



Role of DPP (*Phoenix dactylifera* L.) extract on Ameliorating The incidence of hemoglobinoxidation induced by sodium nitrite

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Summary

The effects of ethanolic extract of date palm pollen grains(DPP) was investigated on the ameliorating the oxidative effect of sodium nitrite on hemoglobin in albino mature female rats , twenty four(24) rats weighing 150- 200 g were grouped into four groups two control and two experimental groups, first group(GI) considered as negative control received 0.5 ml distilled water and after 45 minutes received another 0.5 ml of distilled water while the second control group(GII), (positive control) received 0.5 ml of distilled water then after 45 minutes received 100mg/kg Body weight sodium nitrite. While the two experimental groups were treated as follow, first treated group(GIII) received 100mg/kg Body weight crude ethanolic extract of DPP then after 45 minutes received 100mg/kg Body weight sodium nitrite. while second(GIV) treated group received 100mg/kg Body weight crude ethanolic extract of DPP daily for seven days then after 45 minutes from last dose received 100mg/kg B.W. sodium nitrite. Whole blood samples were obtained in heparinized tubes to detect methemoglobin percentage. The results in animals treated with single oral dose (100mg/Kg) of sodium nitrite comprised 45% met-Hb, 45 minutes after nitrite treatment, while animal's group pretreated with ethanolic extract of DPP in a dose of 100mg/kg for seven consecutive days given as single oral dose of sodium nitrite after 45 minutes from last dose (before induction of methemoglobinemia) by nitrite administration revealed an increases in the level of met-Hb percentage in blood compared to that of control group treated with distilled water before sodium nitrite. However the percent inhibition of met-Hb formation over long term treatment was less than that observed with short term treatment.

Conclusions: related to high antioxidant activity of DPP extract then it given good protective against hemoglobin oxidation when treated for long period.

Key word: Methemoglobin, Date Palm Polled, Sodium nitrite.

دور المستخلص الكحولي لحبوب طلع النخيل في التخفيف من اكسدة هيموكلوبين الدم

في اناث الجرذان البالغة المعرضة لتأثيرات الصوديوم

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الخلاصة:

تناول هذا البحث دور المستخلص الكحولي لحبوب طلع النخيل في التخفيف من شدة تاكسد هيموكلوبين الدم الحاصل بفعل نترتيت الصوديوم . استعمل 24 من اناث الجرذان البالغة بعمر شهرين ونصف وقسمت عشوائيا الى اربعة مجاميع متساوية , جرعت المجموعتين الاولى والثانية ماء مقطر وبعد 45 دقيقة جرعت الاولى مرة اخرى ماء مقطر واعتبرت مجموعة سيطرة سالبة وجرعت الثانية بعد 45 دقيقة 100 ملغم / كغم نترتيت الصوديوم واعتبرت مجموعة سيطرة موجبة واعطيت الثالثة جرعة مفردة من المستخلص الكحولي لحبوب طلع النخيل بجرعة 100 ملغم/كغم (معاملة قصيرة الامد) والرابعة اعطيت 100 ملغم/كغم من المستخلص الكحولي لحبوب طلع النخيل لمدة اسبوع وبعد 45 دقيقة من الجرعة الاخيرة اعطيت 100 ملغم / كغم نترتيت الصوديوم (معاملة قصيرة الامد). من قلب الحيوان مباشرة تم جمع نماذج من الدم في انابيب تحوي مانع تخثر لغرض قياس النسبة المئوية للمتيهيموكلوبين , واوضحت النتائج ان المعاملة ادت الى خفض نسبة المتيهيموكلوبين في جردان المعاملة الطويلة الامد الى 30% وقصيرة الامد الى 44.6% بينما ارتفعت نسبة المتيهيموكلوبين في الجرذان التي لم تتناول المستخلص الى اكثر من 63% . نستنتج من هذه الدراسة بان للمستخلص دور في خفض نسبة اكسدة هيموكلوبين الدم .

