

The effect of aqueous preparation of *Allium Cepa* (onion) and *Allium Sativa* (garlic) on erythrocyte osmotic fragility in wistar rats: *in vivo* and *in vitro* studies

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Summary: *Allium cepa* (onion) and *Allium sativa* (garlic) are bulbous herbs used as food item, spice and medicine in different parts of the world. The effects of onion and garlic on the osmotic fragility of red blood cells in albino rats were assessed *in vivo* and *in vitro*. In the *in vivo* studies, five albino rats weighing between 150 – 200g composed each of three study groups. Group A were administered 150mg/Kg body weight aqueous onion preparation; Group B 75mg/Kg body weight aqueous onion and 75mg/Kg body weight garlic preparations; and Group C served as the control and were administered distilled water. The treatment regimens were orally administered thrice a week, for a period of four weeks by gavages. The *in vitro* erythrocyte osmotic fragility was also evaluated in 12 wistar rats that were not pre-treated with either onion alone or onion and garlic. The animals were divided into three groups. Blood samples from group A rats were treated with 150mg onion while blood from group B rats was treated with 75mg onion and 75mg garlic extracts. Group C served as the control and were treated with normal saline and osmotic fragility assays were carried out. The degree of haemolysis was greater ($P<0.05$) in the treatment group compared to control and the percentage haemolysis was greater in blood samples with onion and garlic compared to the onion group ($P<0.05$). The same observation was made in the *in vitro* study, but the degree of haemolysis was significantly higher ($P<0.05$) in *in vitro* than the *in vivo* experiments. It is concluded that onion and garlic increase the osmotic fragility of red blood cells in albino rats.

Keywords: *Allium cepa* (onion), *Allium sativa* (garlic), Osmotic fragility, Red blood cells, Membrane stability, % Haemolysis.

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Manuscript Accepted: January, 2012

INTRODUCTION

Allium cepa (onion) and *Allium sativa* (garlic) are widely used as flavouring vegetables for their aroma and taste in various types of food worldwide. Therapeutic and medicinal values are also attached to these *Alliums*, most of which are scientifically validated (Amagase *et al*, 2001; Huddein *et al*, 2007). In the Northern part of Nigeria, onion is widely cultivated and consumed in cooked or raw form. Consumption of raw onions is widespread as it is used with 'suya' (berbecue) to add to its taste and aroma, while the majority of the people that consume raw garlic do so for therapeutic reasons (Silagy and Neil, 1994; Umar *et al*, 1996; Dubravka and Ilona, 2003).

Allium cepa and *Allium sativa* were reported to have hypoglycaemic, antidiabetic (Eidi *et al*, 2006; Vishnu *et al*, 2009), antimicrobial (Adetumbi and Lau, 1983), anti-inflammatory, anti-carcinogenic,

hepato-protective (Hussein *et al*, 2007), antithrombotic (Steiner and Li, 2001), antihypertensive, and cardio-protective effects (Ernest, 1987; Singh and Singh, 2008) and as an antioxidant (Dubravka and Ilona, 2003). Phytochemical studies show that *Alliums* are a rich source of important organic compounds, which include steroid, saponins and flavonoids, and are characterized by a high content of organo-sulphur compounds that are well absorbed through the gastrointestinal tract and metabolized to highly reactive oxidants (Singh and Singh, 2008).

Chronic and unregulated intake of garlic and onion was reported to cause haemolytic anaemia in rats (Umar *et al*, 1996) and in dogs (Yamoto and Maeds, 1992). Acute toxicity studies reveal the LD₅₀ of *Allium cepa* to be 3000mg/Kg body weight (Shenoy *et al*, 2009) in rodents and *Allium* toxicosis is consistently noted in animals that consume more than 0.5% of their body weight in onions at one time.

Clinical signs of toxicity manifest following a single dose with pathology revealing intra- and extra-vascular haemolysis, Heinz body anaemia, haemoglobinaemia, methaemoglobinaemia and haemoglobinuria (Burrows, 2001).

