بسم الله الرحمن الرحيم

MONA MAGHRABY

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

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شبكة المعلومات الجامعية

التوثيق الإلكتروني والميكروفيلم

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

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The Possible Role of Orthoboric Acid on Adipogenesis In Rats (*In Vitro* and *In Vivo* Models) Histological and Immunohistochemical Study

Thesis

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بسم الله الرحمن الرحيم

قالوا

سبحانك لا علم لنا إلا ما علمتنا إنك أنت العليم الحكيم

صدق الله العظيم

سورة البقرة الآية: ٣٢

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Dedication

To:

My Mother,

My Belooed Daughters (Hania & Malaika)

For their endless love, support,
and continuous care

ABSTRACT

Background: obesity is a worldwide problem which leads to increased morbidity and mortality. Clinical use of some therapeutic options for the prevention of overweightness is limited due to their dangerous side effects. Orthoboric acid is a new safe and beneficial treatment for prevention of high fat diet induced obesity. **Aim**: study the effect of orthoboric acid on adipogenesis *in vitro* and *in vivo*. **Materials and methods:** Fifty adult male albino rats were included in this study: Thirty rats for experimental grouping of average weight 200-250 gm. Ten rats for preparation of bone marrow derived mesenchymal stem cells (BM-MSCs). Ten rats for preparation of platelet rich plasma (PRP). The experiment has been carried out ***In vitro*** and ***In vivo***: **1. *In vitro* group (Group I):** This group was subdivided into three subgroups: Subgroup **Ia** (negative control): consisted of 15×10^4 BM-MSCs which was seeded in each well of 6-well culture dishes and treated with 5% fetal bovine serum (FBS) for 8 days. Subgroup **Ib** (positive control) "platelet lysate (PL) treated": consisted of 15×10^4 BM-MSCs which was seeded in each well of 6-well culture dishes and treated with 10% PL at the same time of seeding for 8 days. Subgroup **Ic** (Orthoboric acid treated): It consisted of 15×10^4 BM-MSCs which was seeded in each well of 6-well culture dishes and treated with 10% PL concomitantly with 1 mg/mL orthoboric acid at the same time of seeding for 8 days. After 8 days, cells were fixed with 4% paraformaldehyde and stained with 0.1% Oil Red O staining. **2. *In - vivo* group:** The rats were divided into 4 main groups. Group **II** (control) Subgroup **IIa** (control 8 weeks), **IIb** (control 12 weeks). Group **III** (High Fat Diet ″HFD″ group): **IIIa** (HFD 8 weeks), **IIIb** (HFD 12 weeks). Group **IV** (HFD and orthoboric acid group) for 8 weeks. Group **V** (HFD for 12 weeks but starting from the 8th week the rats were given orthoboric acid daily for 4 weeks). Orthoboric acid was used at dose of 2.5 mg/rat given orally via intragastric tube. Body weights of all rats were recorded weekly during the experiment. Adipose tissues specimens were collected at the end of experiment and processed for H&E, oil red O stain and antiβ - catenin immunohistochemistry. Histomorphometric and statistical analysis were also done. **Results: 1. *In vitro*** group, subgroup **Ib** showed conformational change from spindle shaped fibroblast like cells into spherical shaped cells (adipocytes) with significant increase in its leptin concentration compared to subgroup **Ia**. Meanwhile, subgroup **Ic** maintained their fibroblast like shape and showed a significant decrease in leptin concentration compared to subgroup **Ib**. **2. *In - vivo*** group, Group **III** showed a significant increase in body weight and in size of adipocytes compared to group **II**. Meanwhile, group **IV** and group **V** showed a significant decrease in body weight and size of adipocytes relative to group **III**. As regard effect of orthoboric acid on adipogenesis, group **IV** and group **V** showed positive immunohistochemical reaction for β- catenin with subsequent decrease in lipid accumulation confirmed with less optically dense oil red O staining compared to group **III**. **Conclusions:** Orthoboric acid inhibited PL induced adipogenesis in BM-MSCs. Moreover, low dose of oral orthoboric acid was able to reduce body weight in rats and decrease mean size of adipocytes and mean optical density of oil red O together with increase in mean optical density of β- catenin. Therefore, it can be considered as a suitable treatment for obesity due to its vital role in inhibition of adipogenesis.

Key wards: orthoboric acid, adipogenesis, high fat diet, in vitro, in vivo.

