

EFFECT OF GARCINIA KOLA SEED ON PLASMA C-REACTIVE PROTEIN IN HUMAN SUBJECTS

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ABSTRACT

Garcinia kola seed (*Garcinia afzelii*; also called bitter kola seed) is a common Africa snack fruit from a species of flowering plant in the Guttiferae family. Over the years, Africa traditional medicine and ethno pharmacology have attached invaluable roles to Garcinia kola seed (GKS) as a potent anti-hepatotoxic, anti-diabetic, antioxidant and antilisterial. To consolidate these roles, we focus our study on the effect of GKS on C-reactive protein (CRP) in human subjects. Sixty students volunteers (n=60; 30 female & 30 male) from Faculty of Basic Medical Sciences, University of Ilorin, Nigeria, were used for this study. The screened volunteers were subjected to an hour rest for acclimatization. This was followed by collection of about 2.5 ml of blood through vene puncture. The subjects were then asked to ingest 100mg/kg of GKS and after 6 hours, another 2.5ml of blood sample was collected from each subject. The level of CRP was assay in the two sets of blood samples. The result shows 66.31% significant decrease in the mean level of basal CRP of the female ($p < 0.05$). Contrast to this, in male, there is 72.27% significant increase from the mean level of basal CRP ($p < 0.05$). In conclusion, this study shows GKS as a potent anti-inflammatory fruit for female subjects but not for male subjects. GKS anti-inflammatory effects are thus gender based. With these observations, it can be said to be more beneficial to female but not to male subjects. Lastly, the clinical practise of using CRP level in the blood to predict the pathogenesis of a disease, track of a disease progress or measure of effectiveness of a treatment will be misleading in habitual GKS consuming patients.

Keywords: Blood, C-reactive protein, Garcinia kola, human subjects

INTRODUCTION

Garcinia kola (*Garcinia afzelii*), commonly called bitter kola is one of species of flowering plant in the Guttiferae family. The plant bears medicinal nuts known as Garcinia kola seed (GKS), otherwise called bitter kola seed or bitter kola which over the years has been attached to some aspect of spiritual healing. GKS is usually egg in shape with tapering ends. Depending on the moisture content of GKS, it is about 3-4cm long and 1.4-2cm in circumference. It has a unique bitter taste with a slight sweetness when chewed (Iwu, 1993; Igbozulike and Aremu, 2009; Olajide and Adeyemi, 2011).

The value of GKS in Africa traditional medicine and as a local fruit taken regularly among Africa folks is inestimable. Tremendous efforts from various scientists reported GKS to be potent anti-hepatotoxic (Farombi *et al.*, 2009; Farombi and Owoeye, 2011; Nwokoeha *et al.*, 2011).

Antilisterial (Dambudzo and Anthony, 2012), anti-diabetic (Emeka *et al.*, 2012) antioxidant and anti-lipoperoxidative (Chiagoziem and Ebenezer, 2012; Ebenezer *et al.*, 2013).

Other out-standing benefits of GKS includes its ability to increased the activities of the enzymes lactate dehydrogenase and glucose-6-phosphate dehydrogenase in animal (Olajide and Adeniyi, 2011), its protective effects on reproductive toxicity induced by cadmium and gamma-irradiation in rats (Farombi *et al.*, 2012; Adaramoye *et al.*, 2012), its protective effects against heavy metal accumulation in the liver (Nwokocho *et al.*, 2011) and its preservative ability in preventing beer spoilage (Oguntola, 2009). In addition to this, GKS has been reported to have shown promising signs in fighting and slowing down multiplication of infectious diseases such as gonorrhoea, Ebola, HIV and other viral diseases (Oguntola, 2009; Adefule-Ositelu *et al.*, 2010).

Recent surge in evidences that linked chronic inflammation to major known disease processes has increased the interest of researchers in C - reactive protein (CRP) as an accepted marker of systemic inflammation (Ryuichi *et al.*, 2011). CRP is the member of the class of acute-phase reactants in the inflammatory processes occurring in the body. It is synthesized by the liver in response to markers or indicators released by fat cells and macrophages (Pepys, 2003; Lau *et al.*, 2005). CRP was the first pattern recognition receptor (PRR) to be identified that is believed to enhance phagocytosis of the microbes by binding to its phosphocholine (Mantovani *et al.*, 2008). Past studies showed that high-sensitivity C-reactive protein is a valuable precursor of the metabolic syndrome, type 2 diabetes and an important predictor of early stage cardiovascular disease (Ridker *et al.*, 1998; Ford, 2003; Tomiyama *et al.*, 2005; Anan *et al.*, 2010; Sabanayagam *et al.*, 2011; Wu *et al.*, 2011). The above fact is again buttressed by reports which claimed that individual with CRP > 10 mg/L is at highest risk of future vascular events in several cohorts that include middle-aged men and women, older men and women, and healthy middle-aged women (Muir *et al.*, 1999; Cushman *et al.*, 2005; Hamer *et al.*, 2009). Also, a study in year 2012 assumed that CRP values > 10mg/L indicates chronic inflammation in a large proportion of individuals; this is believe to be true particularly in people with known risk factors for chronic inflammation such as obesity and smoking (Ishii *et al.*, 2012). Apart from this, high sensitivity CRP has been tagged not only as an independent risk factor for coronary artery spasm development but also has been shown to interact with haemoglobin and platelet in woman with coronary artery spasm (Ming-Yow *et al.*, 2013). In another revelation, CRP is claimed to be associated with lipid responses to low-fat and high-polyunsaturated fat diets, and people with high CRP due to genetic variation had no increased risk of cardiovascular disease when compared to those with a normal or low CRP (Zacho *et al.*, 2008; St-Onge *et al.*, 2009).

