

# In Vitro Anti-Inflammatory Activities of *Coturnix japonica* Egg Components and Poultry Feed

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## Abstract

This study was designed to evaluate the in vitro anti-inflammatory activities of acidified methanolic (80%) extracts of *Coturnix japonica* egg components-yolk and albumen, and ascertain the contribution of the quail egg "layers" dietary feed to the pharmacological potency of the egg. The anti-inflammatory activities were evaluated by stabilization of erythrocyte membranes and inhibition of denaturation of albumin. The results revealed that yolk extract exhibited significant, potent and appreciable anti-inflammatory activity than the albumen and poultry feed which were concentration-dependent.

**Keywords:** Anti-inflammatory, Antioxidant, Membrane stability, Ibuprofen, Denaturation, *C. japonica* egg yolk and albumen.

## INTRODUCTION

The large generation of free radicals, particularly reactive oxygen species and their high activity play important roles in the progression of a great number of pathological disorders like inflammation, atherosclerosis, stroke, heart disease, diabetes mellitus, multiple sclerosis, cancer, Parkinson's and Alzheimer's diseases (Aina and Oyedapo, 2013; Mensor *et al.*, 2001; Ozgen *et al.*, 2006). The advent of stress in the biosystem is envisaged to release corticosterone that is synthesized in response to adrenocorticotrophic hormone, stimulating the circulation of high-energy compounds such as glucose, free amino acids, and free fatty acids, initiating cellular proliferation (Ibukun and Oladipo, 2016) leading to these disorders, which are characterized by inflammation.

Inflammation is a complex process, which is frequently associated with pain and involves occurrences such as: the increase of vascular permeability, increase of protein denaturation and membrane alteration (Leelaprakash and Mohan Dass, 2011). Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues. Chronic inflammation is characterized by malignant growth. When tissue cells become injured they released kinins, prostroglandins and histamine. These work collectively to cause increased vasodilation (widening of blood capillaries) and permeability of the capillaries leading to increased blood flow to the injured sites. These substances also act as chemical messengers that attract some of the body's natural defense cells a mechanism known as chemotaxis.

Quail egg had been reported as viable remedy for treating sickle cell anemia and diabetes in Nigeria. This study was conducted to give definition to the activities of the egg components and investigate the contributing effect of the feed to the egg activities against cellular proliferation by investigating the in-vitro anti-inflammatory activities of *Coturnix japonica* egg yolk and albumen.

## METHODOLOGY

Reagents and Chemicals used in this experiment were obtained from different sources such as British Drug House (BDH) and Sigma limited and were all of good analytical grades. All the solutions, buffers and reagents were prepared using glass distilled water. Ibuprofen was purchased from a registered pharmaceutical store in Akure, Nigeria.

Extraction of bioactive compounds involved (Ibukun and Oladipo, 2016); approximately 100g of freeze-dried egg yolk was soaked in 500 mL of 80% methanol (80:20, vol/vol) adjusted to pH 1.5 with 1 M HCl. Albumen extract was gotten by deproteination using cyanogen bromide, formic acid and then urea for complete denaturation of the albumen and isolation of anti-inflammatory amino acids. The mixture was then mixed thoroughly using a vortex mixer for 2 min and centrifuged at 6000g for 10 min at 4°C. The supernatant was collected, freeze-dried and reconstituted with distilled water.

The evaluation of *in vitro* anti-inflammatory activity is a quantitative analysis, all assays were performed in triplicate. Each test performed involved various concentrations (100-500µg/ml) of the extracts and standard-ibuprofen, prepared using the serial dilution method.

### Inhibition of Denaturation of Albumen Activity Assay

Different concentrations of the extracts were assayed for the ability to inhibit denaturation of albumen following the method of Aina and Oyedapo (2013) with slight modifications. The reaction mixtures contained 0.5 ml (1.5 mg/ml albumen) and different concentrations of the extracts, followed by incubation at 37 °C for 20min. The reaction mixtures were heated at 57 °C for 3 min and 2.5 ml of 0.5 M phosphate buffer, pH 6.3 was added. From each of the reaction mixtures, 1 ml was pipetted into clean dried test tubes in triplicates followed by the addition

of Copper-Alkaline reagent (1 ml) and 1.0 ml of Folin-Ciocateu's Phenol reagent (1:10). The reaction mixtures were incubated at 55 °C for 10 min. The tubes were cooled and the absorbance as read at 650 nm against reagent blank.

