Honey as an Antioxidant in Children and Adolescents with Epilepsy

Thesis

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Summary

This study was conducted in 4 weeks duration on 20 children and adolescents with idiopathic epilepsy treated with VPA monotherapy divided into two groups each group included 10 patients. Group A (Intervention /Control- I/C) included 10 patients who received a calculated daily dose of honey 1.2ml/kg/day for 2 weeks (Intervention-I) to explore the effect of its intake on GPx serum levels, followed by another 2 weeks without receiving honey (Control-C) to explore honey's long lasting effect as well as the effect of stoppage of its intake. Group B (Control/Intervention- C/I) included 10 patients who did not receive honey for the 1st 2 weeks of the study (control-C) to examine whether the change was due to honey ingestion later or not, followed by another 2 weeks in which they received honey as in the protocol of group A to further elucidate the effect of Honey intake on them too. The patients were recruited from the regular attendants of the children in the out-patients neurological pediatric clinic of Ain Shams university hospital.

All patients were subjected to: **1)** Full history taking including seizure type, seizures' frequency (/ and2 weeks), history of sodium valproate therapy. **2)** Data from each patient's file was accurately recorded as regards the epilepsy type according to the international classification of the International League against Epilepsy (ILAE), duration of disease and

List of Contents

Page No.

List of Abbreviations

AEDs : Antiepileptic Drugs.
AF : Atrial fibrillation
AIDS : Acquired immune deficiency syndrome.
AIF : Apoptosis Inducing Factor
ALT : Alanin transeferase
AMPA : a-amino-3-hydroxy-5- methylisoxazolepropionic acid
ANS : Autonomic Nervous System.
AOC : Antioxidant capacity
BPM : Beat per min
C/I : Control/intervention
CAPE : Caffeic acid phenyl ester
CAPE : Caffeic acid phenethyl ester
CD : Conjugated diene
CDC : Centers for Disease Control and Prevention.
CHD : Coronary heart disease
CI : Confidence interval
CNS : Central Nervous System.
CRP : C-reactive protein
CSF : Cerebrospinal fluid.
CT : Computerized tomography.
CuOOH : Cumene hydroperoxide
CVS : Cardiovascular System.
DPPH-1 : 1-diphenyl-2-picrylhydrazyl
ECG : Electrocardiogram.
EEG : Electroencephalogram.
EPR : Electron paramagnetic resonance
ER : Endoplasmic reticulum
ERK : Extracellular signal-regulated kinase
ERK : Extracellular signal-regulated kinase
FET : Fisher's Exact Test.
FLE : Frontal lobe epilepsy.
FOS : fructooligosaccharides
FRAP : Ferric reducing/antioxidant power
GABA : Gamma amino butyric acid.
GEFS+ : Generalized epilepsy with febrile seizures plus.

GPx : Glutathione peroxidase
GSH : Glutathione
GSSG : Oxidized glutathione
H2O2 : Hydrogen peroxide
HDAC1 : Histone Deacetylase 1
HDL-C : High-density lipoprotein-cholesterol
HF : High frequency.
HIV : Human immunodeficiency virus
HMF : Hydroxymethylfurfural
HR : Heart rate
HRV : Heart rate variability
HS : Highly Significant
I/C : Intervention/control
I/R : Ischemia-reperfusion
IGE : Idiopathic generalized epilepsy.
ILAE : International League against Epilepsy.
iNOS : Inducible NOS
iPS : Induced pluripotent stem
IQs : Intelligent quotient
JNK : Jun N-terminal kinase
LDH : Low density lipoprotein
LDL-C : Low-density lipoprotein-cholesterol
MAP : Mitogen-activated protein
MAPK : Mitogen-activated protein kinase
MDA : Malondialdehyde
mEH : Microsomal Epoxide Hydrolase
MGO : Methylglyoxal
MI : Myocardial infarction
MMP : Mitochondrial membrane potential
Mo : Monthes
MRI : Magnetic resonance imaging.
MRP1 : Multidrug resistance-associated protein 1.
MRSA : Methicillin resistant staph.aurous
NMDA : N-methyl-d-aspartate
nNOS : Neuronal nitric oxide synthase
NO : Nitric oxide
NO2 : Nitrogen dioxide,
ORAC : Oxygen radical absorbance capacity
PKC : Protein Kinase C