The presence of peroxidizable polyunsaturated fatty acids and malonyl dialdehyde in *Allium* impaired erythrocyte membrane stability (Wagner *et al* 1988; Mansour and Mansour, 2009). Other studies show that injection of *Allium* increased susceptibility of erythrocyte to oxidative stress and their destruction (Surai, 2001). On the other hand, erythrocytes are endowed with efficient antioxidants such as superoxide dismutase and antioxidant free radicals that counter the persistent effect of reactive oxygen species (Rai *et al*, 2009). In addition, antioxidants are also important components of *Allium* that could contribute to erythrocyte resistance to oxidative damage (Stajner and Varga, 2003). In view of all these findings, the present study aims to evaluate the behaviour of erythrocyte membrane subject to sub-toxic, acute repeated exposure to onion and garlic through erythrocyte osmotic fragility which have been used as an indirect assessment of oxidative stress (Chihurailaf *et al*, 2002). The results obtained from *in vivo* studies will also be compared with erythrocyte assay with onion and garlic. This will give a clear picture on the direct effect of *Allium* on the erythrocyte membrane when subjected to various concentrations of hypotonic saline solutions.

MATERIALS AND METHODS

Plant Materials: Onion and Garlic were purchased from the market and authenticated by plant taxonomists in the Department of Biological Sciences, University of Maiduguri, Nigeria.

Animals: Twenty-seven (27) adult albino rats weighing between 100 – 200g were obtained from the disease and germ free animal facility of the National Veterinary Research Institute (NVRI) Vom, Plateau State, Nigeria. The animals were kept in plastic cages at room temperature of 24±2 °C and less than 30% relative humidity under a 12 hour light-dark cycle. They were fed standard pellet food (Sanders SPEEC Feed PLC, Jos, Nigeria) and had access to water *ad libitum*.

Preparation of Extract: Onion was weighed; 3g was then pulverized and mixed in 20ml of distilled water. 1.5g onion and 1.5g garlic were also pulverized and mixed in 20ml of distilled water. The preparations served as stock, and were stored at 4°C.

Experimental Design: The animals were randomly selected into three groups of five rats each; appropriate concentrations were prepared from the stock solutions and administered to the animals thrice a week, (based on traditional medicinal usage and observed mode of onion consumption with roast meat) for a period of four weeks which corresponds with the occurrence of maximal anaemia in rats fed with onion and garlic (Umar *et al*, 1996). The route of administration was by oral gavages: Sub-toxic doses (5% body weight of experimental animals) were administered (OECD, 2008). Group A received 150mg/Kg body weight aqueous onion preparation, Group B 150mg/Kg of a mixture of onion and garlic and Group C served as control. At the end of the experimental period, the animals were sacrificed by isolating and transecting the jugular vein and 4ml of blood was collected into heparinized sample bottles. Heparin was chosen as an anticoagulant because it affects levels of ions to a lesser degree than EDTA (Baglin *et al*, 2006). In assay experiments, three groups of four rats each were used. These rats were not administered onion or onion and garlic. They were sacrificed by isolating and transecting the jugular vein and 4ml of whole blood was collected into heparinized sample bottles from each rat to prevent coagulation.

Determination of Erythrocyte Osmotic Fragility: The *in vitro* erythrocyte osmotic fragility was evaluated in all the rats in each group using the method described by Faulknet and King (1970) as modified using different amounts of sodium chloride (pH 7.4) from 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.9 and 1.0g/L of distilled water. Freshly obtained whole blood from each rat was pipetted (0.02ml), using a micropipette into the test tubes containing varying concentrations of sodium chloride. The content was mixed by inverting the tube and incubated for thirty minutes at 36°C - 38°C. The test tubes were then centrifuged at 1500rpm for 10 minutes. The supernatants were transferred into glass curvettes and the absorbance of the supernatants were measured colorimetrically using a Corning Colorimeter 252 (Corning Ltd. ALSTEAD, Essex England) at 540nm wavelength. The percentage haemolysis for each sample was calculated and plotted against sodium chloride concentration.