الكلمات الافتتاحية : متهيموكلوبين , حبوب طلع النخيل , نترتيت الصوديوم

Introduction

Methemoglobin (MHb) is an abnormal hemoglobin in which the iron moiety of unoxygenated hemoglobin is in the ferric (Fe^{3+}) state rather than the ferrous (Fe^{2+}) state. then it became unable to carry oxygen. Acquired methemoglobinemia can result from exposure to a wide range of drugs and chemicals, including nitrites and other oxidants (Su *et al.*, 2012). The most common causes of MHb is ingestion or skin exposure to an oxidizing agent. Oxidizing agents can be divided into those that directly oxidize hemoglobin and those that indirectly oxidize hemoglobin. Direct oxidizers react directly with hemoglobin to form MHb. Indirect oxidizers are actually powerful reducing agents that reduce oxygen to the free radical super oxide dismutase (O^{\bullet}), or water to H_2O_2 , which in turn oxidizes hemoglobin to MHb. Many drugs that produce MHb are not themselves the causative agents. Instead these drugs are metabolized to an oxidative free radical. For example, aniline is metabolized by the cytochrome P-450 system to a free radical phenyl hydroxylamine, which, like nitrite, reacts with O_2 to form

oxygen free radicals and then MHb (Wright *et al.*, 1999).

Red blood cells have multiple mechanisms to maintain the normal concentration of methemoglobin at less than 1%. Under normal circumstances, the most important reductive system involves (NADH), a byproduct of cellular glycolysis. This enzyme system enables clears more than 95% percent of the methemoglobin formed under normal circumstances (Amir Miodovnik, 2009).

Date palm pollen (DPP) application in traditional and herbal medicine, They contain concentration of photochemical and nutrients and are rich in carotenoids flavonoids and phytosterols (Broadhurst, 1999). Moreover, they are good source of protein ,amino acid ,vitamins ,dietary fiber, fatty acid , enzymes ,hormones and minerals (Alferz and Campos, 2000). it contained of alkaloids and saponins (Abedi *et al.*, 2013). Treatment with DPP counteracted the increases in antioxidant systems as assessed by restoration of reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT), (Hassan *et al.*, 2012). The antioxidant activity by the DPPH method indicated that the DPP

extract showed a strong antioxidant against DPPH radicals (Ammar *et al.*, 2009).

Then this study was listed to detect the protective effects of DPP extract against hemoglobin oxidation

Materials and methods

Animals

A total of 24 mature albino female rats weighing 150-200g animals were housed in plastic cages in conditioned room (25-30 °C) in the animal house of scientific College of Wasit University. They were left for two weeks for adaptation with the experimental conditions. Animals had free access to water and pellet diet along the experimental period. animals exposed to light evening for 10 – 12 hours / day.

Plant sample

Preparation of plant material

Pollen grains of date palm (*Phoenix dactylifera* L.) was collected from Alzubaidia district 90 Km southern east of Baghdad in Wasit province ,Iraq country.in period through last March and along April 2014 and left to air dried under dark condition then stored in frozen until use.

Preparation of ethanol extract of DPP grains (*Phoenix Dactylifera*)

The air dried powdered were extracted in soxhlet extractor successively with 70% ethanol for 16 hrs. using double-thickness cellulose extraction thimbles. The successive extracts were evaporated by a rotary evaporator at temperature below 45c[□] the crude extract was kept at -20c[□] till use. (Jiheel,2010)

Experimental design

There were two controls and two experimental groups, (six animals each for mature female albino rats), treated as flow: negative control (GI) received 0.5 ml DW and after 45 minutes

received another 0.5 ml of DW then, positive control (GII) received 0.5 ml of DW then after 45 minutes received 100mg/kg B.W sodium nitrite orally by gavage needle. were first treated group (GIII) received 100mg/kg B.W crude ethanolic extract of DPP daily for seven days then after 45 minutes from last dose received 100mg/kg B.W. sodium nitrite. while second (GIV) treated group received 100mg/kg B.W crude ethanolic extract of DPP then after 45 minutes received 100mg/kg B.W. sodium nitrite. All animals received treatment materials (which mention for each group) orally by gavage needle.

Blood sample collection

Blood samples were collected from heart animals after anesthetized by the ketamine and Xylazine, intramuscular injection of Ketamine (90 mg/Kg B.W.) and Xylazine (40 mg/kg B.W.) (Al-Mzaeni, 2009). whole blood samples were obtained in heparinized tubes to detect methemoglobin concentration.

