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Effects of The Extraction of Sugarcane Wax on The Adrenal Cortex and Thyroid Hormones in Hyperlipidemic Female Rats

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Abstract— Hypercholesterolemia is usually known as the presence of high levels of cholesterol in the blood, it closely related with the hazard of coronary heart disease and a possible indicator for early development of atherosclerosis. Octacosanol is one of policasanol components , policasanol is a mixture of higher aliphatic alcohols produces from isolation and purification of sugar cane wax (Saccharum officinarum)is one of the cholesterol-lowering drugs. Statins are the inhibitors of hydroxymethyl glutaryl coenzyme A (HMG CoA) reductase. They are mostly used to treat hyperlipidaemia. Thirty sixhealthy adult female rats. Weights and age of animals were (200-250 gm) and (10-12) weeks respectively they are Inducted to be hypercholesterolemicof 200 mg/dl .octacosanol extracted from Sugar cane plant (Saccharum officinarum)and determined by using gas chromatography – mass spectrometry (GC/MS).Animals were divided into 6 groups (6 rats per group) : all animals were treated orally for 8 weeks as the following: The first group (Control): animals were given Dimethyl Sulphoxide (DMSO) orally only 0.2 ml/ animal per day. The second group were only hypercholesteroled and given 0.2 ml/ animal per day of Dimethyl Sulphoxide (DMSO). The third group were hypercholesteroled and given Atorvastatin at dose (0.02 mg/ animal per day. The fourth group were not hypercholesteroled and given only Atorvastatin at dose (0.02 mg/ animal per day). The fifth group were hypercholesteroled and given Standard octacosanol at dose (0.02 mg/ animal per day). The sixth group animals were hypercholesteroled and given Extracted octacosanol at dose (0.02 mg/ animal per day).
Serum levels of aldosterone , cortisol, T3 and T4 were determined by radio immunoassay. Results showed significant decrease of aldosterone and cortisol in hyperlipidemic group than control group , Also aldosterone levels in hyperlipidemic group that treated with atorvastatin were significantly decreased than those of control group. Results of T3 and T4 in groups treated with octacosanol –both the extracted and standard - were significantly decrease than those in animals of control group, while in groups treated with atorvastatin the levels of these hormones were modulated than those in control group.In conclusion both octacosanol and atorvastatine modulate aldosterone, cortisol, T3 and T4 hormones in female rats.

Keywords: Rats- Octacosanol Extraction- Atorvastatine- Cortisol- Aldosterone, T3,T4.

Introduction

Hypercholesterolemia is usually known as the presence of high levels of cholesterol in the blood. Cholesterol is an amphipathic lipid and is naturally existed in the tissues and the plasma. In the plasma, it is carried in lipoproteins. These lipoproteins are divided into four important groups: High Density Lipoprotein-Cholesterol (HDL-C), Low Density Lipoproteins- Cholesterol (LDL-C), Very Low Density Lipoprotein- Cholesterol (VLDL), and chylomicrons. The last three groups are closely related with the hazard of coronary heart diseaseand a possible indicator for early development of atherosclerosis whereas (HDL-C) is not, (Frederick, et al., 2010). [1]

Octacosanol is one of policasanol components , policasanol is a mixture of higher aliphatic alcohols produces from isolation and purification of sugar cane wax (Saccharum officinarum) is one of the cholesterol-lowering drugs[2]. Octacosanol is the major effective component of policosanol forming about (62.9%)[3] , it has antioxidative, lipid lowering, antithrombotic (antiplatelet) effects and provides protection against free radical associated diseases sugarcane wax contains (60-70%) octacosanol [4]. Octacosanol may protect against cerebral ischemia by reducing theTxA2/PgI2 ratio [5].

Statins are the inhibitors of hydroxymethyl glutaryl coenzyme A (HMG CoA) reductase. They are mostly used to treat hyperlipidaemia [6]. Statins that currently carried for clinical use include Atorvastatin, Fluvastatin, Lovastatin, Pravastatin, and simvastatin. Lowering of low-density lipoprotein (LDL) plasma levels has been shown to reduce primary and secondary cardiovascular events including myocardial infarction (MI), stroke, and all-cause mortality [7]. They also have favorable effects on platelet activation, endothelial function, inflammation, and coagulation cascade [8,9].

