

Effect of *Grewia asiatica* fruit on Glycemic index and phagocytosis tested in healthy human subjects

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Abstract: The *Grewia asiatica* (commonly known as Phalsa or Falsa) is a shrub or small tree found in southern Asia. It produces purple to black color fruit when ripe. In folk medicine the edible *Grewia asiatica* fruit is used in a number of pathological conditions. The current study described the effects of *Grewia asiatica* fruit on glycemic index (GI) and phagocytosis in healthy non-diabetic human subjects. The results showed that *Grewia asiatica* fruit has low GI value of 5.34 with modest hypoglycemic activity. Luminol-enhanced chemiluminescence assay was carried out to determine the production of reactive oxygen species (ROS) in the oxidative burst activity of whole blood. ROS production was found to be significantly affected, having the 78.3, 58.6 and 30.8% when the subjects were fed with D-glucose, mixture of D-glucose and *Grewia asiatica* fruit and *Grewia asiatica* fruit alone respectively as compared to the control. The aqueous, methanolic and butanolic extracts of *Grewia asiatica* fruits were found to produce a stimulatory effect on ROS production however; the chloroform, hexane and ethanol-acetate extracted exerted significant inhibitory effect. These results demonstrated that *Grewia asiatica* fruit has desirable effects on blood glucose metabolism manifested as low glycemic response and modulation of ROS production.

Keywords: Reactive oxygen species, blood glucose level, Nutraceuticals, glycemic response.

INTRODUCTION

Fruits and vegetables exhibit their health-promoting properties by delaying the ageing process and by reducing the risk of various diseases including cardiovascular disorders, cancer, rheumatoid arthritis, lung diseases, cataract, Parkinson's or Alzheimer's disease (Szajdek and Borowska, 2008). It is believed that the phytochemicals and vitamins largely responsible for those protective effects. Their activity is manifested by the scavenging ability of reactive oxygen species (ROS), such as hydroxyl, peroxide radicals, etc. (Rice-Evans *et al.*, 1995). *Grewia asiatica* (commonly known as Phalsa or Falsa) is a species of *Grewia* native to southern Asia from Pakistan to Cambodia. It is a shrub or small tree with the fruit of 5-12 mm diameter, having purple to black color when ripe. In Indian folk medicine, the edible phalsa fruit is used to alleviate inflammation, respiratory, cardiac, and blood disorders, as well it is used as a digestive and antipyretic (Poonam and Singh, 2009). The phalsa fruit extract has been used in polyherbal Ayurvedic preparations in traditional Indian system of medicine (Gupta *et al.*, 2010). However, scientific literature contains a few reports on phalsa fruit. Free radical scavenging and neuro-protective properties against radiation have been found in phalsa fruit extract (Sharma and Sisodia, 2009; Sisodia and Singh, 2009). The bark of

Grewia asiatica plant showed hypoglycemic effect on alloxan diabetic male rabbits (Dogar *et al.*, 1988).

Plasma glucose level and reactive oxygen species (ROS) has important effect on various physiological metabolic processes (Cathcart, 2004). Carbohydrates containing foods influence the release of different metabolic hormones such as insulin (Wolever, 2000). In order to rank different carbohydrates; a concept of Glycemic Index (GI) has been introduced. GI is calculated as 'the area under the blood glucose response curve for each food expressed as a percentage of the area after taking the same amount of carbohydrate as glucose (Jenkins *et al.*, 1981; Augustin *et al.*, 2001; Monro, 2005). The production of free reactive oxygen species (ROS) is important integral part of immune system (Mitra and Abraham, 2006). Elevated plasma glucose level can adversely suppress the activity of innate cellular immune response including neutrophils and macrophages that finally leads to containment of phagocytosis and production of ROS (Touyz, 2004). We have determined the effects of phalsa fruit on blood glucose level and reactive oxygen species (ROS) in healthy non-diabetic human subjects.

MATERIALS AND METHODS

Study Protocol

In present study, fresh phalsa (*Grewia asiatica* L.) fruits were purchased from market in Karachi, Pakistan. The

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study was performed on three consecutive days on same healthy group of subjects. Fifteen healthy human subjects (7 non-pregnant females and 8 males; mean age of 28.6 ± 9.3 years) were recruited from University of Karachi, Karachi, Pakistan. These subjects were nonsmokers, and did not consume any medications in the last 2 weeks. Fully informed written consents were taken from all subjects. On day-1, the participants were given D-Glucose (1 g/kg) dissolved in 250 ml of water. On day-2, the participants were given mixture of D-Glucose (1 g/kg) and phalsa fruit extract (1g/kg) in 250 ml of water. On day-3, the participants were given only phalsa fruit extract on the basis of their body weight (1g/kg) in 250 ml of water.

