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RESEARCH ARTICLE

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THE POTENTIAL EFFECT OF TIGERNUT OIL ON SOME HAEMATO-BIOCHEMICAL BLOOD INDICES IN MALE ALBINO RATS

ABSTRACT

The present work which is considered as an experimental study aimed to assess the effect of tigernut (*Cyperus esculentus*) oil on some haematological and biochemical parameters in normal adult rats to evaluate tigernut oil supplementation and its possible use as a therapeutic agent. Tigernut oil was extracted from tigernut tubers and injected intraperitoneally to rats at two different doses (0.1 ml/Kg bw and 0.5 ml/Kg bw) for successive six weeks. Some haematological and biochemical parameters were assayed. The studied haematological parameters including RBCs, WBCs and platelets counts, as well as haemoglobin, haematocrit, MCV, MCH and MCHC values revealed a positive change in rats treated with tigernut oil particularly at the higher tested dose (0.5 ml/Kg bw). Concerning the biochemical studies, the results revealed that the treatment with tigernut oil reduced serum glucose level at the two studied doses. Also, the data of liver and kidney functions including serum ALT and AST enzymes activity, as well as total bilirubin, urea, and creatinine levels recorded positive changes in rats that treated with tigernut oil and the changes were dose-dependent especially in serum total bilirubin. Moreover, serum total cholesterol showed significant decrease while serum HDL-c and HDL-c/ Total cholesterol ratio revealed significant increases at both treated doses of tigernut oil. However, the results of serum metals (sodium, potassium, calcium, and magnesium concentrations) showed non-significant change, while serum iron concentration was significantly increased. In conclusion, these data clarified that tigernut oil exhibits a good agent for maintenance and improvement the haemato-biochemical parameters in healthy rats. Thus, the supplementation with this oil has benefits and safety. Furthermore, it is hoped that further work goes in this direction to high light the potential use of tigernut oil for overcoming some healthy problems.

Key words : Tigernut (*Cyperus esculentus*) oil – Haemoto-biochemical parameters – Rats.

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INTRODUCTION

A large number of plants and their isolated constituents have been shown to modulate healthy problems (Kyo *et al*., 2001; Rohdewald, 2002). Some medicinal plants including tigernut (*Cyperus esculentus*) are the major source of fat in the traditional Mediterranean diet that may be associated with positive health effects (Fischer *et al.,* 1997; Abuharfeil *et al.,* 2001; Salem and Hossain, 2002; Salim and Fukushima, 2003; Lemaure *et al.,* 2007). Tigernut is a member of the grass family Cyperaceae. It is one of the common herbs in Egypt. Moreover, tigernut tubers are daily ingredients of the diet of many people in North Africa and Spain (Okladnikov *et al.,* 1977). The tubers contain about 27% oil, which are resistant to peroxidation, 50% digestible carbohydrates, 4% protein and 9% crude fiber (Shilenko *et al*., 1979; Emmanuel and Edward, 1984; Ezebor, 2005).

The extracted tigernut oil is rich in fatty acids including significant concentration of dominant saturated fatty acid; miristic acid and dominant unsaturated fatty acids such as oleic acid (72.00%), linoleic acid (9.40%), palmic acid (13.20%) and stearic acid (3.90%) (Eteshola and Oraedu, 1996; Chowdhury *et al.,* 2005). Tigernut oil fatty acids composition is comparable with that of olive oil (Mokady and Dolev 1970; Coskunerm *et al.,* 2002). Numerous studies document the beneficial effects of the ingestion of unsaturated fatty acids-enriched diet on health through its health maintenance and improvement properties as well as its role in the protection of the body against chronic diseases (Yokoyama and Origasa, 2003). It is well known recorded that tigernut oil had blood pressure lowering effects, in addition it can promote bile secretion, increase bile flow as well as it can inhibit the contraction in the intestinal tract of rabbits (Liu *et al*., 1989). At the same time, it acts as a hepatoprotective in the intoxicated experimental rat (Johnson and Mullinix, 2003). Furthermore, the oil can be suggested as a candidate agent for natural

preservative in the cosmetic and/or food industries, and as active compound in medical preparations such as chaemopreventive drugs in cancer therapy due to their antimutagenic activity (Gupta *et al.,* 1971).

However, although this oil is cheap and available with its characteristic lovely tastes, there are very rare reports about its nutritional value and its uses in the medical field. Therefore, it seems of interest in the present study to evaluate the effect of tigernut (*Cyperus esculentus*) oil at two different doses on some haematological and biochemical parameters in male albino rats to asses its benefits and safety.

MATERIAL AND METHODS

Materials

Tigernut **(***Cyperus esculentus* L**)** tubers were obtained from the local market at Tanta city, Egypt.