The quantity of protein left was calculated using the expression:

$$\frac{(\text{Abs of sample}-\text{Abs of blank})}{(\text{Abs of standard}-\text{Abs of blank})} \times 100$$

The percentage inhibition was calculated using the expression,

$$\frac{\text{Quantity of protein left}}{\text{Total protein}} \times 100$$

### Membrane Stabilizing Activity Assay

The membrane stabilizing activity assay method was based on the procedure described by Oyedapo *et al.* (2004) with little modification. The assay mixture consisted of hyposaline (1ml), 0.1M phosphate buffer, pH 7.4 (0.5 ml), varying concentrations of extracts (100- 500 µg/ml) and 0.5 ml of 2% (v/v) erythrocyte suspension in a total volume of 3 ml. The control was prepared as above without the drug while the drug control (3 ml) lacked erythrocyte suspension. The standard anti-inflammatory drug for the assay was ibuprofen. The reaction mixtures were incubated at 56° C for 30 min. The absorbance of the released haemoglobin was read at 560 nm against reagent blank. The percentage membrane stability was estimated using the expression:

Percentage membrane stability

$$100 - \frac{(\text{Abs of test drug}-\text{Abs of drug control})}{\text{Abs of blood control}} \times 100$$

The blood control represented 100% lysis.

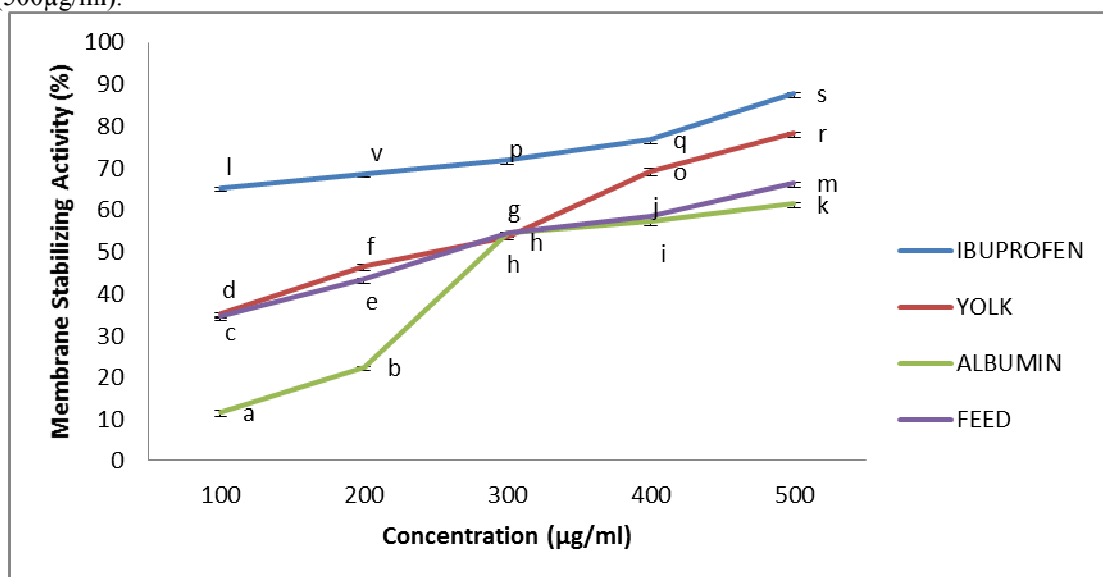
## RESULT

### Inhibition of Denaturation of Protein

The ability of the extracts and a known anti-inflammatory drug (ibuprofen) to inhibit denaturation of protein was shown in Fig. 1. The results revealed that there was inhibition at the different concentrations (100-500 µg/ml) for all the extracts. At 500 µg/ml, it was observed that yolk extract (84.68 ± 0.386) offered the highest protection compared to albumen extract (58.63±0.13) and competed favorably with ibuprofen (88.06±0.125).