After intake of test material, the participants were then required to remain seated at the study centre and refrain from eating or drinking during next 2 hours. Blood samples were taken at 0 time, 30, 90, and 120 minutes after consumption of test material. The plasma glucose level was determined by the strip method employing a glucometer (Roche Diagnostics Ltd., Mannheim, Germany). Blood samples for chemiluminescence assay was obtained by clean vein-puncture (every day at zero time and after 120 minutes) into heparin containing tubes and used immediately.

Extraction and Fractionation of *G. asiatica* fruit

Fruits of *G. asiatica* (1.5 kg) were soaked in 70% aqueous methanol for three days. The extract was filtered and evaporated at room temperature to dryness using rotary evaporator to obtain crude methanolic extract. The crude extract (30 g) was partitioned with n-hexane to remove hexane soluble part. The n-hexane insoluble part was dissolved in water and then subjected to solvent-solvent extraction using ethyl acetate, chloroform, and water (please elaborate) as solvents which resulted in three fractions i.e. ethyl acetate (0.5 g), chloroform (0.3 g) and water soluble fraction (14 g). The water soluble fraction was further partitioned with butanol. Consequently, six extracts were obtained for chemiluminescence assay, namely crude methanolic extract, n-hexane soluble part, ethyl acetate soluble part, chloroform soluble part, water soluble part and butanol soluble part.

Chemiluminescence assay

Human blood samples (10 ml) were drawn from healthy volunteers into heparinized tubes and mixed with Hank's balance salt solution with calcium and magnesium (HBSS⁺⁺ buffer). This was followed by addition of Ficoll-Paque at a ratio of 1:1 and allowed to sediment. The buffy layer was taken out and centrifuged at $400 \times g$ at 22°C for 20 minutes. The resulting neutrophils pellet was mixed with distilled water to lyse the RBCs and centrifuged at $300 \times g$ at 4°C for 10 minutes to obtain neutrophils. The neutrophil pellet was suspended in HBSS⁺⁺ buffer to give final neutrophils concentration of 1×10^6 cells/mL (Saeed *et al.*, 2007). Luminol-enhanced chemiluminescence

assay was performed according to Helfand *et al.*, (1982). Briefly, 25 μ L of whole blood (diluted 1:50) or neutrophils (1×10^7 cells/mL) suspended in HBSS⁺⁺ buffer was mixed with 25 μ L of (20 mg/mL) zymosan and 25 μ L of (7×10^{-5} M) Luminol. 25 μ L of HBSS⁺⁺ buffer was added to adjust the final volume to 0.1 mL. Chemiluminescence peaks were recorded with the Luminometer (Luminoscan RS Labsystem, Finland).

RESULTS

During the present research, the effects of *Grewia asiatica* fruit on physiological glycemic response and ROS scavenging activity were analyzed.

Effect of *G. asiatica* fruit consumption on plasma glucose level

Fig. 1 shows plasma glucose levels at fasting (i.e. 0 time) and after 30, 90 and 120 minutes of intake of glucose (GG), phalsa fruit extract (PG) and a mixture of glucose and phalsa fruit extract (GPG). The cumulative mean base line fasting glucose level of all 3 groups (that is, D-glucose consumers group, GG; phalsa fruit consumers group, PG; and phalsa+D-glucose consumers group, GPG) was 68.7 mg/dL. Table 1 showed comparative PGL in fasting as well as at 60, 120, and 180 min after test meal consumption by the GG, GPG, and PG groups. Compared to fasting plasma glucose level (PGL), consumption of D-glucose resulted in 90 and 34% increase in PGL at 30 and 120 minutes respectively. However, considerably decreased PGL was recorded after 120 min (20%) showing typical glucose tolerance phenomenon. On the other hand, consumption of a mixture of glucose and phalsa fruit extract (GPG) resulted comparably low PGL after 30 minutes (80%), 90 minutes (21%) and 120 minutes (6.4%). Hence, phalsa fruit exhibited modest hypoglycemic effect. The present data are, for the most part, consistent with previous studies on glycemic response of phalsa (Dogar *et al.*, 1988). The glycemic index of phalsa fruit was also estimated using the PGL after intake of phalsa fruit (PG).

The glycemic index (GI) of a food is defined as the area under the two hour blood glucose response curve (AUC) following the ingestion of a fixed portion (usually 50 g) (Frost *et al.*, 1999). For GI calculation, the AUC of the test food (i.e. phalsa fruit extract) is divided by the AUC of the standard (Glucose) and multiplied by 100 (Liu *et al.*, 2000; Salmeron *et al.*, 1997). Results showed that phalsa fruit has low GI value of 5.34.

