

Anti-inflammatory and analgesic effects of egg yolk: a comparison between organic and machine made

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Abstract. – OBJECTIVES: Yolk composition may be influenced by the bird's dietary intake. Quality of egg yolk from different sources is also varied. Especially egg yolk from organic sources, those prepared by the hen and duck were naturally fed. Traditional medicine users in north of Iran believe the oil extracted by direct heat from egg yolk has analgesic activity. In this work anti-inflammatory and antinociceptive activities of organic and machine made hen yolk and duck yolk were evaluated.

MATERIALS AND METHODS: Three various sources of egg yolk (organic hens, duck and machine made) were chosen. Each yolk was extracted by two methods, solvent extraction (hexane) and direct heating. In order to investigate the analgesic and anti-inflammatory activity, carageenan, hot plate and writhing tests were carried out.

RESULTS: The organic hen and duck oil extracted by direct heat method showed the highest activity compared to other types. Organic sources may contain some special components which are not existed in machine made eggs. Oil samples obtained by direct heat from three different kinds of egg yolk showed significant anti-inflammatory activity at 100-300 mg/kg compared to controls ($p < 0.001$).

All samples indicated analgesic properties at all doses in writhing test. The highest activity was seen at 300 mg/kg of organic hen egg yolk oil prepared by heat (HO) and hexane extract of duck egg yolk (ED) which was similar to 5 mg/kg morphine ($p > 0.001$). In hot plate test all of the oil samples did not show significant difference with controls. Hexane extract of organic egg yolk (EO) (300 mg/kg) and ED (200 and 300 mg/kg) showed significant analgesic activity in hot plate test.

CONCLUSIONS: This study confirms anti-inflammatory and analgesic scheme of yolk. We can also claim environmental and dietary condition is very important in yolk contents. In order to improve life quality of human beings and bet-

ter efficiency for egg yolk components we suggest considering the way of feeding for poultry. We can propose organic yolk oil prepared by direct heat as an alternative remedy for inflammatory joint conditions.

Key Words:

Antiinflammatory activity, Analgesic effect, Egg yolk, Carageenan, Hot plate, Writhing test.

Introduction

Yolk represents a major source of active principles usable in medical, pharmaceutical, cosmetic, nutritional and biotechnological industries¹. The main components of yolk are lipid and protein but minerals and vitamins². One of the health benefit aspect of eggs is their contribution to the diet as a source of choline. Choline is a key component of many fat-containing structures in cell membranes, whose flexibility and integrity depend on adequate supplies of choline. Eggs' choline also reduces inflammation. People whose diets supplied the highest average intake of choline (found in egg yolk and soybeans), and its metabolite betaine (found naturally in vegetables such as beets and spinach), have levels of inflammatory markers at least 20% lower than subjects with the lowest average intakes^{3,4}. Each of these markers has been linked to a wide range of conditions including heart disease, osteoporosis, cognitive decline and Alzheimer's, and type-2 diabetes. Eating eggs may help lower risk of a heart attack or stroke by helping to prevent blood clots. Proteins in egg yolk are not only potent inhibitors of human platelet aggregation, but also prolong the time it takes for fibrinogen, a protein present in blood, to be converted into fibrin⁵. Lutein, a carotenoid thought to help prevent age-related mac-

ular degeneration and cataracts, may be found in even higher amounts in eggs than in green vegetables. Natural lutein esters found in eggs are as or even more bioavailable as the forms of the nutrient offered in purified lutein products⁶. Zeaxanthin existing in yolk may reduce the risk of developing age-related macular degeneration (AMD)⁷. *Gamma-linolenic acid* (GLA) is an *omega-6* fatty acid that is naturally present in egg yolk. Human and animal studies have confirmed the anti-inflammatory properties of GLA. Although its exact mechanism of action is not well understood, GLA acts in several ways to exert its effects, including the modulation of eicosanoids (prostaglandins, leukotrienes) and cytokines, and by regulating genes that affect apoptosis and cell growth⁸⁻¹⁰. Palmitoyl ethanolamide (PEA) is an endogenous cannabinoid found in brain, liver, and other mammalian tissues¹¹. PEA has also been isolated from egg yolk. PEA and its analogs found to have anti-anaphylactic and anti-inflammatory activities *in vitro*^{12,13}.

