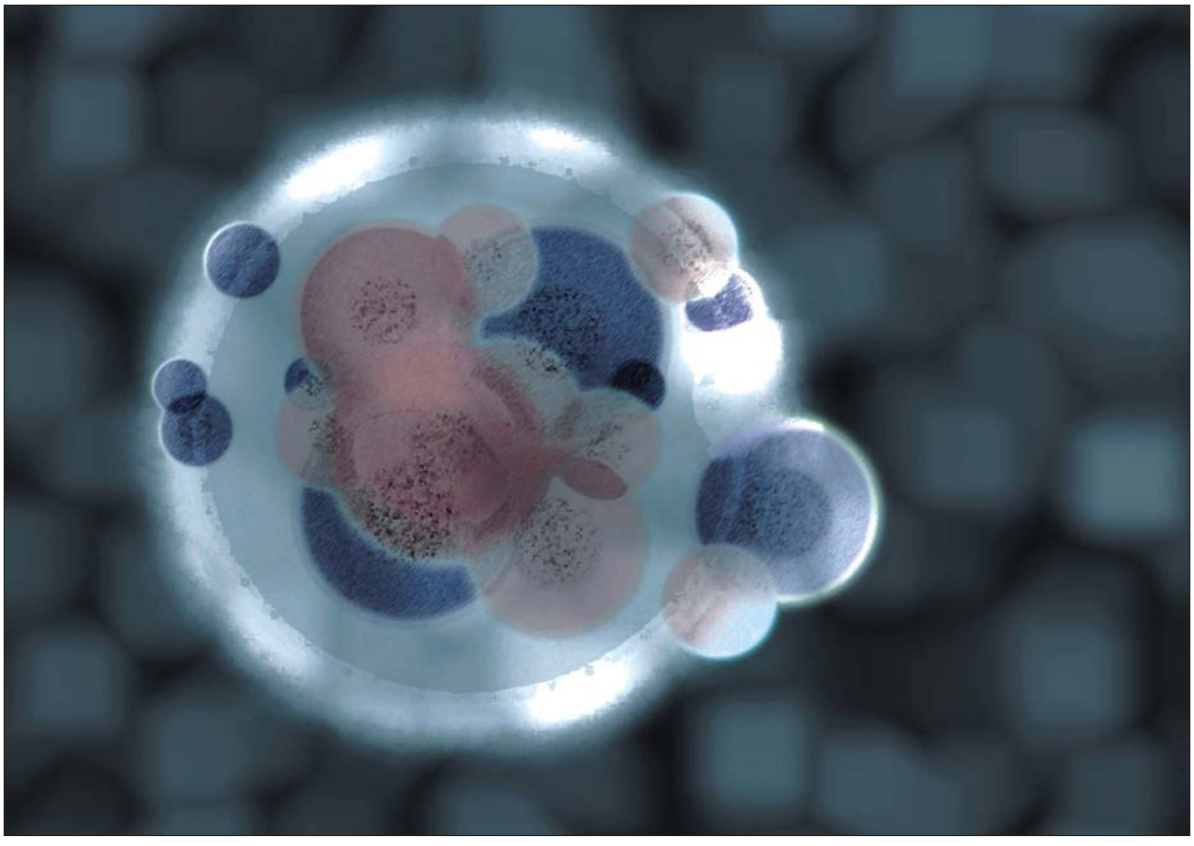

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Dose-related Influence of Esculetin (6,7-dihydroxy-coumarin) on Some Liver and Prostate Function Markers of Male Wistar Rats