METHOD

Sixty healthy students volunteers (n=60; 30 female & 30 male) from Faculty of Basic Medical Sciences, University of Ilorin, Nigeria, were used for this study. The female subjects are 17 - 23 years in age with an average weight of 62kg and height that range between 1.55 - 1.82 metres. The male subjects on the other hands are between 19 - 26 years in age with an average weight of 63kg and height that range between 1.71 - 1.86 metres. The sample size was limited to these few numbers since consumption of GKS is within a few cohorts. For instance, out of over 250 ethnic groups in Nigeria, GKS consumption is favoured by just few of these tribes. In addition to this, other studies where less than sixty subjects were used include Gangarosa *et al.*, 1989, Owoyele *et al.*, 2005 and Salim, 2012. The protocol for this study was well explained to the subjects and they gave their informed consent. The research was approved by the Departmental Ethic Committee and conducted in accordance with the institutional standards for human studies, these standards are similar to the international guide lines for human subject study. Subjects were subjected to one hour acclimatizing rest before GKS consumption and restricted movement was imposed after consumption of GKS.

As stated above, this study started with the subjects comfortably seated and rested for an hour. After this, about 2.5 ml of blood was collected separately from each volunteer into a lithium heparinised bottle through venepuncture using a separate syringe and needle. The level of CRP in these samples represents baseline and the control group.

The subjects were subsequently asked to ingest 100mg/kg of GKS obtained locally from a dealer in a local market of Ilorin, Nigeria. It was identified and confirmed at appropriate quarters to be the species *Garcinia afzelii*. After 6 hours of ingestion, another 2.5ml of blood sample was taken from each subject using the same method as above. The level of CRP in these samples represents the test group.

MEASUREMENTS OF CRP

Having separated the plasma from the blood cells by centrifuging the blood sample in a centrifuging machine, CRP concentrations in the plasma were analysed using an enhanced latex-agglutination turbidimetric immunoassay with human anti-CRP antibody (Sekisui Medical, Tokyo). The detection limit was 150 mg/L.

Measurements: Predictors of CRP alteration

At the beginning of this study, subjects were screened for predictors that could result into alteration of CRP as it was claimed in the literature review. These predictors include socio-demographic variables, health related variables and dietary inclination or habit (Ishii *et al.*, 2012; Buchwald *et al.*, 2013).

For socio-demographic variables predictor, the subjects used for this study were of the same race (Nigerian), have similar education background and level, collect relatively similar monthly allowance and have parents with closely related annual income.

On health related variables predictor, volunteers were asked about their doctors-diagnosed health related problem particularly those that had been associated with CRP in the literature review. These include high blood pressure, diabetes, high cholesterol, asthma, chronic obstructive pulmonary disease and cancer.

Finally, to verify for dietary inclination or habit that can be predictor, subjects were verbally asked for their smoking status, alcohol consumption, the last 48-hour consumed food that had been associated with CRP in the literature review, and pregnancy, use of oral contraceptive pills and hormone replacement therapy. These screenings are important because all the above had in the past tagged as predictors that could result into alteration of CRP in human (Ishii *et al.*, 2012; Buchwald *et al.*, 2013). Only subjects that were cleared of these predictors were finally used for this study.

STATISTICAL ANALYSIS

Statistical analyses were performed using SPSS 17 (SPSS Inc., Chicago, IL, USA). All data are presented as means $\pm$ standard error of the mean (SEM) for 30 human subjects. Independent t-test analysis was used to compare the mean values of CRP level between the pre-and-post consumption of *Garcinia kola* seed. Value with $p < 0.05$ compared with control were considered as significant.

RESULTS AND DISCUSSION

The result (Table 1) showed that the mean value of female BMI is significantly higher than that of male; additional predictors, age, weight and height that are associated with CRP were equally shown in the table with no significant differences between the male and female subjects.

When comparing the level of CRP before and after consumption of GKS, the mean value of CRP at post consumption was significantly decreased in female subjects and significantly increased in male subjects (Table 2).