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List of Abbreviations

Abb.		Full Term
AF-1	:	Activation Function 1.
AP-1	:	Activation of activating protein-1.
AT	:	Adipose tissue
ATGL	:	Adipose Triglyceride Lipase.
BAT	:	Brown adipose tissue.
BeAT	:	Beige adipose tissue.
BMDCs	:	Bone marrow - derived cells.
BMI	:	Body mass index.
BMP4	:	Bone morphogenetic protein 4.
Brite	:	Brown in white.
C/EBPs	:	CCAAT/enhancer-binding proteins.
CD	:	Cluster differentiation.
ECM	:	Extracellular matrix.
HDL	:	High-density lipoprotein.
HSL	:	Hormone Sensitive Lipase.
KLF	:	Krüppel-like factor.
LBDs	:	Ligand-binding domains.
Myf 5	:	Myogenic factor 5.
PL	:	Platelet Lysate.
PPARγ	:	Peroxisome proliferator-activated receptor γ.
Pref-1	:	Preadipocyte factor-1.
PRP	:	Platelet Rich Plasma.
RER	:	Rough endoplasmic reticulum.
Runx2	:	Runt-related gene 2.
SD	:	Standard deviation.
SER	:	Smooth endoplasmic reticulum.
SMAD	:	Small mother against decapentaplegic.

List of Abbreviations (Cont.)

Abb.		Full Term
SREBP-1	:	Sterol response element–binding protein-1.
STATs	:	Signal transducers, activators of transcription.
TG	:	Triglycerides.
TGF-β	:	Transforming growth factor β.
UCP-1	:	Uncoupling protein-1.
WAT	:	White adipose tissue.
WHO	:	World Health Organization.
Wnt	:	Wingless-type MMTV integration site.
ZFP423	:	Zinc- finger transcription protein 423.

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Introduction

Overweight or obese people were found to estimate about 2.1 billion people worldwide, and 2.8 million deaths are caused by obesity every year **(Smith and Smith, 2016).** Obesity can be reached when the body mass index (BMI) exceeds 30 kg/m2and abdominal obesity is considered if the waist circumference exceeds 102 cm for men and 88 cm for women **(Giralt and Villarroya, 2013).** In humans, there are two main sites for distribution of adipose tissue: visceral; around the internal organs and subcutaneous; just under the skin around the belly, thighs, and rear. It has been noticed that visceral abdominal fat is more dangerous than subcutaneous fat as it contributes to insulin resistance and inflammation reducing 8 years from the life expectancy **(Baglioni et al., 2012).** In developed countries obesity leads to increased morbidity and rate of deaths as it causes serious associated diseases, such as type II diabetes, high blood pressure, cardiovascular disorders and metabolic syndrome **(Ahima and Lazar, 2013; Farr et al., 2014).** Metabolic syndrome or prediabetes can be defined as a complex of metabolic abnormalities including obesity especially visceral obesity with its associated comorbidities including, insulin resistance, hyperlipidemia, hypertension, prothrombotic and proinflammatory states **(Pérez-Martínez et al., 2017).**

As regard development of obesity, uncontrolled increased number and size of adipocytes thought to be

contributing factors in process of adipogenesis **(Bak et al., 2010).** Although several therapeutic options have been offered to the market for the treatment of obesity, their clinical use is restricted due to their dangerous side effects including high blood pressure, cardiovascular complications, liver diseases and psychiatric illnesses **(Derosa and Maffioli, 2012).** Therefore, it was necessary to discover a new, safe, and efficient alternative treatment for obesity. The new approaches in treatment of overweightness concentrate on aiming certain pathways involved in adipogenesis to decrease storage of lipid and adipocyte proliferation.

Boron compounds are naturally occurring elements and found in various human tissues. They are present in two forms; Orthoboric acid which is hydrogen borate and Borax which is sodium borate. There are several types of foods rich in these compounds specially orthoboric acid, including fruits like raw red apple with peel and raw banana, nuts like almonds and peanuts, cereal grain products like enriched white bread and instant white rice. They are involved in several pathways, including psychological activities, hormonal regulation, bone development **(Nielsen, 2014)**, and wound healing **(Demirci et al., 2015)**. Orthoboric acid is a weak inorganic acid with antiseptic properties. It′s also called **boric acid** or boracic acid. Low concentrations of boric acid don't pose any toxicity. However, boric acid is poisonous if ingested or inhaled in large quantities.