Thus, Percentage haemolysis:

$$= \frac{\text{Optical Density of Test Solution}}{\text{Optical Density of Standard Solution}} \times 100$$

Assay: Erythrocyte osmotic fragility assay was carried out using the method described by Adalgisa *et*

al (2008). Whole blood samples from these animals were incubated with onion (150mg) for group A, onion and garlic (75mg each) for group B and normal saline for group C (control) for a period of 60mins at room temperature. These were gently mixed and centrifuged to separate plasma from RBCs. RBC aliquots from these samples were then mixed with graded concentrations (0.1-1.0%) buffered phosphate saline and also centrifuged at 1500 rpm for 10 minutes and the supernatants were subjected to measurement in a spectrophotometer at 540nm.

Statistical Analysis

Values expressed as mean ± SEM were subjected to one-way analysis of variance (ANOVA) and Tukey test using GraphPad Prism version 4.0 for windows from Graphpad software, San Diego, California, U.S.A (www.graphpad.com). Values of P <0.05 were considered significant.

RESULTS

In vivo

The mean fragility values after oral administration of onion, and onion and garlic for 4 weeks are presented in Fig.1. The osmotic fragility decreased as the concentration of NaCl increased in both treatment and control groups. However, the percentage decrease in osmotic fragility was greater in the control than treatment groups. The analysis of the result showed a significant decrease (P<0.05) in osmotic fragility of treated rats in the interval II (0.4 – 0.6%NaCl). Significant differences were observed at NaCl concentrations of 0.5 and 0.55% while in the interval

III (0.6 -0.9% NaCl), the increased osmotic fragility was significant (P<0.05) at all concentrations of NaCl studied in animals that received onion and garlic when compared with onion treated and control groups.

Assay

The means of the erythrocyte osmotic fragility at different saline concentrations obtained after treatment with onion and onion plus garlic in vitro are presented in figure 2. The percentage fragility also decreases as the concentration of NaCl increased in treatment and control groups. Analysis of the result showed a significant increase (P<0.05) of osmotic fragility of erythrocytes incubated with onion or a combination of onion and garlic at all isotonic interval concentrations of NaCl when compared with the control. Comparison of osmotic fragility curve observed in both studies showed that while the osmotic fragility decreased in the interval II (0.30 – 0.60% NaCl), the treatment groups of onion or onion and garlic showed complete haemolysis. In the interval III (0.60 – 0.90% NaCl) the haemolysis was significantly higher (P<0.001) at all concentrations of NaCl studied in treated rats.

Analyses of the result showed a significant increase (P<0.05) of osmotic fragility of erythrocyte incubated with either onion or a combination of onion and garlic at all isotonic interval concentration of NaCl when compared with the control. A higher degree of haemolysis was also recorded in the in vitro compared with the in vivo studies (Table 1). These differences were significant (P<0.05).

