

*Full length Research Paper*

# Time and dose dependent effects of esculetin on some routine parameters of biochemical function in male wistar rats

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Accepted 16th May, 2012

The study investigated the time and dose dependent effects of esculetin (6, 7-dihydroxy-coumarin) on some routine parameters of biochemical function in male Wistar rats. Five groups of rats (n = 6) were *per orally* administered with 6.0, 12.0 and 24.0 mg/kg body weight of esculetin; 0.2 ml of the vehicle control (10 % dimethylsulfoxide (DMSO)), and 0.2 ml of the normal control (distilled water (DW)), respectively every 24 hours for 28 days. On comparison with the controls, exposure to esculetin caused a dose dependent decrease in food intake (day 7), in body weight (days 7, 14, and 28), and in water intake (days 7, 14 and 21). However, the exposure caused a time dependent increase in water intake (when compared with the normal control), and in body weight (as against that of the rats at baseline or day 1) whereas rats exposed to 12 mg/kg of esculetin had a time dependent decrease ( $p < 0.05$ ) in food intake on days 7 and 14. Overall, esculetin caused a dose dependent, but time independent, decrease in body weight, food and water intakes of male rats. Thus, caution should be exercised in the use of esculetin over time in male rats.

**Keywords:** Esculetin, coumarins, water intake, food intake, body weight.

## INTRODUCTION

Coumarins, including esculetin, belong to a diverse group of naturally occurring non-nutrient phenolic phytochemical known as benzo- $\alpha$ -pyrones (Leung et al., 2005; Kostova, 2005). Synthetic 7-hydroxy coumarins are used in sunscreen cosmetics (to absorb ultra violet rays) and also in the synthesis of drugs, including anti-cancer drugs (Kostova, 2005). Generally, coumarins possess anti-edema, and anti-inflammatory activities (Hoult and Paya, 1996; Aoife and Richard, 2004). Like other coumarins, esculetin possesses anti-inflammatory activity (Tubaro et al., 1988).

Coumarins are common in foodstuffs, including soybeans and cruciferous vegetables, and are widely used as flavour-enhancing agents for many different

types of foods (Leung et al., 2005). In particular, esculetin (6,7-dihydroxycoumarin, Cichorigenin, Esculetol, 6,7-dihydroxy-2-benzopyrone, 6,7-dihydroxy-2-benzopirona or 6,7- dihydroxybenzopyran-2-one) - a coumarin derivative was isolated from plants, including *Artemisia capillaries*, *Citrus limonia*, *Aesculus hippocastanum* and *Euphobia lathyris* (Masamoto et al., 2003).

It is estimated that the average western diet contains approximately 1.0 g per day of mixed benzopyrones, including esculetin (Aoife and Richard, 2004). It is estimated that the average western diet contains approximately 1.0 g per day of mixed benzopyrones, including esculetin (Aoife and Richard, 2004). Dietary exposure to benzopyrones may be significant, since they are found in vegetables, fruits, seeds, nuts, coffee, tea and wine (Aoife and Richard, 2004). Thus, research into the time and dose dependent effects of esculetin, a

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**Table I** Effects of esculetin on food Intake of male rats

Treatment Group	Day 7	Day 14	Day 21	Day 28
I (6.0 mg/kg)	115.17 ± 18.54	114.82 ± 9.71*	100.33 ± 10.12	99.30 ± 10.79
II (12.0 mg/kg)	100.32 ± 10.00*	94.37 ± 11.05*	89.68 ± 14.86	85.85 ± 16.02
III (24.0 mg/kg)	100.33 ± 24.06*	134.62 ± 20.76	114.20 ± 18.26	93.12 ± 14.59
IV (Vehicle control (DMSO))	118.90 ± 20.83	118.17 ± 19.36	100.20 ± 18.26	95.00 ± 16.76
V (Normal control (DW))	133.68 ± 29.60	147.67 ± 25.24	98.82 ± 13.89	95.85 ± 12.06

\* Statistically significant difference ( $p < 0.05$ ) between the controls and test groups. Results expressed as mean ± SD for  $n = 6$ .

benzopyrone derivative, on some routine parameters of toxic effect in animal model (male rat) is necessary.

body weight change, feed intake and water intake of the rats were assessed weekly.