Oil extraction

For the preparation of tigernut powder, adequate quantity of mature brown tubers of tigernut were cleaned, washed and dried in a stream of hot air for an hour. The dried tubers were milled using a laboratory electric mill to pass through a 40-mesh sieve. After that, the extraction procedure described by Barminas *et al*. (2001) using petroleum ether at between 40-600° C for 12 hours in a Sohxlet apparatus to obtain the tigernut oil.

Animals groups and treatment:

The healthy adult male albino rats weighing 130±20 g were used in this study. Rats were housed at a constant temperature (24 ± 20 °C) with alternating 12-hour light and dark cycles and were given food and water *ad libitum*. They were randomly divided into three groups. The 1st group served as control. The 2nd and 3rd groups were injected intraperitoneally by 0.1 or 0.5 ml tigernut oil/ Kg body weight for six weeks, respectively (Liu *et al.,* 1989).

Sampling of blood and serum:

From each rat, two blood samples were collected at the end of the treatment. The 1st blood sample was taken on EDTA as anticoagulant for the determination of haematological parameters such as count of red blood cells (RBCs), white blood cells (WBCs) and blood platelets (PLT). Also, the haematocrit value (HCT), haemoglobin content (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were estimated. These parameters were determined using an automated haematological analyzer (Haemocel, 1600) according to Dacie and Lewis (1991). The 2nd blood sample was put into a clean centrifuge tubes and serum was separated by centrifugation for biochemical analysis.

Biochemical analysis:

Serum glucose (Trinder, 1969), urea (Patton and Grouch, 1977), creatinine (Henry, 1974), total bilirubin (Walter and Gerade, 1970), HDL-C (Burstein *et al.,* 1970) and total cholesterol (Allain *et al.,* 1974) levels were measured using Stanbio Kits (Stanbio Laboratory, INC. 2930 East Houstion Street San Antonio, Texas, USA). On the other hand, serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes activity (Reitman and Frankel, 1957) were estimated using a commercially available reagent kits obtained from Randox Lab Ltd, U.K. The serum electrolytes Na^{+}, K^{+}, Ca^{+2}, Mg^{+2} and Fe^{+2} were estimated by an atomic absorption spectrophotemeter (Zettner and Seligson, 1964).

Statistical analysis:

All results were expressed as means ± SE and % of change of five rats. The obtained data were statistically evaluated using the Student *t*-test. The differences between groups were considered non significant when P>0.05, significant at P<0.05 and highly significant at P<0.01 (Sokal and Rahif, 1981).

RESULTS

I- Haematological data:

Table 1 showed RBCs, WBCs and PLTs counts, haemoglobin, haematocrit, MCH and MCHC values in control and tigernut oil treated rats. In the tigernut oil treated rat groups and as compared to the control one, the obtained data revealed a highly significant increase in most of the above mentioned haematological parameters especially at the higher tested dose level with the exception of WBCs and PLTs counts, where the increase was insignificant at the two studied doses. Concerning the obtained MCV value the data recorded a marked decrease at both doses of tigernut oil.

Table 1. Haematological parameters in control and tigernut oil treated rat groups

Parameters \ Groups	Control	Tigernut Oil (0.1ml/kg bw)	Tigernut Oil (0.5 ml/kg bw)
RBCs (10^6/ml) *% of change*	6.4 ± 0.4	7.1 ± 0.7* *+10.9*	9.0 ± 0.6*** *+40.6*
WBCs (10^3/ml) *% of change*	9.0 ± 0.3	9.1 ± 0.2* *+ 1.1*	9.5 ± 0.3* *+ 5.6*
PLTs (10^3/ml) *% of change*	330 ± 2.5	332 ± 3.1* *+ 0.61*	336 ± 2.9* *+ 1.8*
Hb (g/dl) *% of change*	7.3 ± 0.4	8.9 ± 0.7** *+ 21.9*	11.8 ± 0.3*** *+ 61.6*
HCT (%) *% of change*	40.0 ± 0.6	42.5 ± 0.5*** *+ 6.3*	46.6 ± 0.7*** *+ 9.6*
MCV (µ3) *% of change*	62.5 ± 0.7	59.9 ± 0.6*** *- 4.2*	51.8 ± 0.5*** *- 17.1*
MCH (pg/dl) *% of change*	11.4 ± 0.04	12.5 ± 0.04*** *+ 9.6*	13.1± 0.06*** *+ 14.9*
MCHC (%) *% of change*	18.3 ± 0.06	20.9 ± 0.1*** *+ 14.2*	25.3 ± 0.08*** *+ 38.3*

Data are expressed as mean ± SE of five rats.
*: Non-significant P>0.05 **: Significant P<0.05
***: Highly significant P<0.01.