The hen and duck egg yolks obtained from village breeders extensively used as an anti-inflammatory and analgesic in northern parts of Iran. The breeders believe the eggs of organic hens and ducks contains more useful ingredients than machine made ones. With all above information about egg yolk we decided to plan a research to investigate yolk anti-inflammatory and analgesic activities in laboratory animals.

Materials and Methods

Three different kinds of eggs, natural organic hen eggs (the hens which were bred by local farmers in a natural environment) (O), machine made hen eggs (M) and duck eggs (D) were prepared from the local farmers and food shops. Any necessary solvents and materials were purchased from Fluka (Fluka Chemie GmbH CH-9471, Buchs, USA) and Merck companies (Darmstadt, Germany). Diclofenac, Morphine (M) and olive oil (O) were used as positive and negative standards in the tests.

Preparation of Yolk Oil and Extract

Egg yolks were prepared from fresh eggs. The shelling of the eggs and the separation of the yolk from the albumen were performed manually. Yolk of each egg kind was directly heated about 4 hours gently and filtered by a suitable cloth filter. The filtrate was used as yolk oil prepared by direct heat in the study (HO: organic

hen egg yolk oil prepared by Heat, HM: machine made hen egg yolk oil, HD: duck egg yolk oil). Also hexane extract of each kind of egg yolk was prepared by solving the yolks in hexane and stirring for 30 min. After separation of hexane phase and removing the solvent, yolk extract was obtained (EO, EM, and ED for hexane extracts of each kinds of egg yolk).

Animals

Swiss albino mice (weighing 25-30 g) of either sex, 6-7 weeks of age and bred in Animal House of Mazarandani University of Medical Sciences, Sari, Iran, were used in hot plate and writhing tests. The animals were housed under standard laboratory conditions with food and water provided *ad libitum*. Six mice were used in each experiment. Wistar strain of albino rats (200-250 g) of either sex were used for this carrageenan experiment. The animals were housed in standard metal cages and provided with food and water *ad libitum*. 10 animals were used in each experiment. All the experimental procedures were conducted in accordance with the NIH guidelines of the Care and Use of Laboratory Animals.

Writhing Test

Intraperitoneal (i.p.) injection of 0.6% acetic acid (10 ml/kg) induced abdominal constriction half an hour after the samples administration. Control group was treated by olive oil (10 ml/kg) and the extracts of different yolk (100, 200, 300 mg/kg, i.p.) were administered separately to the animals for the tests. In the reference group diclofenac (50 mg/kg, i.p.), a nonsteroidal anti-inflammatory agent, and morphine (5 mg/kg), an opioid analgesic used as reference drugs for comparison. The number of abdominal constrictions was counted between 8 and 20 min after acetic acid injection¹⁴⁻¹⁶.

Hot Plate Test

The extracts were administered at 100, 200, 300 mg/kg respectively. The mice were placed on a hot plate set at $55 \pm 0.5^\circ\text{C}$. The endpoint was licking or kicking of fore or hind paws. Mice scoring below 12 over 18 s in the preset were rejected. An arbitrary cut-off time of 45 s was adopted. Morphine (10 mg/kg) used as reference drug¹⁴⁻¹⁶.

Carrageenan induced Paw Edema

Edema was induced by subcutaneous injection of 0.1 ml of 1% freshly prepared suspension of

carrageenan into the hind paws of the rats. Following the administration of carrageenan, the volume of the injected and counter-lateral paws was measured hourly for 10 hours (h) using a plethysmometer. The test groups were administered with yolk extract (100, 200 and 300 mg/kg, i.p.) or a standard drug, diclofenac 100 mg/kg, 30 min before administration of carrageenan. The control group received only the olive oil¹⁷⁻¹⁸.

Results

All oil samples obtained by direct heat from three different kinds of egg yolk (HO, HM and HD) showed significant anti-inflammatory activity at 100, 200 and 300 mg/kg compared to negative standard (olive oil) ($p < 0.001$) (Figure 1). Hexane extracted oils did not give significant difference to olive oil as a negative standard at lower doses but it was significant at doses more than 100 nmg/kg for EO, EM and specially ED ($p < 0.001$). Activity in this test was similar to diclofenac anti-inflammatory effect at 100 nmg/kg. HO and EO showed the highest notable activity even more than diclofenac ($p < 0.001$). It seems the oils obtained from direct heat contains have more anti-inflammatory components than solvent extracted ones.

