Effect of *Phoenix dactylifera* Ethanolic Extract on Induced-heat stress in Wistar Albino Rats

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**ABSTRACT**

Heat-induced and heat-related illnesses remain highly prevalent in man working and living environments, specifically during a sudden increase of ambient temperature. This study is designed to determine the effect of fruits of *Phoenix dactylifera* ethanolic extract on some biochemical parameters (plasma glucose, total protein, lipid profile, urea and uric acid) of induced-heat stress in Wistar albino rats. Wistar albino rats were exposed to heat stress until the rectal temperature reached 40 °C after treated with extract at doses 250 mg/kg and 500 mg/kg for two days, then biochemical parameters were measured from plasma and compared with two groups as control. Heat stress elevates levels of total cholesterol and plasma glucose (p<0.01) and reduced the level of plasma total protein (p<0.01), triglyceride, HDL, LDL, urea and uric acid. *P. dactylifera* ethanolic extract caused (p<0.01) reduction on plasma glucose at dose of 500 mg/kg and elevation (p<0.01) on triglyceride at dose 250 mg/kg after stress. It is concluded that *P. dactylifera* extract may have the potentiality to counteract changes in biochemical parameters affected by stress.

**INTRODUCTION**

It has been known that during a sudden elevation in the environmental temperature or during heat waves, heat stress will proceed and eventually cause heat stroke and other ailments and this is known to be highly prevalent in various working and living environments (Bouchama and Knochel, 2002). In addition to heat stroke, other illnesses were observed (heat syncope, heat rash, heat cramps and heat exhaustion) (Alzeer et al., 1997) and cited as either the underlying cause or contributing cause of death for 3,332 (31%) of death in U.S. during 2006-2010 (Berko et al., 2014). On the other hand, heat-stress is known to produce Reactive Oxygen Species (ROS) (Tsong and Su, 1999) from metabolism of oxygen as a byproduct of cell respiration (Slimen et al., 2014). When body temperature and rate of metabolism are high the production of ROS increased (Subasini et al., 2013) resulting in free radical mediated chain reactions (Stadtmen and Levine, 2000) targeting various cellular molecules such as poly saccharides (Kaur and Halliwell, 1994), proteins, lipids and nucleic acids causing cellular injury (Wells et al., 1997) also triggering the intrinsic pathway of apoptosis (Gu et al., 2014) and variety of diseases such as (Iwu eke et al., 2010) cancer, Alzheimer’s and autoimmune diseases (Slimen et al., 2014). thermo-regulator, acute-phase proteins and heat shock proteins play an important role in protecting cells from the stress, however thermo-regulator process takes several weeks and involves enhancement of cardiovascular performance, salt conservation by sweat glands and kidneys and expansion of plasma volume to acclimatize heat (Bouchama and knouchel, 2002).
Date palm (Phoenix dactylifera) has long been one of the most important and oldest fruit in the arid regions of North Africa, and the Middle East (Atteq et al., 2013). The inspiration for the pursuit of studying the medicinal properties of the date derives from Islamic Prophetic traditions mentioning dates, including some that point directly to its medicinal properties and historic Islamic medical literature. Dates have been found to contain extremely high levels of phenolic, hypothesized to have formed due to exposure to extreme temperature and climate in comparison to other fruit and have the capacity to act as potent scavengers of reactive oxidative species generated by chemical agents (Mousa et al., 2015). The current study was performed to determine the effect of ethanolic extract of Phoenix dactylifera fruits on plasma glucose, total protein, lipid profile, urea and uric acid in induced heat stress in Wistar albino rats.

MATERIALS AND METHODS

Preparation of the extract:

Fruits of Phoenix dactylifera were purchased from markets in Omdurman, Sudan. The extraction was carried out according to method described by Sukhdev et al., (2008). About 250 g of the Phoenix dactylifera fruits were ground down with mortar and pestle and extracted by soaked in 80% ethanol at room temperature for about 72 h with slight filtration and evaporation. Solvent was evaporated under reduced pressure to dryness using rotary evaporator apparatus (Buchi Switzerland) and the extracts were lyophilized by using freeze dryer until dryness.

Experimental Animals:

Twenty healthy Wistar albino rats (Animal House of Medicinal and Aromatic Plants Research Institute-National center for Research, Sudan) were kept separately in individual cages in a temperature of 22 ± 3 °C. They were fed with a standard laboratory diets and given drinking water ad libitum.

Experimental Design:

Rats were randomly divided into 4 groups, 5 rats in each; rats of the first group were not exposed to heat stress or fruit extract (Negative Control), whereas the rats of the second group were exposed only to heat-stress (Positive Control). Rats of third and fourth group were received an extract at the doses 250 and 500 mg/kg, respectively for two consecutive days then exposed to heat-stress. On the day 3, all groups except the first one were exposed to heat in hot chamber until their rectal temperature reached 40 °C. Blood was collected from retro orbital plexus for each group (Subasini et al., 2013).