Table 1: Age, weight, height and BMI of the human subjects

Parameters	Female (Mean $\pm$ SEM)	Male (Mean $\pm$ SEM)
Age (year)	20.20 $\pm$ 1.28	21.67 $\pm$ 0.46
Weight (Kg)	62.30 $\pm$ 1.72	63.47 $\pm$ 1.04
Height (M)	1.63 $\pm$ 0.01	1.98 $\pm$ 0.06
BMI (K/G/M ²)	23.33 $\pm$ 0.55	6.11 $\pm$ 1.58

*p<0.05 confidence interval; student t-test

Table 2. Level of CRP before and after consumption of GKS with their percentage differences

Parameters SEM)	Female (Mean $\pm$ SEM)	Male (Mean $\pm$
CRP Before Consumption (mg/l)	29.89 $\pm$ 5.09	8.45 $\pm$ 1.06
CRP After Consumption (mg/l)	10.07 $\pm$ 2.70*	14.50 $\pm$ 1.50*
Percentage Difference	66.31%	72.27%

*p<0.05 confidence interval; student t-test

Our results showed an association between female gender, high BMI and elevated CRP in our basal CRP levels measurement. This is not surprising because past studies had associated low income and female gender with greater levels of perceived stress and the resulted chronic stress is a factor for inflammation (Macintyre and Hunt, 1997; Black, 2003). This is in addition to the fact that adipose tissue is an accepted source of CRP in human (Calabro et al., 2005), this tissue is found more readily in females. Recently, Ishii *et al.*, (2012) confirmed female gender, low income, high BMI and the use of sex hormone as chronic factors that are associated with elevated CRP. On the other hand, male in the absent of significant clinical inflammation processes tends to have low basal CRP levels compared with their female counter-parts (Nazmi *et al.*, 2008). This again reflected in our results for basal CRP measurement.

Having taken GKS however, CRP level in the female subjects drastically reduced by 66.31% from the basal level despite the fact that CRP elevated predictors (female gender, high BMI, adiposity) are still present. Surprisingly, there is 72.27% increased in CRP from basal level in male subjects. Though, the mechanism of action and the observed gender variation are still under

investigation, we however assumed GKS might have a gender based receptors or second messengers hence different mechanism of actions. This is true for Spearmint tea, a plant documented to have antiandrogenic properties in females with hirsutism (Akdogan *et al.*, 2007; Grant, 2010). Another example is phytoestrogens seen in some plants such as red clover which enhances female animals' fertility but reduced that of male animals. This gender based action is believed to be the plant defensive mechanism against fecundity of the animals that pasture on such plants (Hughes, 1988; Masole-taylor and Bentley, 2000; Johnston, 2003; Yildiz, 2005). In our chain of assumptions, depending on the gender, we also assumed that GSK might be equipped with phytochemicals that could stimulate or inhibit predictor factors that might lead to increased or decreased in CRP synthesis in the liver. Such predictors include IL-6 & some cytokines that are released mainly by macrophages and adipocytes (Pepys and Hirschfield, 2003; Lau *et al.*, 2005), estrogen level (Lakoski *et al.*, 2005), adipocytes metabolism (Calabro *et al.*, 2005, Cartier *et al.*, 2009) and BMI (Nazmi *et al.*, 2008; Khera *et al.*, 2009; Ishii *et al.*, 2012). Lastly, we also assumed that it might be that GKS increased CRP level in the female at first but because of the high basal level of CRP, this makes it to quickly reach a level that can initiate a negative feedback mechanism, a process that resulted into decreased synthesis of CRP in the liver since there is no clinical significant inflammation present. This can explain why the CRP level in male subjects remains high as at when blood was taken. It is possible that the level of CRP in male could not quickly reach the level that can initiate negative feedback because the male basal level of CRP was low.

Using information from the literature however, one may conclude that since GKS had been shown to contain the following phytochemicals, flavonoids, cardiac glycosides, steroids, reducing sugars anthraquinones, free anthraquinones, saponins and tannins (Adegboye *et al.*, 2008; Adejumo *et al.*, 2011), and flavonoids have been widely claimed to be potent antibacterial, antiviral, antiallergic, and anti-inflammatory alkaloid, then it might be responsible for CRP reduction in female subjects. This however cannot explain the observation in male subjects. Hence, thorough study is further needed to establish not only the mechanism of action but also the gender based action of GKS.

In conclusion, this study shows that GKS is a potent anti-inflammatory fruit for female subjects but not for male subjects. Anti-inflammatory of GKS effects are thus gender based. With these observations, it can be deduced that GKS is more beneficial to female subjects than male subjects. Since CRP had been claimed to exacerbate ischemic necrosis in a complement-dependent fashion, male patients with inflammatory conditions should take caution while consuming the fruits. Lastly, the clinical practise of using CRP level in the blood to predict the pathogenesis of a disease, track of a disease progress or measure of effectiveness of a treatment will be misleading in habitual GKS consuming patients.

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