Measurement of methemoglobin in blood

Aliquot (0.2 ml) of fresh blood was lysed in a solution containing 4.0 ml of buffer (Phosphate buffer, pH 6.8) and 6.0 ml of non-ionic detergent solution (Triton X-100, 1%); the lysate was divided into two equal volumes (A and B). The absorbance of A was measured using a spectrophotometer at 630 nm (D₁). Then 1.0 drop of potassium cyanide solution (50 gm/L) was added to A and after mixing the absorbance was measured again (D₂). To solution B add 1.0 drop of potassium ferricyanide solution (50 gm/L) was added, and after 5 minutes, measure the absorbance at 630nm (D₃). Then add 1.0 drop of potassium cyanide solution to B and after mixing read the absorbance to get the final

reading (D_4). All the measurements are made against a blank containing buffer and detergent in the same proportion as present in the sample (Rodkey, 1979). The level of Met-Hb then expressed as a percentage according to the following formula: $\text{Met-Hb\%} = (D_1 - D_2 / D_3 - D_4) \times 100$. (Sulaiman, 2012; Dacie, 1997; & Rodkey, *et al.*, 1979)

Statistical analysis

The values were recorded as mean \pm standard error of mean. Statistical analysis was done by Kruskal Wallis test (ANOVA). The comparison of means between control and each experimental group (one way ANOVA). $P < 0.05$ was regarded as significant. GenStat software and Excel 2010 were used for analyzing.

Results and discussion

Table (1) showed that animals treated with single oral dose

(100mg/Kg) of sodium nitrite (GII) comprised 63% met-Hb, 45 minutes after nitrite treatment, and this percent was significantly reduced ($P < 0.05$) when animal groups pretreated with ethanolic extract of DPP in a dose of 100mg/kg, given as single oral dose 45 minutes before induction of methemoglobinemia by nitrite (GIII). Pretreatment of animals with oral dose of DPP extract (100mg/kg) for seven consecutive days before nitrite treatment (GIV group) significantly ($P < 0.05$) decreases the level of met-Hb measured in blood compared to that of control group treated with distilled water before sodium nitrite oral dose ($P < 0.05$); however, the percent inhibition of met-Hb formation over short term (GIII) treatment was less than that observed with long term (GIV) treatment

Table (1) Effect of pretreatment with oral dose (100mg/kg) of ethanolic extract of DPP for different time intervals before sodium nitrite oral dose (100mg/Kg) on percentage met-Hb blood level in rats.

Group	GI	GII	G III	GIV
Percentage of Met - HB	2.7 ± 0.04 D	63 ± 1.6 A	44.63 ± 1.05 B	30.15 ± 1.43 C

L.S.D = 1.49 N=6 large letters denote significant ($P < 0.05$) differences between group

GI: negative control received 0.5 ml DW orally by gavage needle and after 45 minutes received another 0.5 ml of DW then

GII: positive control received 0.5 ml of DW then after 45 minutes received 100mg/kg B.W sodium nitrite orally by gavage needle.

G III: received 100mg/kg B.W crude ethanolic extract of DPP orally by gavage needle then after 45 minutes received 100mg/kg B.W. sodium nitrite.

G IV: received 100mg/kg B.W crude ethanolic extract of DPP orally by gavage needle daily for seven days then after 45 minutes from last dose received 100mg/kg B.W. sodium nitrite.

discussion

Data represents mean \pm Stander Error (SD); significant compared to nitrite treated group ($P < 0.05$). Values with non-identical superscripts are significantly different ($P < 0.05$).

In living tissues, hemoglobin exists basically in the reduced form (Fe^{2+}) either saturated with an oxygen molecule (oxyhemoglobin), or desaturated of oxygen molecules (deoxyhemoglobin). After death, the concentration of oxy- and deoxy-hemoglobin progressively decreases as it is converted to methemoglobin which cannot bind oxygen. Therefore methemoglobin is the likely hemoglobin species to be involved in peroxidation of *post mortem* tissues (Kristinova *et al.*, 2014).

Methemoglobin is formed by the interaction of hemoglobin with nitric oxide and nitrites. (Umbreit, 2006 and Buehler & Alayash, 2005). MHb formed when ferrous iron (Fe^{2+}) of deoxyhemoglobin is converted to the ferric iron (Fe^{3+}) on exposure of erythrocytes to oxidizing agents and free oxygen radicals, (Callister, 2003).

Methemoglobinemia results from either inadequate enzyme activity or too much MetHb production. MetHb is continuously being formed in the normal red blood cells by the process of auto oxidation of ferrous ion of the hem complex to the ferric form. At the same time, MetHb is rapidly reduced to hemoglobin by intra erythrocytic MetHb reductase (Shihana *et al.*, 2011).