RCS : Reactive chlorine species
RNS : Reactive nitrogen species
ROS : Reactive oxygen species
ROS : Reactive oxygen species
RS : Reactive species
S : Significant
SD : Standard Deviation
SE : Status epilepticus
sEPSCs : Spontaneous postsynaptic excitatory currents
SLE : Systemic lupus erythematosus.
SOD : Superoxide dismutase
SOD : Superoxide dismutase.
SPSS : Statistical package for Social Science
SUDEP : Sudden unexpected death in epilepsy.
T : t test for independent samples
TBARS : Thiobarbituric acid reactive substances;
TG : Triglycerides
TPK : Tyrosine Protein Kinase
Trx : Thioredoxin
USA : United States of America.
VPA : Valproic acid
VSMC : Vascular smooth muscle cell
WHO : World Health Organization.
Wk : Weeke
XO : Xanthine oxidase

Introduction

Valproic acid (VPA) is a chemical compound that has found clinical use as an anticonvulsant and mood-stabilizing drug, primarily in the treatment of epilepsy and bipolar disorders ***(Levy et al., 2002).*** A serious adverse reaction of VPA therapy is a rare, idiosyncratic, and potentially fatal hepatotoxicity characterized by liver steatosis and necrosis ***(Dreifuss et al., 1989).*** The underlying mechanism responsible for the hepatotoxicity is still not well understood, but various hypotheses have been proposed, including oxidative stress ***(Thomas et al., 2006)***. These changes in the antioxidant system were associated with an elevation in serum lipid peroxides in children on chronic VPA therapy ***(Pippenger and Yuksel, 2000)***. There are a number of studies suggesting that excessive generation of free-radical intermediates are associated with VPA possibly as a consequence of VPA biotransformation, alterations in glutathione homeostasis, and/or depletion of cofactors required for antioxidant defense ***(Klee et al., 2000).***

Lipid peroxidation is a well-established mechanism of cellular injury in both plants and animals, and is used as an indicator of oxidative stress in cells and tissues Glutathione, the most abundant low molecular weight thiol compound synthesized in cells, plays an important role in anti-oxidant defense and detoxification reactions. It is primarily synthesized in the liver by the transsulfuration pathway. Glutathione

peroxidases catalyze the reduction of H_2O_2 or organic hydroperoxides to water or corresponding alcohols using reduced glutathione ***(Margis et al., 2008)***. Deficits in glutathione have been implicated in aging and many of diseases including Alzheimer's disease, Parkinson's disease, cardiovascular disease, cancer, Down syndrome and autism ***(Forman et al., 2008; Reed et al., 2008)***. *So mesuerment glutathion peroxidase is used as antioxidant marker.*

The therapeutic properties of honey, once considered a form of folk or preventive medicine, are acquiring importance for the treatment of acute and chronic free radical - mediated diseases (atherosclerosis, diabetes and cancer) ***(Beretta et al., 2007).*** The antioxidant capacity of honey is a result of the combined activity of a wide range of compounds including phenolics, peptides, organic acids, enzymes and possibly other minor components. The phenolic compounds contribute significantly to the antioxidant capacity of honey but are not solely responsible for it ***(Gheldof et al., 2002)***.

Beretta et al (2007) studied the protective activity of a honey of multifloral origin in a cultured endothelial cell line subjected to oxidative stress and they found that honey showed strong quenching activity against lipophilic cumoxyl and cumoperoxyl radicals, with significant suppression / prevention of cell damage, complete inhibition of cell membrane oxidation, of intracellular reactive oxygen species (ROS) production and recovery of intracellular glutathione (GSH). Phenolic acids and

flavonoids were the main causes of this protective effect. ***Perez et al (2007)*** studied 53 honey samples from Spain and their results showed that PH, acidity, net absorbance, electrical conductivity, and total polyphenolic contents of the honeys showed a strong correlation with the radical scavenging capacity. They also found that the correlation between the radical scavenging capacity of honey and amino acid contents was high with 18 of the 20 amino acids detected, with correlation values higher than those obtained for polyphenolic content.

Aim of the work

The aim of this work is to study the antioxidant effect of honey in children and adolescents with epilepsy on VPA monotherapy.