All samples indicated analgesic properties at all doses in writhing test. The highest activity was seen at 300 mg/kg of HO and ED which was similar to 5 mg/kg morphine ($p > 0.001$) (Figure 1). More than 91% inhibition of nociception was obtained by HO as seen by morphine. Analgesic action of organic yolk is much more than machine made hen yolks.

In hot plate test all of the oil samples did not show significant difference with controls at 15 min. The result of hot plate test after 30 minutes showed HO (300 mg/kg) and ED (200 and 300 mg/kg) had notable activity compared to morphine at 10 mg/kg. EO (300 mg/kg) and ED (200 and 300 mg/kg) showed significant analgesic activity in hot plate test (Table I). At 60 min all samples had good analgesic property like morphine. The highest analgesic activity was observed with 300 mg/kg of the samples at 45 min as morphine (10 mg/kg).

Discussion

People believe that organic egg yolk has better quality and food values. Yolk contains important proteins and fats such as choline and GLA which play critical roles in human health^{3,10}. These components have values in treating of several

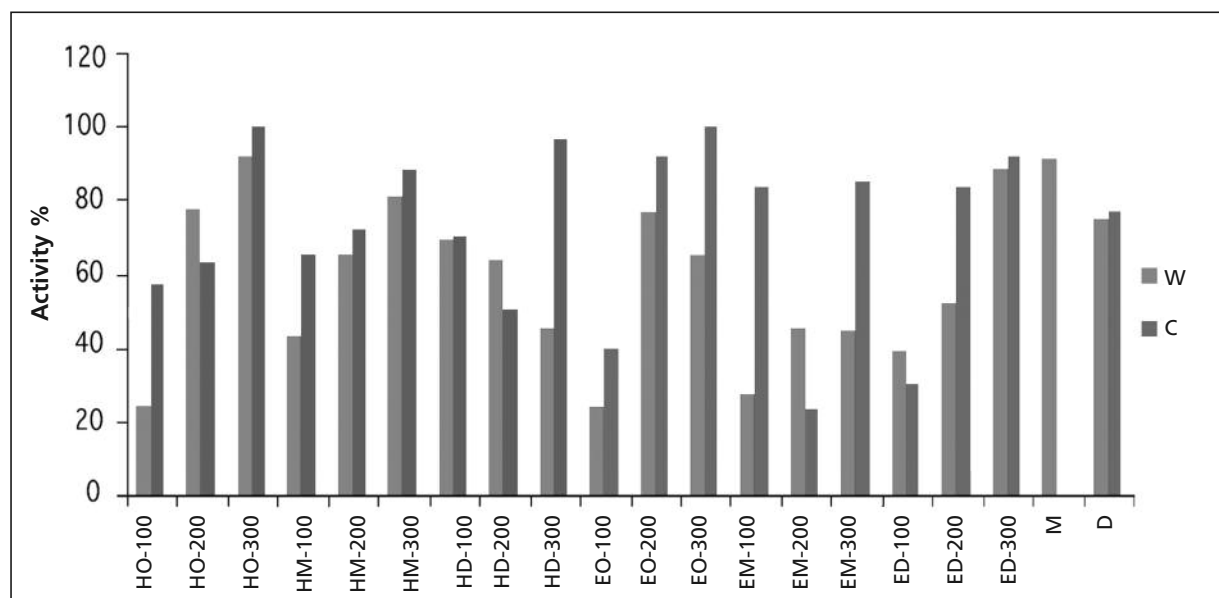


Figure 1. Pharmacological activity of different egg yolk oil samples in writhing (W) and carrageenan (C) tests. Each column represents the Mean \pm SD of 10 observations. (HO: organic hen egg yolk oil prepared by heat; HM: machine made hen egg oil prepared by direct heat; HD: duck egg oil by heat; EO: hexane extracted organic hen egg yolk oil; EM and ED for hexane extracts of each kind of machine- made and duck egg yolk; M: Morphine, D: Diclofenac).

Table I. Antinociceptive activity of different egg yolk oil samples in Hot plate test.