## MATERIALS AND METHODS

### Animals

Thirty male Wistar rats (ten - twelve weeks old) with 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 Science, University of Nigeria, Nsukka, Nigeria.

### Chemicals

All the chemicals used in this research were of reagent grade and were obtained from May and Baker, England; Merck, Germany; BDH Chemicals, UK; and Sigma Chemical Compound, USA. Reagents used for some assays were commercial kits and products of Randox Laboratories Ltd., UK.

### Experimental design

The rats were housed in separate cages, acclimatized for seven days and then randomly assigned to five groups of six rats each. The rats in group I were given the esculetin solution at a dose of 6.0 mg/kg body weight while those in groups II and III were given 12.0 mg/kg body weight and 24.0 mg/kg body weight of esculetin respectively. Rats in group IV received 0.2 ml of 10 % dimethylsulfoxide (DMSO) as vehicle control whereas those in group V were given 0.2 ml of distilled water (DW) as normal control. The administration was *per oral* for twenty eight consecutive days. All the animals were fed *ad libitum* pelletised growers feed manufactured by Grand Cereals and Oil Mills limited, Jos, Nigeria. The

### Statistical analysis

The data were analyzed using one way analysis of variance (ANOVA) followed by Least Significant Difference (LSD) to determine significant difference between groups. The criterion of significance was taken as  $P < 0.05$ . The results were expressed as mean value ± standard deviation of the measured variables in each group.

## RESULTS

### Effects of esculetin on food Intake of male rats

As shown in Table 1, exposure to esculetin for 7 days caused a dose dependent decrease in food intake when compared with the controls. However, the effect was not significant ( $p > 0.05$ ) at the least test dose (6.0 mg/kg) but significant ( $p < 0.05$ ) at the median and highest test doses. Rats exposed to 12 mg/kg of esculetin had a time dependent decrease in food intake which was significant ( $p < 0.05$ ) on days 7 and 14. The highest decrease ( $p > 0.05$ ) in the food intake ( $85.85 \pm 16.02$  g) was recorded in the group given 12.0 mg/kg body weight of esculetin. Generally, a time dependent reduction in food intake was noted in all the groups compared with baseline (day 7) except in group III where an increase was recorded on day 14.

### Influence of esculetin on body weight (g) of male rats

A time dependent increase in body weight was noted in all the groups when compared with the body weight of the rats at baseline (day 1), whereas a dose dependent

**Table 2** Influence of esculetin on body weight (g) of male rats

Treatment Group	Day 1	Day 7	Day 14	Day 21	Day 28
I (6.0 mg/kg)	114.92 ± 18.46	125.33 ± 10.62	138.67 ± 15.02	153.83 ± 15.55	168.00 ± 15.80
II (12.0 mg/kg)	114.77 ± 14.48	123.67 ± 14.47	135.33 ± 22.43	144.33 ± 26.97	154.50 ± 26.30
III (24.0 mg/kg)	114.92 ± 27.57	129.83 ± 29.65	141.33 ± 34.96	153.50 ± 32.75	152.17 ± 21.81
IV (Vehicle control (DMSO))	114.53 ± 20.08	131.50 ± 21.53	143.00 ± 26.09	153.17 ± 27.57	170.50 ± 27.54
V (Normal control (DW))	114.78 ± 20.17	133.67 ± 22.85	152.67 ± 21.52	163.83 ± 20.61	179.00 ± 19.52

Results expressed as mean ± SD for n = 6.

**Table 3** Effects of esculetin on water Intake (ml) of male rats

Treatment Group	Day 7	Day 14	Day 21	Day 28
I (6.0 mg/kg)	131.67 ± 21.21*	151.58 ± 12.85*	175.00 ± 18.93	198.52 ± 19.90
II (12.0 mg/kg)	119.21 ± 15.03*	145.83 ± 17.05*	148.33 ± 24.57	157.83 ± 29.50
III (24.0 mg/kg)	146.17 ± 35.07	159.47 ± 24.63	163.93 ± 30.76	191.00 ± 29.29
IV (Vehicle control (DMSO))	149.73 ± 26.25	175.00 ± 28.65	182.90 ± 33.36	184.02 ± 32.29
V (Normal control (DW))	179.52 ± 39.74	188.08 ± 32.16	183.33 ± 25.87	176.82 ± 22.23

\* Statistically significant difference ( $p < 0.05$ ) between the controls and test groups. Results expressed as mean ± SD for n = 6.

decrease in body weight was observed on the 7<sup>th</sup>, 14<sup>th</sup>, and 28<sup>th</sup> days of treatment with esculetin on comparison with controls (Table 2). The observed effects, however, were not significant ( $p > 0.05$ ).