Aldosterone is the main mineralocorticoid steroid hormone, produced by the zona glomerulosa of the adrenal cortex in the adrenal gland, it is essential for sodium conservation in the kidney, salivary glands, sweat glands and colon[10-11]. It plays a central role in the regulation of the arterial blood pressure, plasma sodium (Na+), and extracellular potassium (K+) levels. It does so mainly by acting on the mineralocorticoid receptors in the distal tubules and collecting ducts of the nephron [11].It influences the reabsorption of sodium and excretion of potassium (from and into the tubular fluids, respectively) of the kidney, thereby indirectly influencing water retention or loss, blood pressure and blood volume [12].
Cortisol , the most potent glucocorticoid steroid hormone , is produced by the zona fasciculata of the adrenal cortex in the adrenal gland . It is synthesized from cholesterol and its production is stimulated by pituitary adrenocorticotropic hormone (ACTH) which is regulated by corticotrophin releasing factor (CRF) [13]. ACTH and CRF secretions are inhibited by high cortisol levels in a negative feedback loop. In blood a majority of cortisol is bound with high affinity to corticosteroid binding globulin (CBG). Cortisol acts through specific intracellular receptors and affects numerous physiologic systems including immune function, glucose counter regulation, vascular tone, and bone metabolism. [14].

The thyroid hormone is well known for controlling metabolism, growth, and many other body functions. The thyroid gland, anterior pituitary gland, and hypothalamus comprise a self-regulatory circuit called the hypothalamic-pituitary-thyroid axis. The main hormones produced by the thyroid gland are thyroxine or tetraiodothyronine (T4) and triiodothyronine (T3). Thyrotropin-releasing hormone (TRH) from hypothalamus, thyroid-stimulating hormone (TSH) from the anterior pituitary gland, and T4 work in synchronous harmony to maintain a proper feedback mechanism and homeostasis. [15].
The present study was designed to compare the effects of octacosanol on hormones of the adrenal gland (aldosterone and cortisol) as well the thyroid hormone T3 and T4 in female rat model with atorvastatin that currently present in market with certain side effects like rhabdomylysis, muscle weakness and affect the liver enzymes.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS:

Thirty sixhealthy adult female rats. Weights and age of animals were (200-250 gm) and (10-12) weeks respectively. Animals were housed in plastic cages with metal covers, containing bedding materials of fine wood which was kept dry and changed twice weekly. The animals were maintained under controlled optimum conditions light dark cycle (12/12) hours, at a temperature (25±4°C). The diet was offered ad Libitum, and presented with tap water.

STANDARD DRUG :

Standard octacosanol obtained from USA.

PLANT MATERIAL COLLECTION AND PREPARATION OF THE EXTRACTION

Sugar cane plant (Saccharum officinarum)were collected from Mesan province , peels were manually scrapped and dried at 60°C for 24 hours and stored in air tight container, after that grinding sugar cane then extracted the policosanol, and determined its contents – particularly octacosanol- by using gas chromatography – mass spectrometry (GC/MS).

INDUCTION OF HYPERCHOLESTEROLEMIA

Induction of hypercholesterolemiawas done by addition of 1% cholesterol (El Gomhorya Co., Egypt), 0.5% saturated fat and 0.5% cholic acid to rat chow diet and 0.01% thiouracil in drinking water and supplementation to rats for eight weeks [16,17].Rats which achieved a serum cholesterol level greater than 200 mg/dl were selected for this study [18].