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Abstract: The study aimed to ascertain the effect of esculetin (Esc) on some liver and prostate function markers of male Wistar rats. Thirty male Wistar rats were divided into five groups (n = 6). Groups A, B and C were administered with 6.0, 12.0 and 24.0 mg kg^{-1} b.wt. of esculetin, respectively. Groups D and E were administered with 0.2 mL of the vehicle control (10% Dimethyl Sulfoxide (DMSO)) and 0.2 mL of the normal control (distilled water (DW)), respectively. Administration was per oral after every 24 h for 28 days. On comparison with the controls, serum enzymes; aspartate aminotransferase (AST), alamine aminotransferase (ALT) and Alkaline Phosphatase (ALP), activities of the esculetin-fed rats decreased in a dose dependent manner. The decrease was significant ($p<0.05$) except that of ALP activity (79.12±11.82 IU L^{-1}) that was not significant ($p>0.05$) at the 6.0 mg kg^{-1} dose level. Esculetin exposure in the rats induced a dose dependent decrease in Total Acid Phosphatase (TACP) and Prostatic Acid Phosphatase (PACP) activities of the rats serum. However, the decrease was not significant ($p>0.05$) except that of PACP activity (0.76±0.28 IU L^{-1}) that decreased sgnificantly ($p<0.05$) in the group treated with 24 mg kg^{-1} b.wt. of esculetin. The results of this study suggest that esculetin caused a dose dependent improvement of these markers. Thus, repeated exposure to esculetin may not impair the functional capacity of the associated organs, particularly the liver and prostate, of the male rats, irrespective of dose.

Key words: Esculetin, dose related, liver function, prostate function, coumarins

INTRODUCTION

Esculetin (6,7-dihydroxy coumarin) is a coumarin derivative that is present in several plants, including *Citrus limonia, Aesculus hippocastanum, Euphorbia lathyris, Fraxinus rhynchophylla* and *Artemisia capillaris* (Masamoto *et al*., 2002; Tien *et al*., 2011). Coumarins are group of non-nutrient phenolic phytochemicals known as benzo-α-pyrones (Leung *et al*., 2005; Kostova, 2005). Coumarins, including esculetin, are common in foodstuffs and are widely used as flavour-enhancing agents for many different types of foods (Leung *et al*., 2005). They (coumarins) possess diverse biological and pharmaceutical properties, including anti-edema, anti-inflammatory and anti-tumour activities (Hoult and Paya, 1996; Lacy and O'Kennedy, 2004) hence, are used to synthesize drugs (Kostova, 2005).

The average western diet contains approximately 1.0 g per day of mixed coumarins, including esculetin, (Lacy and O'Kennedy, 2004). In particular, esculetin possesses biological activities, including anti-inflammatory activity (Tubaro *et al*., 1988), anti-proliferative activity (Huang *et al*., 1993; Pan *et al*., 2003), tyrosinase inhibitory activity (Masamoto *et al*., 2002) and suppressive activity on oxidative damage to DNA (Kaneko *et al*., 2003; Tahara *et al*., 2005). Esculetin induced the reduction of glutathione in rat liver cells (Chang *et al*., 1996; Martin-Aragon *et al*., 1998) hence, is an antioxidant used in the treatment of a variety of diseases (Surse *et al*., 2011).

Although, dietary intervention could play a significant role in managing chronic diseases (Ezeanyika and Egbuonu, 2011), the dietary and therapeutic exposure of animals to esculetin may be quite significant, warranting a study to assess the possible effect of repeated esculetin exposure in animals. Thus, this study aimed at investigating the dose-related influence of esculetin on some markers of liver and prostate function of male rats. This is because the liver is a major organ involved in the metabolism of foreign compounds, including drugs (Egbuonu, 2010) and agent-induced adverse influence on high metabolic organs including the liver (Egbuonu *et al*., 2010b; Egbuonu and Osakwe, 2011) and prostate (Egbuonu *et al*., 2010a; Egbuonu *et al*., 2010c) have been reported.

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MATERIALS AND METHODS

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: Chemicals used in this research were obtained from 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.

Experimental design: The rats were housed in separate cages, acclimatized for seven days and then randomly assigned to five groups (A, B, C, D and E) of six rats each. The rats in group A, B and C were given esculetin solution at a dose of 6.0, 12.0 and 24.0 mg kg^{-1} b.wt., respectively while those in group D received 0.2 mL of 10% Dimethyl Sulfoxide (DMSO) as vehicle control whereas those in group E 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.

Methods: Serum aspartate aminotransferase, AST was measured with Randox Commercial Enzyme Kit based on the method of Reitman and Frankel (1957). In principle, the aspartate aminotransferase (AST) activity was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine.

Serum alamine aminotransferase (ALT) activity was measured with Randox Commercial Enzyme Kit based on the method of Reitman and Frankel (1957) by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine.