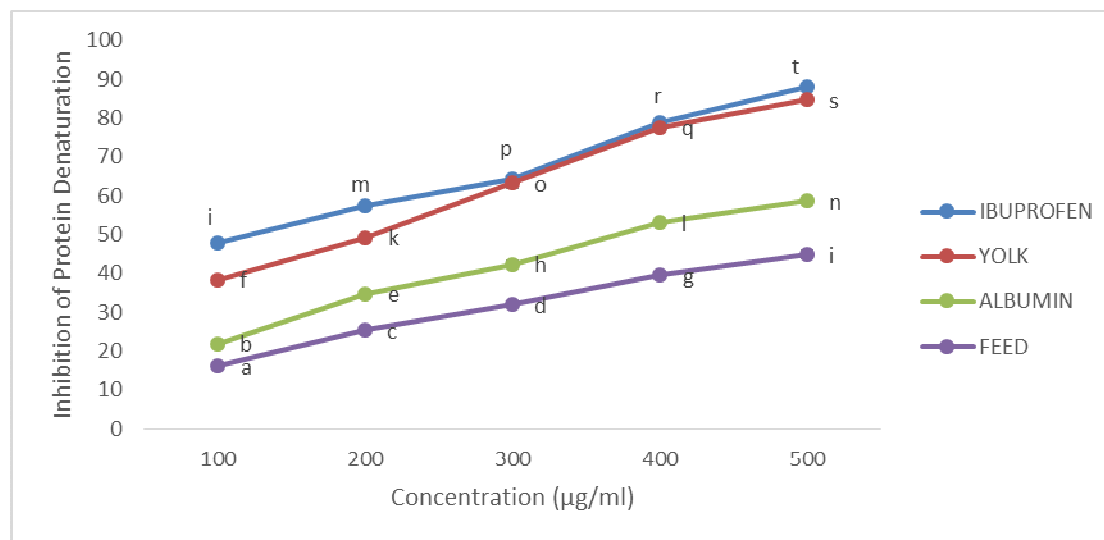
### RBC Membrane Stability Activity

The stabilizing effects of the extracts of *C. japonica* egg yolk and albumen on red blood cell membranes exposed to both heat and hypotonic lyses was shown in Fig. 2. *In vitro* assessment on membrane stability showed that extracts inhibited heat and hypotonic-induced lyses of red blood cells to varying degrees with yolk (78.42 ± 0.058%) offering the highest protection (at 500µg/ml) (P<0.05). The mode of protection exhibited by the fractions showed a favorable activity for the albumen (61.48±0.157%) compared to ibuprofen (87.8 ± 0.069%) (500µg/ml).



**Figure 1:** Inhibition of protein denaturation activities of extracts of yolk and albumen.

Values are expressed as mean ± standard deviation (n=3). Values with different superscript are significantly different (P<0.05).



**Figure 2:** Membrane stability activities of extracts of yolk, albumen and feed.

Values are expressed as mean  $\pm$  standard deviation (n=3). Values with different superscript are significantly different (P<0.05).

## DISCUSSION

Ibuprofen was an effective non-steroidal anti-inflammatory drug but its toxicological profile evaluation in wistar albino rats established a significant disruption in hematological indices and biochemical markers of hepatic and renal functions at ibuprofen concentrations of 20 and 40 mg/kg/day administered orally (Aprioku *et al.*, 2014). The implication of Aprioku *et al.* (2014) study was that chronic use of ibuprofen could affect hepatic, renal and hematological functions in the rat; and duration of exposure may promote ibuprofen toxicity relative to dose.

The RBC membrane stabilization has been used as a method to study the in-vitro anti-inflammatory activity because the erythrocyte membrane is analogous to the lysosomal membrane (Leelaprakash and Mohan, 2011; Shenoy *et al.*, 2010; Gandhidasan *et al.*, 1991) and its stabilization implied that the extracts might well stabilize lysosomal membranes. Stabilization of lysosomal is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil, such as bacterial enzymes and proteases, which causes further tissue inflammation and damage upon extra cellular release. The lysosomal enzymes released during inflammation produce a various disorders. The extra cellular activity of these enzymes are said to be related to acute or chronic inflammation. The non-steroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane (Leelaprakash and Mohan, 2011; Rajendran and Lakshmi, 2008)

This experiment had indeed shown that the extracts exhibited lysosomal membrane stabilizing activities. However, the deductions from these results was that the activities of the yolk extract are significantly different from the albumen and poultry feed. Previous report showed that dietary sinapic acid (4-hydroxy-3,5-dimethoxycinnamic acid) affects egg quality characteristics and sinapic acid was detected in egg yolk (Johnson *et al.*, 2008), this might be a contributing factor the anti-inflammatory activity of the yolk, however the phenolics composition of the layers feed ration extract could be said to be responsible for its lysosomal membrane stabilizing activity. Which is a possibility that the bioactive components of the feed could contribute to the bioactivity of the egg components, especially the yolk.

Studies had shown that the antioxidant activities of free amino acids in the yolk, and credited the structural resemblance of tyroxine to p-coumaric acid, a phenolic acid with high antioxidant activity and tryptophan whose antioxidant property was due to its indole group (Galisteo and Herraiz, 2004), apart from being an essential amino acid and a precursor of neuromediators such as serotonin, tryptophan may exert some important biological functions through its high antioxidant activity. Although, the study was conducted on hen egg but to the best of our knowledge no study had been done on the study of tyroxine and tryptophan in quail egg yolk. The denaturation of the quail egg albumen was supposed to expose the R-groups of its amino acids for their several functional purposes.

The denaturation of proteins is a cause of inflammation and rheumatoid arthritis, this evaluation was used to ascertain the properties of the extracts in stabilizing the protein from the denaturation process. This study revealed competitive activities of the yolk and ibuprofen, though ibuprofen had significantly higher activity than the yolk (P<0.05). The most essential amino acid (EAA) of quail egg albumen were leucine, valine and lysine (Tanasorn *et al.*, 2013) which could be responsible for the anti-inflammatory activity.

## CONCLUSION

This study revealed that the extracts of the yolk and albumen have anti-inflammatory activities which are significantly different from the “layers” poultry feed. Ibuprofen had been used as a standard anti-inflammatory drug, however, the multi-nutritional quail egg could be used as supplement for anti-inflammatory as one of the many dietary importance. The activities of the extracts of the egg components, had contributed to the knowledge and reason behind the acceptability of quail egg as the best pharmacological poultry products.

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