	Dose (mg/kg)	Time (min)				
		0	15	30	45	60
HO	100	7.25 ± 0.24	10.10 ± 1.50	11.20 ± 0.56**	9.50 ± 0.89	8.00 ± 1.40
	200	6.35 ± 0.85	10.25 ± 1.20	8.43 ± 1.16	8.24 ± 1.02	7.00 ± 0.91
	300	7.00 ± 0.38	10.75 ± 1.66*	12.38 ± 0.72***	11.25 ± 0.48***	10.70 ± 1.43**
HM	100	6.20 ± 0.15	7.95 ± 0.13	8.40 ± 0.24	7.80 ± 0.13	7.20 ± 0.11
	200	5.73 ± 0.25	8.73 ± 0.40	9.60 ± 0.19	7.50 ± 0.18	7.20 ± 0.45
	300	6.63 ± 0.47	8.25 ± 0.63	9.50 ± 0.70	9.33 ± 0.78	8.80 ± 0.87
HD	100	6.50 ± 0.12	7.10 ± 0.90	7.40 ± 0.82	6.80 ± 0.07	6.90 ± 0.46
	200	5.73 ± 0.25	8.73 ± 0.40	9.60 ± 0.19	7.55 ± 0.45	7.20 ± 0.18
	300	6.63 ± 0.43	9.38 ± 0.55	11.30 ± 1.10**	8.88 ± 1.30	7.50 ± 0.73
EO	100	7.73 ± 0.39	8.75 ± 0.75	10.40 ± 0.48*	9.88 ± 0.13*	9.00 ± 0.99
	200	6.63 ± 0.55	10.50 ± 0.65*	11.10 ± 0.49**	11.10 ± 1.50***	10.20 ± 1.70*
	300	7.00 ± 0.20	9.05 ± 0.66	10.98 ± 0.72**	12.50 ± 0.35***	9.25 ± 0.72
EM	100	7.15 ± 1.33	8.75 ± 0.85	9.9 ± 0.75	9.5 ± 0.78	8.75 ± 0.98
	200	7.35 ± 0.58	9.10 ± 2.10	11.20 ± 0.92	10.50 ± 0.75**	9.60 ± 1.40
	300	7.38 ± 0.63	9.50 ± 1.80	11.13 ± 0.83**	11.40 ± 0.88***	9.10 ± 0.66
ED	100	7.48 ± 0.50	9.72 ± 0.86	11.15 ± 0.60**	11.90 ± 1.23***	9.80 ± 1.60*
	200	7.73 ± 0.17	9.00 ± 0.29	13.25 ± 1.40***	12.20 ± 0.86***	10.7 ± 1.50**
	300	6.63 ± 0.30	10.40 ± 0.35	11.70 ± 1.10***	14.5 ± 0.35***	10.25 ± 2.00*
M	10	7.80 ± 0.20	9.80 ± 0.66	15.20 ± 0.14***	15.30 ± 0.75***	10.2 ± 0.83*

*** $p < 0.001$, ** $p < 0.01$ respect to control. For abbreviations see Figure 1

disorders like arthritis and cataract^{3,6}. Studies showed concentration and profile of carotenoids in the egg yolk are dependent to a large extent on the dietary intake of these components by birds¹⁹⁻²¹. It was suggested that the potential effect of environmental conditions on the concentration and profile of yolk dietary antioxidants should not be ignored when analyzing yolk composition in different bird species²². Carageenan test is one of the most sensitive methods for non steroidal anti-inflammatory drugs evaluation. Anti-inflammatory response was calculated after three hours of injection. All oil samples obtained by direct heat from three different kinds of egg yolk (HO, HM and HD) showed significant anti-inflammatory activity at 100-300 mg/kg compared to negative standard (olive oil) ($p < 0.001$) (Figure 1). Hexane extracted oils did not give significant difference to olive oil but it was significant at doses more than 100 mg/kg for EO, EM and specially ED ($p < 0.001$). HO and EO showed the highest notable activity even more than diclofenac ($p < 0.001$). It seems the oils obtained from direct heat contains more anti-inflammatory components than solvent extracted ones.

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This study confirms anti-inflammatory and analgesic scheme of yolk. Egg yolk obtained from organic and duck has more activity than machine made ones as compared to the other study²¹. We can also claim environmental and dietary condition is very important in yolk contents. Village breeders give chance to their poultry to use natural environment and food to grow up. In order to improve life quality of human beings and better efficiency for egg yolk components we suggest considering the way of feeding for poultry. As some people use yolk oil for

arthritis like pain and the confirmation of anti-inflammatory and analgesic activity of yolk oil in this study we suggest organic yolk oil prepared by direct heat as an alternative remedy for inflammatory joint conditions.

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