Serum alkaline phosphatase (ALP) activity was measured with Randox Enzyme Kit based on the optimized standard method according to the recommendation of the German Society of Clinical Chemistry (Bessey *et al*., 1972). In principle, the enzyme, alkaline phosphatase, hydrolyzes the p-nitrophenylphosphate substrate to produce inorganic phosphate and p-nitrophenol. The quantity of p-nitrophenol released under standardized conditions of time, temperature and pH is measured (by the absorbance of the yellow colour it assumes in alkaline solution) at 405 nm.

The serum acid phosphatase (ACP) activity was measured with Randox Enzyme Kit based on the methods of Andersch *et al*. (1947) and Fishman and Learner (1953). The principle is that acid phosphatase could hydrolyze the organic phosphate ester, p-nitrophenylphosphate in acid medium (pH 4.8-6.0) to produce p-nitrophenol and an inorganic phosphate. The quantity of p-nitrophenol released under standard condition is measured by the absorbance in the acid medium at 405 nm. To determine the acid phosphatase of prostatic cell origin, sodium tartrate was used to inhibit acid phosphatase other than the prostatic type.

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

Alanine aminotransferase (ALT) activity: ALT activity (IU L^{-1}) is presented in Table 1, exposure to esculetin for 28 days caused a significant ($p<0.05$) decrease in the serum ALT activity of the rats in a dose-related manner on comparison with the controls. The ALT activity of the group of rats exposed to 6.0 mg kg^{-1} b.wt. of esculetin was the least (17.17±0.7 IU L^{-1}) when compared to the controls and the other test groups.

Aspartate aminotransferase (AST) activity: Exposure to esculetin for 28 days caused a significant ($p<0.05$) decrease in the serum AST activity (IU L^{-1}) of the rats in a dose-related manner on comparison with the controls. The AST activity of the group of rats exposed to 6.0 mg kg^{-1} b.wt. of esculetin was the least (45.0±7.75 IU L^{-1}) in comparison with the controls and the other treatment groups (Table 1).

Alkaline phosphatase (ALP) activity: The ALP activity (IU L^{-1}) of the rats following esculetin exposure is shown in Fig. 1. When compared with the controls, the result shows a significant ($p<0.05$) decrease in the alkaline

Table 1: Influence of esculetin on the ALT, AST, TACP and PACP activities of the male rats

Groups	ALT	AST	TACP	PACP
	(IU L^{-1})			
A	17.17±0.71*	45.0±7.75*	7.07±2.71	1.43±0.93
B	17.46±1.11*	52.5±14.75*	6.06±1.24	1.43±0.93
C	18.34±1.46*	55.0±9.49*	5.81±1.35	0.76±0.28*
D	30.85±1.43	72.5±11.29	7.41±1.65	1.68±0.83
E	30.26±0.90	70.0±16.43	7.58±1.06	1.69±0.59

Results expressed as Mean±SD of sample, n = 6 rats. *Significant difference at $p<0.05$

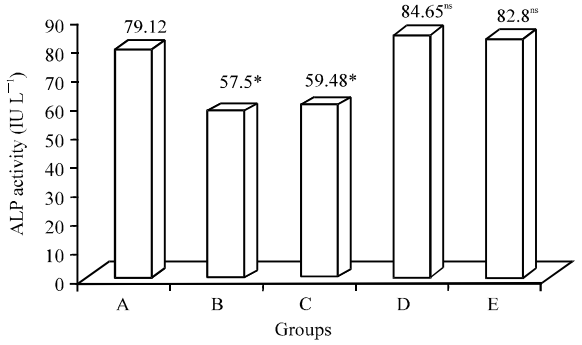

Fig. 1: Effect of esculetin exposure on the serum ALP activity of the rats, n = 6 rats, of *Significant at $p<0.005$ ns: Non significant with respect to control

phosphatase activity of the groups of rats exposed to 12.0 and 24.0 mg kg^{-1} of esculetin but a non-significant ($p>0.05$) of decrease in the group exposed to 6.0 mg kg^{-1} b.wt. of esculetin. The rats exposed to 12.0 mg kg^{-1} b.wt. of esculetin showed the least alkaline phosphatase activity (57.50±6.85 IU L^{-1}).