Chapter (1): Epilepsy

Introduction:

As a result of advances in technology and enhanced medical knowledge, children with chronic diseases that were previously fatal in early childhood now survive to be young adults. Chronic childhood illnesses have, therefore, become one of the primary health priorities that need intensive research. These diseases vary widely in their nature and severity, their influence on the child's behavior, daily activity, normal growth, and their interference with scholastic achievement. Among the important childhood chronic diseases is epilepsy ***(Isaacs and Sewell, 2003).***

Historical aspects:

In ancient times, many ancient writers considered seizures to be the work of supernatural forces and the most vivid example of demonic possession. Many of history's greatest men suffered from seizures: Julius Caesar, Alexander the Great, Napoleon Bonaparte, Van Gogh, Isaac Newton, and Dostoievski ***(Schachter, 2006).***

The earliest description of seizures appears in Egyptian hieroglyphics prior to 700 B.C ***(Fisch, 1996).*** The first fundamental scientific step on the subject is attributed to Hippocrates about 400 B.C in his book "on the sacred disease" and recognized epilepsy as an organic process of the brain ***(Keith, 1963).*** Al-Razi was the first to use the term El-Sarr'e in

his book "El-Hawi" and the term epilepsy could be considered as the Latin version of the former term ***(Mahdi, 1984).***

In fact, the word epilepsy derived from a Greek word meaning "to be seized by forces from without" ***(Brockhaus and Enger, 1995).***

Definition:

Multiple definitions have been set for both seizures and epilepsy.

- Seizures

Freeman and Vining (1992) defined a seizure as a paroxysmal electrical discharge of neurons in the brain resulting in alteration of function or behavior.

Johnston (2008) mentioned that a seizure is a paroxysmal, time-limited change in motor activity and/or behavior that results from abnormal electrical activity in the brain.

- Epilepsy:

Epilepsy can be defined as recurrent seizures unrelated to fever or to an acute cerebral insult ***(Haslem, 2000).***

French and Pedley (2008) defined epilepsy as tendency to develop two or more seizures that are not provoked by other illnesses or circumstances.

While all patients with epilepsy experience seizures, not all individuals with seizures have epilepsy. Seizures may be

acute symptomatic or unprovoked. Acute symptomatic seizures are seizures occurring at the time of a systemic insult or in close temporal association with a documented brain insult. Unprovoked seizures are seizures occurring in the absence of precipitating factors and may be caused by a static injury (remote symptomatic seizures) or a progressing injury (progressive symptomatic seizures). Unprovoked seizures may be single or recurrent (epilepsy) ***(Hauser and Beghi, 2008).***

During a seizure, the epileptic activity in the brain can be seen as a series of "spikes" or "spikes and waves" in electroencephalographic recordings. These "spikes" or "spikes and waves" are called the "electrographic" seizure. The behavior of the patient during the epileptic attack is called the "clinical" seizure. If the clinical seizure involves muscle spasms, it is called a "convulsion" ***(Burnham, 2002).***

Epidemiology and morbidity indices:

- ***Incidence by seizure type***

Seizure types vary in incidence. Generalized tonic clonic or various types of partial seizures dominate about 75% of childhood epilepsy syndromes and partial seizures seemed to occur more often than generalized seizures ***(Kotsopoulos et al., 2002).*** Absence epilepsies account for approximately 15% and other generalized epilepsies account for only 10%. This latter group consists of the majority of the catastrophic syndromes, including West syndrome, Lenox-Gastaut syndrome, and severe myoclonic epilepsy of infancy ***(Camfield et al., 1996).***

● ***Worldwide distribution***

Around 50 millions people worldwide have epilepsy with nearly 90% of them are found in developing regions ***(WHO, 2009).*** In developed countries the incidence of epilepsy varies between 50 and 100 per 100, 000 persons per year ***(Kobau and Price, 2003).***

The incidence rates are higher in developing countries due to exposure to higher risks of permanent brain damage like CNS infection, head trauma, perinatal complications and malnutrition in addition to different population demographic characteristics, poor antenatal care, and lower standards of epilepsy care ***(Hopkins and Shorvon, 1995; Leary et al., 1999; Tellez-Zenteno et al., 2004)***. However, seizure prevalence may be under-reported because of reluctance to disclose a potentially stigmatizing condition ***(Jacoby, 2002).***

In Egypt, ***Mekky (1981)*** studied the epidemiology of epilepsy and reported a prevalence of 4.1 per 1000 population and the highest prevalence was in the age group 10-19 years reaching 7.4 per 1000. ***El-Afify (1981)*** studied epilepsy in El-Sahel Teaching Hospital and reported a prevalence of 9.8 per 1000 population, whereas ***El-Khayat et al., (1994)*** studied the epidemiology of epilepsy among Egyptian infants and children and reported a prevalence rate of 3.5 per 1000.

In Upper Egypt, Assiut Governorate, the prevalence of epilepsy was reported by ***Shawki (1995)*** to be 12.9 per 1000 population, while ***Massoud (1997)***, in his study on 195 school children in Cairo, reported a lower overall prevalence of 1.9 per 1000.