Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(8):6-9

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

The effect of *Anethum graveolens* L.(dill) extraction on PAPP-A protein in healthy and epileptic mice

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INTRODUCTION

Anethum graveolens L. (dill), an important member of the Umbelliferae family native to southwest Asia or southeast Europe, is broadly used for flavoring foods and beverages due to its pleasant spicy aroma. Cultivated since antiquity, dill is a hardy annual or biennial plant and has a single stem with a terminal or primary umbellate flower. Its pharmacological properties, such as its antibacterial activity, as well as antihyperlipidemic and antihypercholesterolemic effects, have been reported. As a traditional medicine, dill increases milk production and promotes menstruation in females. Some studies have shown that dill seed oil suppresses some spoilage fungi. Dill is also widely distributed in the Xinjiang Uyghur Autonomous Region of China and has been commonly used as medicine and food flavor. In China, dill seeds have known pharmacological effects, such as promoting appetite and alleviating pain effects [1].

Down syndrome, or trisomy 21, is the most widespread autosomal chromosome abnormality in live births and the most ordinary cause of mental retardation. The occurrence is approximately 1 in 700 newborns, with a higher incidence in older-maternal-age populations owing to a higher incidence of the main pathogenetic event—meiotic nondisjunction in the oocyte—in older women. This mechanism gives rise to an extra copy of chromosome 21 in the zygote in 95% of cases [10].

Screening for Down syndrome during the first trimester helps address the previous concerns. accruing research and prospective studies from several countries reveal the potential of this new screening regimen. Instead of screening from 15 to 22 weeks, screening would be conducted at 10 to 14 weeks of process of early development with an ultrasound scan and a serum sample. Because the second trimester markers AFP, uE3, and inhibin A do not discriminate between affected and unaffected pregnancies at this earlier time, a new set of markers is required. The markers most widely projected include pregnancy-associated plasma protein A (PAPP-A), free β-hCG, and nuchal translucency (NT). Their combination has been termed the first trimester combined test [10]. In maternal serum samples from women in the first trimester of Down syndrome pregnancies, PAPP-A levels were about half those of normal pregnancies [12]. The first trimester combined test presents the most favorable balance between performance and patient satisfaction[12].PAPP-A mRNA and protein levels do not differ between trisomic and normal pregnancies, indicating that lower levels result from alterations in posttranslational events (intracellular protein stability, release, impaired transport across placenta, and/or modified serum stability)[13].

As suggested by its name, pregnancy-associated plasma protein-A (PAPP-A) plays an important role in pregnancy and fetal development. On the opposite end of life's spectrum, recent studies using genetically engineered mice indicate a recently recognized role for PAPP-A in and in the development of age-related disease [17]. PAPP-A is expressed in a variety of tissues and cell types, and is potently up- controlled by pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β[17].

Proteins that participate in the process of transforming a stable plaque to a vulnerable plaque are potential markers now being investigated [20]. One of these proteins is pregnancy-associated plasma protein A (PAPP-A) that has been believed as a biomarker of plaque rupture [5]. In the past several years, a plurality of the in vivo studies on PAPP-A have been focused on PAPP-A's function, rather than its mechanism of action. One study attempted to conclude the effect of IGF-II and PAPP-A interaction on somatic growth [20]. PAPP-A's actions in growth and development are completely caused by increased IGF bioavailability [18].

PAPP-A is a zinc-binding metalloproteinase and its known substrates in humans are insulin-like growth factor binding proteins (IGFBP)-4 and -5 [11,12]. The biological activities of insulin-like growth factors (IGFs) are controlled by six IGFBPs [13]. The gap of IGFBP-4 and -5 by PAPP-A increases free bioactive IGFs for interactions with IGF receptors [14, 15]. Pregnancy-associated plasma protein (PAPP)-A is a macromolecular glycoprotein discovered in the blood of pregnant women by Lin et al. The analysis of cloned cDNA has displaied that the PAPP-A subunit is a 1547-residue polypeptide [16].PAPP-A contains a putative Zn motif, HEIGHSLYH (amino acids 482– 492), which is basically identical to the active-site Zn motif of the matrix metalloproteases and very similar to those of other metalloproteases [2]. The circulating form of PAPP-A is a heterotetrameric complex collected of two 200- to 250-kDa PAPP-A subunits disulfide-bridged to two 50- to 90-kDa molecules of the pro form of the eosinophil major basic protein (proMBP)[3].

The interaction between heparin and PAPP-A led to a made easy purification scheme for PAPP-A, and using absorbed polyclonal antibodies this formed the basis of the first sensitive and specific PAPP-A radio immunoassay (RIA) [9]. Afterward, the rapid accumulation of data on PAPP-A in early pregnancy and its complications led to the introduction of serum PAPP-A as a essential biochemical marker in first trimester screening of Down syndrome [7]. PAPP-A is sometimes included among the battery of tests performed in the first trimester screening for fetal Down syndrome [4, 6]. PAPP-A has been usually measured during pregnancy in maternal blood for the fetal diagnosis of Down syndrome. During pregnancy, PAPP-A is produced by placental tissue, reaching serum concentrations ranging from 985–3655 mIU/l (95th interval) [19]. However, circulating concentrations of PAPP-A were later shown to be existing in lower concentrations (3.8–46.6 mIU/l) in both men and non-pregnant women [3].