Plasma glucose was measured using enzymatic colorimetric method GOD-POD (BioMed- Glucose L.S). Total plasma protein was measured using colorimetric, endpoint (BioMed-Total Protein). Total plasma cholesterol was measured using enzymatic colorimetric method CHOD-PAP (BioMed- Cholesterol- L.S). Plasma HDL-cholesterol was measured using Precept.Reagent (BioMed-HDL- cholesterol). Plasma triglyceride-cholesterol was measured using enzymatic colorimetric method GPO-PAP (BioMed- Triglycerides L.S). Plasma LDL- cholesterol level was calculated as follow;

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\text{LDL Cholesterol (mg/dl) = Total Cholesterol} - \frac{\text{Triglycerides}}{5} - \text{HDL Cholesterol}
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Plasma uric acid was measured by using enzymatic colorimetric method (BioMed- Urea). Plasma uric acid was measured by using enzymatic colorimetric method (BioMed- Uric acid L.S).

Data collected was analyzed by using SPSS version 20 (Two pair T-test).

RESULTS AND DISCUSSION

The current study revealed that, heat stress caused elevation in level of total cholesterol of group II compared with group I, this finding is similar to study carried out by (Handekari and Bondade, 2012) who reported that the elevation of total cholesterol by stress is uncertain but it may be to increase in level of lipolytic hormones which mobilize lipid stores of adipose tissue and liver to meet the extra caloric requirement of tissue. 250 and 500 mg/kg of Phoenix dactylifera ethanolic extract caused a non significant reduction in plasma total cholesterol level of group III and group IV compared to group II (Figure 1A).

The triglyceride level was observed to be decreased significantly (p<0.01) in group II compared with group I that may be one of the mechanisms for stress induced elevation of serum triglycerides. However, Handekari and Bondade (2012) reported that triglyceride showed a highly significant increase in heat stress pigs. A significant (p<0.01) elevation in the triglyceride level observed in group III (250mg/kg) compared to group II (Figure 1B).
Heat stress caused a non-significant reduction in the levels of High-density lipoprotein (HDL) as well as Low-density lipoprotein (LDL) of group II. The two doses of the extract caused elevation in the HDL and LDL levels of group III and IV but none significantly (Figure 1C & D).

Figure 1: Effect of heat stress (Group I), Phoenix dactylifera ethanolic extract at doses 250 mg/kg (Group III) and 500 mg/kg (Group IV) compared with (Group I) on plasma lipid profile; (A) total cholesterol; (B) triglyceride; (C) HDL; and (D) LDL. Result are given as mean ± std error, n=5, ** (P<0.01).

Figure 2 shows that heat stress caused a significant (P<0.01) elevation in the plasma glucose in group II than group I, this finding is similar to study performed by (Gabler et al., 2013) who reported that after 24 h of an acute heat load, heat stress pigs had increased blood glucose, in addition to that studies carried out by (Febbraio et al., 1944), Fink et al., (1975), Rowell et al., (1968) and Yasplekis et al., (1993), plasma glucose levels were found to be increased significantly (p<0.01) in heat stressed positive control rats that may be due to liver output exceeding muscles glucose uptake. 00 mg/kg of the extract showed a significant (p<0.01) reduction in the glucose level of group IV compared with group II.
Moreover, it was found that the significant (p<0.01) reduction of total protein in group II affected by stress when compared with group I which is correlated with finding by (Zuprizal et al., 1993) who observed a significant (p<0.01) reduction in total protein levels of heat stress rats and justified that may be due to a decrease in digestibility of amino acid and proteins in high temperature.

Fig. 2: Level of plasma glucose (mg/dl) among studied groups. Result are given as mean ± std error, n=5, ** (P<0.01).

Fig. 3: Effect of heat stress (Group I), Phoenix dactylifera ethanolic extract at doses 250 mg/kg (Group III) and 500 mg/kg (Group IV) compared with (Group I) on (A) total protein; (B) urea; and (C) uric acid in plasma. Result are given as mean ± std error, n=5, * (P<0.05) and ** (P<0.01).
Two doses of Phoenix dactylifera extract (group III & group IV) expressed an elevation in the total protein level after stress that may be due to the activity of pancreas decreased digestibility of amino acids or anabolism of proteins as reported by Subasini et al., (2013) (Figure 3 A). On the other hand, study showed that heat stress caused a reduction in the urea and uric acid levels (Figure 3 B & C) in group II compared to group I that may be due to reduction in protein metabolism during exercise in heat (Dolhy and Lemon, 1988). 500 mg/kg of Phoenix dactylifera extract contracted this reduction by causing an elevation on these two parameters with a significant (p<0.05) elevation in uric acid levels after stress.

**Conclusion:**

This study concludes in that heat stress elevates the levels of plasma glucose as well as plasma total cholesterol, however reduction was observed in other parameters; triglycerides, HDL, LDL, total protein, urea and uric acid.

The ethanolic extract of Phoenix dactylifera at different doses have a potential effect to counteract the changes in biochemical parameters associated with induced- heat stress in Wistar rats.

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