Total acid phosphatase (TACP) and prostatic acid phosphatase (PACP) activities: As shown in Table 1, esculetin exposure in the rats induced a dose dependent decrease in total acid phosphatase (TACP) and prostatic acid phosphatase (PACP) activities (IU L^{-1}) of the rats. However, the decrease was not significant ($p>0.05$) except that of PACP activity that decreased ($p<0.05$) in the group treated with 24.0 mg kg^{-1} b.wt. of esculetin. The rats given 24.0 mg kg^{-1} b.wt. of esculetin had the least TACP (5.81±1.35 IU L^{-1}) and PACP (0.76±0.28 IU L^{-1}) activities.

DISCUSSION

Esculetin (6,7-dihydroxy coumarin), a coumarin derivative found in various natural plant products, have beneficial biological and biochemical activities (Tahara *et al*., 2005; Surse *et al*., 2011). In this study, the effect of esculetin on some markers of liver and prostate function of male Wistar rats was investigated.

Sub-chronic administration of esculetin led to a dose related and significant decrease ($p<0.05$) in ALT and AST activities of the rats. The observation may indicate esculetin-induced benefit on the liver and, possibly, other organs of the rats irrespective of dose. This finding is in agreement with earlier observation of Lin *et al*. (2000) that pretreatment of rats with esculetin lowered the serum activities of ALT and AST. Furthermore, esculetin (100, 500 mg kg^{-1}) significantly reduced the elevated activities of serum ALT and AST caused by carbon tetra chloride (CCl_4) (Tien *et al*., 2011). Elevated serum activities of ALT and AST were common biochemical markers of liver injury (Ko and Lim, 2006) since any damage to the liver results in the elevation of these transaminases (Sallie *et al*., 1991). We did not explore the possible mechanism for the apparent esculetin-induced benefit on the liver but anti-inflammatory response may be a possible mechanism. Inflammation plays a central role during liver damage (Perez-Alvarez *et al*., 1993; Steele *et al*., 1999) and the anti-inflammatory response of esculetin *in vivo* (Tubaro *et al*., 1988), via the lipoxygenase (Neichi *et al*., 1983) and cyclooxygenase (Sekiya *et al*., 1982) inhibitory effects of esculetin have been reported.

A significant decrease ($p<0.05$) in alkaline phosphatase activity of the 12.0 and 24.0 mg doses of esculetin, suggested absence of obstructive liver disease (Egbuonu *et al*., 2010c). Gilani *et al*. (1998) also reported a reduction of alkaline phosphatase in mice treated with esculetin after paracetamol and carbon tetra chloride (CCL_4) -induced liver damage.

ALT, unlike AST, is a specific marker of liver damage (Kuramitsu *et al*., 1985; Song *et al*., 2004) hence, significant decrease in AST activity following esculetin ingestion to the rats suggest benefit on other high metabolic organs besides the liver (Egbuonu *et al*., 2010c). Since alkaline phosphatase is present in the cells lining many organs, elevation of its activity in plasma (hyperphosphatasemia) may result from liver diseases, bone diseases and hyperparathyroidism (Schide *et al*., 1983).

Elevated TACP and PACP activities are used in the assessment of the functional capacity of the prostate and increased serum TACP activity indicated metastatic carcinoma of the prostate (Egbuonu *et al*., 2010a, c). Thus, the decrease in PACP and TACP activities of the esculetin-fed rats suggest benefit on the prostate function of the rats. Although, acid phosphatase enzyme is abundant in the prostate and seminal fluid, it also occurs in significant quantity in other tissues, including spleen, liver, kidney, red cells and bone (Modder, 1973).

CONCLUSION

The results of this study suggest that esculetin caused a dose dependent improvement of these markers, hence, repeated exposure to esculetin, such as in diets and drugs, may not impair the functional capacity of the associated organs, particularly the liver and prostate, of the male rats, irrespective of dose. Histological examination of organs following higher dose ingestion of

esculetin (and for longer period) should be carried out to ascertain the possible effect of esculetin ingestion on major organs.

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