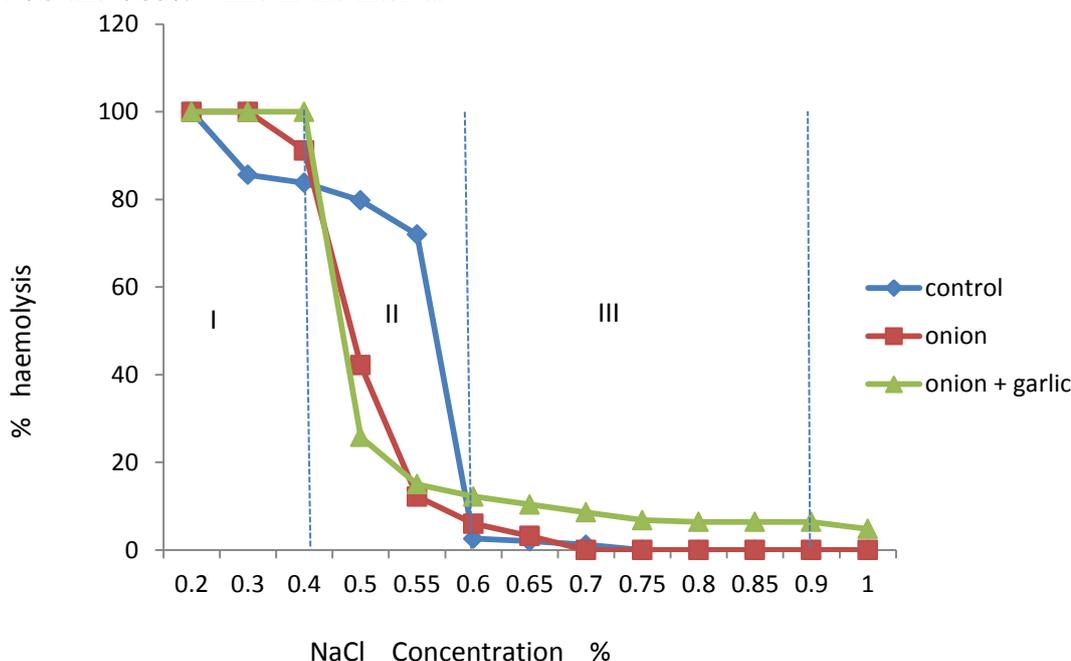


Fig.1. The effect of treatments on red cell osmotic fragility. Compared with the control, the treatments provide greater membrane stability in interval II and less in interval III.

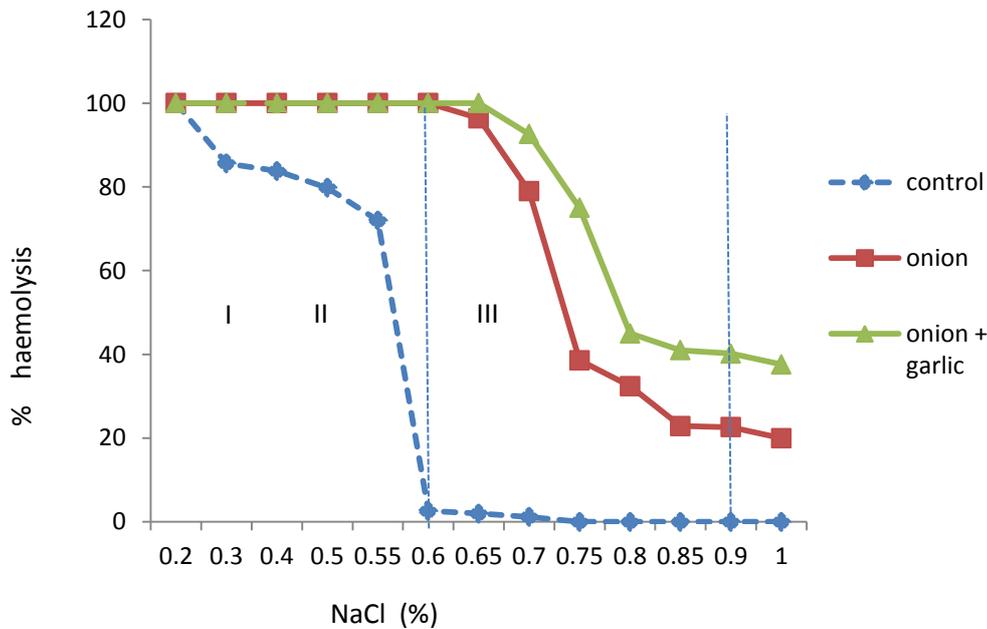


Fig.2. Mean Percentage Haemolysis in Erythrocytes Incubated with Onion Alone and with Garlic Preparations

Table 1. In Vitro Effects of Onion Alone and Onion plus Garlic on Osmotic Fragility of Rats (Mean ± SEM)