Effect of *G. asiatica* fruit consumption on ROS production

Three sets of experiments were designed to monitor the affect of *G. asiatica* fruit consumption on the production of the ROS species. In the first two sets of experiments, subjects were either fed with D-glucose or a mixture of D-

Table 1: Plasma glucose levels (PGL) of subjects before and after consumption of D-glucose (GG), a mixture of phalsa fruit extract+D-glucose (GPG), and phalsa fruit (PG). Percentage increase or decrease in PGLs indicated within parentheses; change (increase/decrease) in the PGL is shown by arrows.

Test/Time	0	30 min.	90 min.	120 min.
GG	69.6	130.2 (89.5%↑)	92.1 (34.1%↑)	69.7 (1.4%↑)
GPG	64.7	124.2 (80.7%↑)	83.5 (21.5%↑)	81.9 19.2%↑)
PG	72	69.7 (1.4%↑)	67.7 (1.4%↓)	73.1 (6.4%↑)

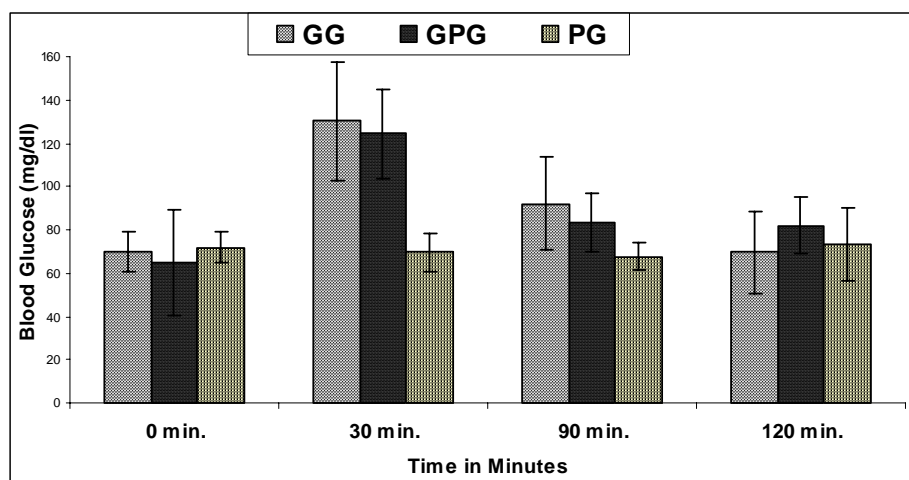


Fig. 1: Blood glucose level at zero time, 30, 90, 120 minutes in all 3 studied groups, that is, Glucose group (GG), Glucose-Phalsa group (GPG); Phalsa Group (PG). Each bar represents mean \pm SD values of the 3 groups studied. The glucose concentrations in blood samples were determined by the strip method employing a glucometer (Roche Diagnostics Ltd., Mannheim, Germany).

glucose and phalsa extract. In the first experiment, glucose was found to exert a significant suppression on the ROS production with an average of 78.3% inhibition (fig. 2A). However this suppressive effect was found to decrease when phalsa was combined with D-glucose in the second experiment (fig. 2B) showing 59.6% inhibition. Additionally in the third experiment when subjects were fed with phalsa only, comparably less inhibition of ROS production was noted (fig. 2C). The effect of glucose on the production of ROS was confirmed with an *in vitro* study on isolated neutrophils as shown in fig. 2E. Glucose is found to exert a dose dependant effect. At higher glucose concentration (32 mg/mL), the inhibition of ROS production by neutrophils was found to be 80.2% compared 9.2% at 2 mg/mL. Figure 2D showed ROS levels in whole blood of seven individuals after 5 days of phalsa fruit ingestion.

Effect of different *G. asiatica* fruit extracts on ROS production

Aqueous, methanolic and butanolic extracts of phalsa fruit were found to produce a stimulatory effect on ROS production. The chloroform, hexane and ethanol-acetate extracts at 100 μ g/mL concentration exerted significant inhibitory effect on the ROS production, while at low concentration (i.e. 1 and 10 μ g/mL) a significant stimulatory effect was monitored in the ROS level (fig. 2F).

DISCUSSION

Integrated physiological mechanisms co-coordinately influence the release of insulin and other metabolic hormones and regulate the plasma glucose level which in turn affects different functions of the body such as phagocytosis and production of reactive oxygen species (Saiepour et al., 2003). Phalsa fruit extract has previously reported to affect glucose metabolism and immune system (Dogar *et al.*, 1988; Sharma and Sisodia 2009). The results of the present study showed that the high glucose concentration suppressed the ROS production; an important part of immune response. The low glycemic index of phalsa fruit indicated that ingestion of fruit would not elevate the plasma glucose level in spite of the sweat taste.