EXPERIMENTAL GROUPS:

Animals were divided into 6 groups (6 rats per group) : all animals were treated orally for 8 weeks as the following: The first group (Control): animals were given Dimethyl Sulphoxide (DMSO)orally only 0.2 ml/ animal per day. The second groupwereonlyhypercholesteroled and given 0.2 ml/ animal per day of Dimethyl Sulphoxide (DMSO). The third group were hypercholesteroled and given Atorvastatin at dose (0.02 mg/ animal per day. The fourth group were not hypercholesteroled and given only Atorvastatin at dose (0.02 mg/ animal per day).The fifth groupwere hypercholesteroledand given Standard octacosanol at dose (0.02 mg/ animal per day). The sixth group animals were hypercholesteroled and given Extracted octacosanol at dose (0.02 mg/ animal per day).

HORMONE MEASUREMENTS

Serum levels of aldosterone , cortisol, T3 and T4were determined by radio immunoassay using assay kits obtained from Accu-Bind, Monobind,USA.

Statistical Analysis:

One-way ANOVA-test was used to determine the significant difference between groups. Differences between data were compared by least significant difference (LSD). All data were expressed as Mean ± Standard deviation. All statistical tests were done by using statistical program SPSS (version 21.0) the level significant set on $p \leq 0.05$ [19] .

RESULTSAND DISCUSSION

In this study the significant decrease of aldosterone and cortisol in hyperlipidemic group than control group was in agreement with a reported study referred that hyperlipidemia has certain effects on benzodiazepine receptors (PBR), translocation of cholesterol to the inner mitochondrial membrane of steroidogenic cells leading to decrease of STAR protein, pregnenolone and in turn other steroidal hormones namely aldosterone, and cortisol [20].

Also aldosterone levels in hyperlipidemic group that treated with atorvastatin were significantly decreased than those of control group, these changes may be attributed tothat Atorvastatin modulates certain cholesterol derived products in rats. This is mainly attributed to decreased production of steroid acute regulatory (STAR) protein level as mediated through down-regulation of STAR gene expression[21,22], Table (1).

Table 1: Comparing the effects of extracted octacosanol and atorvastatin on Aldosterone, Cortisol, T3 and T4 levels in female rats.

Parameters / Groups	Aldosterone (pg/ml)	Cortisol (nmol/L)	T3 (nmol/L)	T4 (nmol/L)
First group Cont.	407.33 ± 17.52 c	151.56 ± 10.27 b	3.55 ± 0.61 b	76.09± 1.76b
Second group HC	360.60 ± 9.94 d	138.8 ± 7.09 c	2.34 ± 0.21 d	67.83± 5.00c
Third group HC+AT	211.53 ± 23.40 e	150.43 ± 8.64 b	3.87 ± 1.37a	68.16± 12.13 c
Fourth group AT	383.06 ± 14.02 cd	172.79 ± 33.07a	3.62 ± 0.85b	82.35± 14.51 a
Fifth group HC+ST. Octa.	476.66 ± 34.44 b	158.4 ± 1.86 b	2.48 ± 0.03c	47.99± 0.81 d
Sixth group HC+EX. Octa.	540.33 ± 26.27 a	93.17 ± 1.50 d	1.25 ± 0.08e	44.72± 0.06d
LSD	46.56	11.6	0.138	6.24

*Different letters refer to a significant differences at ($P \leq 0.05$).no=6 animals/ group.

There were a significant increase in levels of aldosterone in both groups that treated with extracted and standard octacosanol as compared to control group, but significant decreasing in cortisol levels in group treated with extracted octacosanol, these results may attribute to the antioxidant activity of the octacosanol[23].

Thyroid hormones, including T3 and T4, can take part to regulate body metabolism and have two-ways regulation effects to the growth of animals [24].

Results of T3 and T4 in groups treated with octacosanol –both the extracted and standard - were significantly decrease than those in animals of control group, these results were not matched with the study of Le, et al in 2015, who proved that the octacosanol can promote the secretion of T3, in blood of weaning piglets[25].This can be presumed that octacosanol may affect protein deposition and consequently energy balance by decreasing of thyroid hormones, and that may causes utilization of protein and affect lipid oxidation and growth performance of the animal.In conclusion both octacosanol and atorvastatine modulate aldosterone, cortisol, T3 and T4 hormones in female rats

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