NaCl Conc. (%)	Mean ± SEM % Fragility		
	Control	Onion	Onion + Garlic
0.20	100.0± 0.00	100.0± 0.00	100.0± 0.00
0.30	85.6 ±1.78	100.0± 0.00	100.0± 0.00
0.40	83.8 ±1.88	100.0± 0.00	100.0± 0.00
0.50	79.8 ±2.87	100.0± 0.00	100.0± 0.00
0.55	72.0± 0.89	100.0±0.00	100.0± 0.00
0.60	2.6± 0.24	100.0± 0.00	100.0± 0.00
0.65	2.0± 1.05	96.4± 0.68	100.0± 0.00
0.70	1.2± 1.20	79.0± 0.45	92.6 ±1.66
0.75	0.0± 0.00	38.6± 0.60	75.0± 4.29
0.80	0.0± 0.00	32.4 ±0.24	45± 0.20
0.85	0.0± 0.00	2.3± 0.45	41± 0.45
0.90	0.0±0.00	25.8± 0.49	40.2± 0.20
1.00	0.0 ±0.00	20.0± 0.00	37.6 ±0.81

DISCUSSION

The data obtained from *in vivo* and *in vitro* osmotic fragility tests of rats treated with either garlic alone or onion plus garlic in this study indicate that *Allium cepa* and *Allium sativa* could alter the red cell membrane stability in isotonic solutions with NaCl content ranging from 0.1%-0.9% to which a small amount of fresh blood is added. Since erythrocyte osmotic fragility is used as a measure of the tensile

strength of the red cell membrane (Rai *et al*, 2009), the impairment of membrane stability seen as a result of treatment with onion and garlic could be attributed to some of the components of these alliums. This finding is in agreement with earlier reports on *Allium* species that they are readily absorbed through the gastrointestinal tract and metabolized to highly reactive oxidants (Amagase *et al*, 2001) and that cooking and spoilage of *Allium* species does not reduce their potential toxicity (Burrows *et al*,2001). It is therefore suggested that oxidative haemolysis might have occurred due to an increased concentration of oxidants in the erythrocyte as a result of exposure to *Allium* exceeding the capacity of the antioxidant metabolic pathways in the erythrocyte.

This observation was also supported by the presence of anaemia observed in rats fed with garlic (Umar *et al*, 1996) which may be attributed to lipid peroxidation as a toxic mechanism resulting in erythrocyte damage. Other reports indicate that erythrocyte membrane is highly vulnerable to lipid peroxidation because it increases the susceptibility to oxidative stress thereby enhancing free radical attack on the erythrocyte membrane resulting in their destruction (Urano, 1993; Surai, 2001).

Onion and garlic possess well defined antioxidant activity (Stainer and Varga, 2003) corollary with the presence of efficient antioxidant enzymatic systems such as superoxide dismutase, catalase and glutathione-S-transferase as well as antioxidant free radical scavengers in erythrocytes (Rai *et al*, 2009). These would have been expected to counter the effect of oxidative damage caused by *Allium*. It appears that

red cells could have been exposed to highly reactive oxidants in *Allium* than antioxidants naturally endowed in them, resulting in membrane damage. Other studies have shown that flavonoids inhibit peroxidation which could be helpful in stabilizing the integrity of red cell membrane against hypotonic lysis (Chaudhuri *et al*, 2007). However the flavonoid in *Allium* could not reduce the activity of peroxidase and other highly reactive oxidants.

Studies *in vitro* and *in vivo* on the effect of *Allium* showed that though osmotic fragility increases, the effect is more pronounced *in vitro*; suggesting that minimal quantity of reactive oxidants might have been inactivated in the gastrointestinal tract, probably by saponins.

The present study has shown the ability of *Allium* exposure both *in vivo* and *in vitro* to increase erythrocyte membrane fragility due to reactive oxidative damage to the erythrocyte membrane. This could be the result of lipid peroxidation when *Allium* is administered in raw form in albino rats. It is then suggested that ingesting raw allium should be monitored in people with blood diseases.

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