate pathologies in male Wistar rats. Afr. J. Biotechnol., 9: 5950-5954.

Egbuonu, A.C.C. and O.N. Osakwe, 2011. Effects of high monosodium glutamate on some serum markers of lipid status in male Wistar rats. J. Med. Med. Sci., 2: 653-656.

Egbuonu, A.C.C. and L.U.S. Ezeanyika, 2012. Effect of L-arginine on selected markers of metabolic syndrome related to oxidative stress, glucose metabolism and nitric oxide synthesis in female Wistar Albino rats. Int. Res. J. Biochem. Bioinf., 2: 186-192.

Egbuonu, A.C.C., A.E. Ogbu and L.U.S. Ezeanyika, 2012. Dose-related influence of esculetin (6,7-dihydroxy-coumarin) on some liver and prostate function markers of male Wistar rats. J. Biol. Sci., 12: 253-257.

Egbuonu, A.C.C. and L.U.S. Ezeanyika, 2013. L-arginine exposure improves renal function markers of metabolic syndrome in female rats. Am. J. Biochem. Mol. Biol., 3: 50-60.

Egbuonu, A.C.C., A.E. Ogbu and L.U.S. Ezeanyika, 2015. Sub-chronic oral esculetin (6, 7-dihydroxy-coumarin) exposure in male Wistar rats: Effect on some serum functions and organ histology. Asian J. Biochem., 10: 67-77.

Gilani, A.H., K.H. Janbaz and B.H. Shah, 1998. Esculetin prevents liver damage induced by paracetamol and CCl_4. Pharmacol. Res., 37: 31-35.

Guilhermino, L., A.M.V.M. Soares, A.P. Carvalho and M.C. Lopes, 1998. Acute effects of 3, 4-dichloroaniline on blood of male wistar rats. Chemosphere, 37: 619-632.

Kadakol, A., V. Malek, S.K. Goru, A. Pandey, S. Bagal and A.B. Gaikwad, 2015. Esculetin attenuates alterations in Ang II and acetylcholine mediated vascular reactivity associated with hyperinsulinemia and hyperglycemia. Biochem. Biophys. Res. Commun., 461: 342-347.

Kaneko, T., N. Baba and M. Matsuo, 2003. Protection of coumarins against linoleic acid hydroperoxide-induced cytotoxicity. Chem. Biol. Interact., 142: 239-254.

Kim, Y. and J. Lee, 2015. Esculetin, a coumarin derivative, suppresses adipogenesis through modulation of the AMPK pathway in 3T3-L1 adipocytes. J. Funct. Foods, 12: 509-515.

Kostova, I., 2005. Synthetic and natural coumarins as cytotoxic agents. Curr. Med. Chem. Anti-Cancer Agents, 5: 29-46.

Lacy, A. and R. O'Kennedy, 2004. Studies on coumarins and coumarin-related compounds to determine their therapeutic role in the treatment of cancer. Curr. Pharamaceut. Des., 10: 3797-3811.

Leung, K.N., P.Y. Leung, L.P. Kong and P.K. Leung, 2005. Immunomodulatory effects of esculetin (6,7-dihydroxycoumarin) on murine lymphocytes and peritoneal macrophages. Cell. Mol. Immunol., 2: 181-188.

Lin, W.L., C.J. Wang, Y.Y. Tsai, C.L. Liu, J.M. Hwang and T.H. Tseng, 2000. Inhibitory effect of esculetin on oxidative damage induced by t-butyl hydroperoxide in rat liver. Arch. Toxicol., 74: 467-472.

Martin-Aragon, S., J.M. Benedi and A.M. Villar, 1998. Effects of the antioxidant (6,7-dihydroxycoumarin) esculetin on the glutathione system and lipid peroxidation in mice. Gerontology, 44: 21-25.

Masamoto, Y., H. Ando, Y. Murata, Y. Shimoishi, M. Tada and K. Takahata, 2003. Mushroom tyrosinase inhibitory activity of esculetin isolated from seeds of *Euphorbia lathyris* L. Biosci. Biotechnol. Biochem., 67: 631-634.

Nishiyama, T., J. Ohnishi and Y. Hashiguchi, 2001. Fused heterocyclic antioxidants: Antioxidative activities of hydrocoumarins in a homogeneous solution. Biosci. Biotechnol. Biochem., 65: 1127-1133.

Oleforuh-Okoleh, V.U., S.O. Olorunleke and I.J. Nte, 2015. Comparative response of bitter leaf (*Vernonia amygdalina*) infusion administration on performance, haematology and serum biochemistry of broiler chicks. Asian J. Anim. Sci., 9: 217-224.

Prabakaran, D. and N. Ashokkumar, 2012. Antihyperglycemic effect of esculetin modulated carbohydrate metabolic enzymes activities in streptozotocin induced diabetic rats. J. Funct. Foods, 4: 776-783.

Prabakaran, D. and N. Ashokkumar, 2013. Protective effect of esculetin on hyperglycemia-mediated oxidative damage in the hepatic and renal tissues of experimental diabetic rats. Biochimie, 95: 366-373.

Tien, Y.C., J.C. Liao, C.S. Chiu, T.H. Huang, C.Y. Huang, W.T. Chang and W.H. Peng, 2011. Esculetin ameliorates carbon tetrachloride-mediated hepatic apoptosis in rats. Int. J. Mol. Sci., 12: 4053-4067.

Trinder, P., 1969. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann. Clin. Biochem., 6: 24-27.

Wallin, B., B. Rosengren, H.G. Shertzer and G. Camejo, 1993. Lipoprotein oxidation and measurement of thiobarbituric acid reacting substances formation in a single microtiter plate: Its use for evaluation of antioxidants. Anal. Biochem., 208: 10-15.