Potential sources of confusing factors include definition of monoclonal antibodies (Mab), the presence of cross-binding antibodies in serum samples and the effects of dilution steps [20]. In contrast to the original RIA, most of the new assays can discover PAPP-A in serum of non-pregnant women and men [21, 22], and frequently at concentrations well above the detection threshold of the original RIA[7].

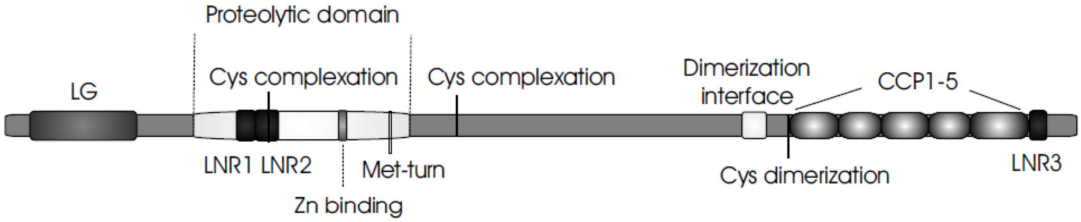

Figure 1. The structure of PAPP-A polypeptide with the practical modules and important amino acids. (CCP1-5, the complement control protein modules 1-5 involved in surface binding; LNR1-3, Lin-Notch repeats important in substrate recognition and activity; LG, laminin G-like module of unknown function)[15]

EXPERIMENTAL SECTION

Plasma samples

The reference interval for PAPP-A was based on serum samples from 40 mice obtained from University of Associated -Medical Sciences of sabzevar. Serum samples from five types of mice (type1;Healthy treated with *Anethum graveolens* 750 mg, type 2; Epileptic treated with *Anethum graveolens* 750 mg, type 3; Epileptic treated with *Anethum graveolens* 500 mg, type 4;Epileptic treated with *Anethum graveolens* 250 mg, type 5; Healthy treated with Valproic acid and *Anethum graveolens* 250 mg). All sera were stored at −20 °C until analysis.

General ELISA procedure

plasma were collected using citrate as an anticoagulant. All ELISA procedures were performed using microtiter plates . Coating with purified monoclonal antibodies, purified PAPP-A or polyclonal anti-PAPP-A (antibody targets conformational epitope rather than linear epitop). Antibodies was performed at 25 °C .The optical density at 450 nm was read on a microtiter plate reader (Biotek, powerwave XS2) and analyses performed using "Microplate Manager" program .

PAPP-A assay

PAPP-A was assessed immunochemically, using the TRACE method (time resolved amplified cryptate emission), based on nonradiating energy transfer. Commercial kit CUSABIO-PAPP-A (Brahms, Germany) was used for determination of the PAPP-A level in the maternal serum. The results are expressed in ng/ml.

RESULTS AND DISCUSSION

In healthy pregnant mice, PAPP-A levels in treated with *Anethum graveolens* of were increased compared to group control. Epileptic mice had increased serum levels of PAPP A. Correlation analysis showed significant relationship between PAPP-A and treated with *Anethum graveolens* in the mice. According to statistical analyses, the equal group variance was accepted via the Leven's test.

($F(4,12) = 1.490$, $P=0.266$)

Table 1: PAPP-A levels in treated with *Anethum graveolens*

	Descriptive Statistics	
Dependent Variable	**mean**	**Std. Deviation**
2	4.768^{abcd}	37.5304
1	6.6356^{abcd}	117.7275
4	6.8361^{abcde}	39.0763
5	7.3954^{abcde}	9.05733
3	8.0409^{ecd}	108.7624

a ; Epileptic treated with Anethum graveolens 750 mg.b ; Healthy treated with Valproic acid and Anethum graveolens 250 mg.c ; Healthy treated with Anethum graveolens 750 mgd ; Epileptic treated with Anethum graveolens 500 mg.e ; Epileptic treated with Anethum graveolens 250 mg

Results of biochemical parameters are expressed as mean±standard deviation (SD). Statistical analysis of group differences was performed by univariate (analysis of variance) followed by post test analysis. Concerning evaluation of PAPP-A, the significances of differences as well as those in the mice treated with dill distribution were tested using the Leven's test. Association between parameters was determined by using Tukey's test. The results were considered as statistically significant at p=0.05.

The mentioned test showed that there was a significant difference in the average amount of protein between the groups; thus, there is a difference between the 'groups'. The differences are as follows: The group of epileptic rats treated with dill 750 mg comparing to the group of epileptic rats treated with dill 500 mg .The group of epileptic rats treated with dill 500 mg comparing to the group of epileptic rats treated with dill 750 mg .

Acknowledgments

We are very grateful to Dr. A. Ghodsi (Department of mathematics, Hakim Sabzevari University)and Faculty of Sabzevar University of Medical Sciences for their constructive cooperation during this project.

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