Our result confirmed the nitrite acute toxicity in animals received single oral dose (100mg/kg) of sodium nitrite, where significant elevation in metHb level, many studies documented the production of oxygenated free radicals along with a decrease in glutathione level within the intracellular medium with consequent

alteration of cell ionic flux the results also agrees with (Gluhcheva *et al.*, 2012) (Rahman, *et al.*, 2009). A major effect in the toxicology of NaNO_2 is the induction of methemoglobinemia. the chemical reactivity of NaNO_2 with hemoglobin may enhance hem- or iron- mediated toxicities. Nitrite is known to cause free radical generation (Kohn *et al.*, 2002), as it can stimulate oxidation of ferrous ions in oxyhemoglobin to form methemoglobin as well as various reactive oxygen species' (ROS) (Baky *et al.*, 2010,).

Oxidizing agents accelerate 100 to a 1,000 times the oxidation of Hb, and eventually overwhelm the capacity of reducing endogenous systems (Roigas *et al.*, 1970), they include several drugs (Manebe *et al.*, 1996). intoxication with pesticides, herbicides, and rtilizers (Gibson, 1984), automobile exhaust fumes (Evelyn and, Malloy 1938), and industrial chemicals . (Price 2002)

Accordingly, in the present study, the protective effect (100mg/kg) of DPP extract was assessed relative to their ability in reducing the level of metHb induced by nitrite. The data plotted in table one showed significant reduction in the level of metHb, suggesting the capacity of DPP extract to fight against the deleterious effect of nitrite by protecting RBCs from free radical-induced oxidative damage, and this result agreed with previous studies that reported the antioxidant activity of some DPP extract composition that present in other plant extraction.

previously reported, dietary DPP extract may prevent the production of oxidants in animal models, and exhibit significant protection against oxidative stress (Abbas and Ateya, 2011). through different mechanisms including inhibition of xanthine

oxidase and chelation of transition metals, inhibit oxidants from attacking the cellular target by electron donation and scavenging activities, block propagation of oxidative reaction by chain breaking antioxidant activity, and reinforce cellular antioxidant capacity; this probably in addition to their anti-inflammatory and modulatory effects on some signaling pathways that finally lead to lower oxidant production, (Akhlaghi and Bandy, 2009).

The NADH-MetHb reductase enzyme reduces MetHb to hemoglobin. This result agreed with previous studies that reported the antioxidant activity of DPP compositions, alkaloids, glycosides, phenols, flavonoids, saponin and tannins, (Alrikabi, 2011), that mention in table 1. And others record in previous report.

Benfotiamine, a vitamin B1 (one of DPP composition) analogue, protects hemoglobin and the plasma membrane of the erythrocytes against oxidation induced by addition of sodium nitrite (Marouf *et al.*, 2010 & Volver *et al.*, 2008). Many study showed that benfotiamine reduces superoxide and hydroxyl radical levels by inducing the activation of pentose phosphate pathway, which regenerates the antioxidant NADPH (Katara *et al.*, 2010). Significant reduction in MetHb% in both short- and long-term benfotiamine approaches followed in the present study might be explained by radical scavenging property of many antioxidants like benfotiamine, which exhibited antioxidant effect by reducing the oxidative stress and genomic damage caused by mitogenic model compounds; such effect is found to be related to its direct antioxidant capacity (Schmid *et al.*, 2008).

Glycolysis involves the breakdown of glucose in addition, reductive power

is generated in the form of NADH which can be used to reduce methemoglobin to hemoglobin (Richard and Wouter, 2005), red blood cells exposed to vitamins A survived for 30 hrs without lysis, oxidation and formation of methemoglobin. (Adaramoye *et al.*, 2005)

The chemical screening showed the presence of polyphenols among which anthocyanins. The biological activity of this plant would be due to these pigments. (Mpiana *et al.*, 2014).

Flavonoids being antioxidant ultimately maintain the hem iron in its ferrous form, which is associated with the production of defective methemoglobin (Ikpeme *et al.*, 2011).

Evaluated the possible ameliorative effect of many phytochemicals like polyphenols, (Koto-te-Nyiwa Ngbolua *et al.*, 2014), polyphenols, flavonoids, tannins, terpenoids, (Petricia Regina Irene *et al.*, 2012), alkaloids, and saponins. (Purushothaman *et al.*, 2011).

Carotenoids (one of DPP component) may exert therapeutical potential to act as a natural antioxidant to prevent ROO•-induced toxicity in human erythrocytes (Chisté *et al.*, 2014 & Rodrigues *et al.*, 2012).

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