The process of phagocytosis and production of free oxygen species are greatly influenced by plasma glucose level (Martha et al., 2004; Touyz 2004). In present study, it was noted that glucose caused suppression in ROS production. The results of day 2 (consumption of a mixture glucose and phalsa fruit extract) and day 3 (consumption of phalsa fruit extract) pointed out a modulatory response in the ROS production which could be a neutralization effect of glucose induced ROS suppression. Results of Chemiluminescence assays after 5 days of phalsa fruit ingestion indicated the persistence of

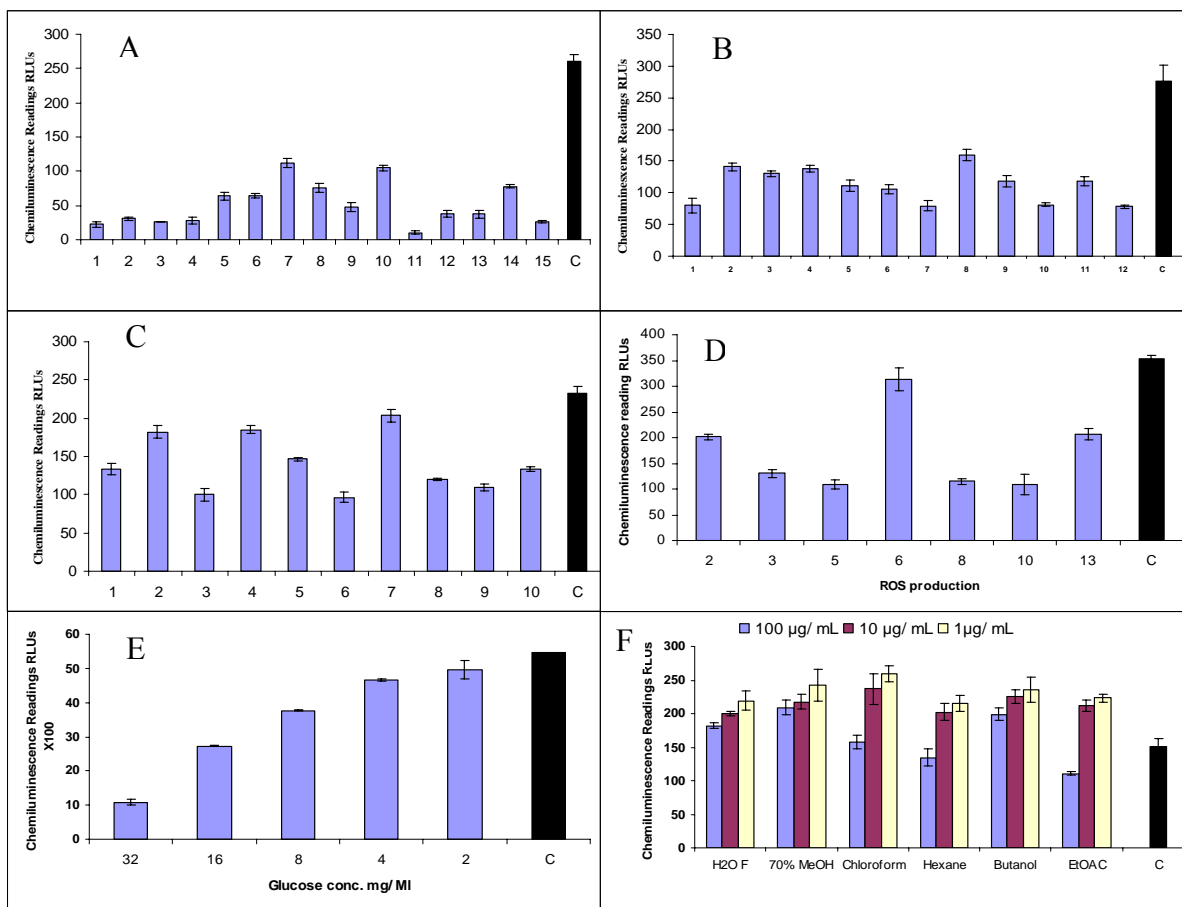


Fig. 2: Effect of phalsa and phalsa combination on oxidative burst. Effect of glucose (A), glucose and phalsa (B), phalsa (C) and GAF extracts (F) on phagocytic ROS production assayed by Luminol CL after 15 min incubation with whole blood; ROS levels after 5 days of ingestion of phalsa (D); Effect of glucose conc.(mg/ MI) on ROS levels of isolated Neutrophils (E). All CL results compared to the control (C) readings.

suppression in ROS production. Different phalsa fruit extracts exerted both stimulatory as well as inhibitory effects on ROS production.

In conclusion, we demonstrated that phalsa fruit has desirable effects on blood glucose metabolism manifested by low glycemic index. Furthermore, when phalsa fruit was tested *in-vitro* with glucose it showed neutralization effects on glucose induced ROS suppression.

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