#### Effects of esculetin on water Intake (ml) of male rats

The results of the study on water as presented in (Table 3) showed a time dependent increase in water intake in the groups compared with the normal control (DW). On the other hand, a dose dependent decrease in water intake noted on days 7, 14 and 21 as against the controls (vehicle and distilled water) was significant ( $p < 0.05$ ) only on days 7 and 14 in the rats exposed to 6.0 mg/kg and 12.0 mg/kg of esculetin.

#### DISCUSSION

Esculetin (6, 7-dihydroxycoumarin), is a coumarin derivative found in various natural plant products. In this study, the time and dose dependent effects of esculetin

on some routine parameters of toxicity in male Wistar rats were investigated.

Changes in body weight have been used as an indicator of adverse effects of drugs and chemicals (Hilaly et al., 2004) and body weight gain following agent-induced stimulation of the receptors in the brain that favour fat deposition (Egbuonu et al., 2010) or enhanced energy balance (He et al., 2008; Van Herpen et al., 2008) were suggested. The result of this study showed a dose dependent decrease ( $p > 0.05$ ) in the body weight of the male rats in all the groups when compared to the controls, suggesting that esculetin may not have enhanced these mechanisms that favour body weight gain in the rats. Decreased feed intake (as noted in this study) may favour decreased energy balance (storable calorie) thereby inhibiting body weight gain.

A general decrease in food intake was observed among the controls and test groups of rats as the experiment progressed. This may be attributed to the handling stressor during oral intubation. Marti et al., (1994) and Harvis et al., (2002) observed that laboratory rats exposed to intermittent stressors of differing intensities, such as handling and immobilization, had a

decrease in their food intake. In particular, exposure to esculetin for 7 days caused a dose dependent decrease in food intake when compared with the controls, indicating suppressed energy balance and possibly diminished caloric storage in the rats. The effect was significant ( $p < 0.05$ ) and highest ( $85.85 \pm 16.02$  g) at the median test dose (Group II), suggesting possible esculetin-induced caloric reduction at 12.0 mg/kg body weight of the rats. The observation was particularly significant and deserves follow up, possibly in human.

Generally, food intake and body weight are interdependent, and in rodents decreases in food intake and body weight are important indication of health deterioration (Ullman-Cullere and Foltz, 1999). Thus, the results (on food intake and body weight) may further suggest that esculetin as used in this study did not interfere with the normal biosynthetic processes of rats.

Water is an electronegative enhancer (Batmanghelidj, 2010) and a key element in wastes removal (Egbonu et al., 2009). It is fundamental to life and the total body water is tightly regulated (Bossingham et al., 2005). For instance, the serum osmolality is closely controlled by water homeostasis, which is mediated by thirst, arginine vasopressin, and the activities of kidney (Horacio and Nicolaos 2000; Egbonu et al., 2009). In particular, thirst (which compels water intake) is of primary importance in body fluid homeostasis (McKinley and Johnson, 2004). Water intake reduced in the esculetin fed rats, indicating absence of thirst following, perhaps, decreased excretion (or enhanced retention) of water, or enhanced antidiuretic hormone effect, as suggested by Egbonu et al. (2009). Esculetin exposure may have interfered with water homeostasis by inhibiting thirst resulting in decrease in water intake observed in the rats. Enhanced release of the antidiuretic hormone, vasopressin inhibited thirst in rats (Fitzsimons, 1998; Szczepanska-sadowska et al., 2003).

Overall, the results suggest that esculetin caused a dose dependent, but time independent, decrease in body weight, food and water intakes of male rats. Thus, caution should be exercised in the use of esculetin over time in male rats.

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