II- Biochemical data:

It is evident from table 2 that serum glucose level was significantly declined in the tigernut oil treated rats at the two tested doses. The maximum significant decrease was recorded at the higher dose of tigernut oil treated rats group in comparison with that of the control group.

On the other hand, the data obtained for the transaminases (ALT and AST) activity and total bilirubin, urea and creatinine levels, the results indicated that the treatment with tigernut oil for six weeks did not induce pronounced change on these parameters, where their recorded values seemed to be normal with the exception of bilirubin level where it recorded significant decrease at the higher tested dose of tigernut oil treatment if compared with control group (Table 2).

Moreover table 2 illustrated that serum HDL-C as well as HDL-c / total cholesterol values showed highly significant increase in tigernut oil treated rats at both tested doses. As a consequence, highly significant decrease in total cholesterol level was observed in rats treated with the same tested doses of tigernut oil.

Table 2. Biochemical parameters in serum of control and tigernut oil treated rat groups

Parameters \ Groups	Control	Tigernut Oil (0.1ml/kg bw)	Tigernut Oil (0.5 ml/kg bw)
Glucose (mg/dl) % of change	98.5 ± 1.8	92.9 ± 1.8** *- 5.7*	77.2 ± 2.4*** *- 21.6*
ALT (U/l) % of change	21.2 ± 0.2	21.0 ± 0.3* *- 0.9*	20.1 ± 0.1* *- 5.2*
AST (U/l) % of change	69.5 ± 1.0	68.3 ± 1.1* *- 1.7*	67.1 ± 1.3* - 3.5
ALT/AST Ratio % of change	0.31±0.03	0.31 ± 0.02* *0.0*	0.30 ± 0.01* *- 3.2*
Total bilirubin (mg/dl) % of change	1.08 ± 0.05	0.99 ± 0.04* *- 8.3*	0.97 ± 0.03** *- 10.2*
Urea (mg/dl) % of change)	20.5 ± 1.2	19.8 ± 2.3* *- 3.4*	18.1 ± 1.8* *- 11.7*
Creatinine (mg/dl) % of change)	0.88 ± 0.01	0.86 ± 0.02* *-2.3*	0.81 ± 0.01* *- 7.95*
HDL-c (mg/dl) % of change	32.5 ± 1.5	43.5 ± 1.2*** *+33.8*	64.1 ± 1.4*** *+ 97.2*
Total cholesterol (mg/dl) % of change	110.4 ± 2.2	98.4 ± 2.1*** *-10.9*	88.1 ± 2.2*** *- 20.2*
HDL-c/Total cholesterol ratio % of change	0.29 ± 0.02	0.44 ± 0.01*** *+ 51.7*	0.73 ± 0.03*** *+151.7*

Data are expressed as Mean ± SE of five rats.
*: Insignificant P > 0.05
**: Significant P < 0.05
***: Highly significant P < 0.01.

Table 3. Serum metals concentration in control and tigernut oil treated rat groups

Parameters \ Groups	Control	Tigernut Oil (0.1ml/kg bw)	Tigernut Oil (0.5 ml/kg b w.)
Sodium (mg/dl) *% of change*	334.3 ± 2.4	331.3 ± 2.3* - 0.84	335. 2± 2.1* + 0.27
Potassium (mg/dl) *% of change*	30.8 ± 0.41	29.9 ± 0.31* *- 2.9*	31.1 ± 0.34* *+ 0.97*
Calcium (mg/dl) *% of change*	4.7 ± 0.22	4.8 ± 0.21* + 2.1	4.9 ± 0.18* + 4.3
Magnesium (ppm) *% of change*	11.9 ± 0.12	11.8 ± 0.21* - 0.84	12.1 ± 0.13* +1.7
Iron (ppm) *% of change*	0.71 ± 0.03	0.79 ± 0.02** *+ 11.3*	0.88 ± 0.02*** *+ 23.9*

Data are expressed as mean ± SE of five rats
*: Insignificant P > 0.05 **: Significant P < 0.05
***: Highly significant P < 0.01

Serum metals (sodium, potassium, calcium and magnesium) concentrations as shown in table 3 were insignificantly affected. On the other hand, serum iron level was significantly increased at the two tested doses of tigernut oil if compared to control group.

DISCUSSION

Unsaturated fatty acids are now a nutritional hot topic, and their presence in foods has attracted both public and industrial interest (Thomsen *et al.,* 1999; MacIntosh *et al.,* 2003; Mozaffarian *et al.,* 2004; Miles, 2006). The primary objective of the study, therefore, was to determine whether tigernut oil has any effect on some haematological or biochemical parameters in healthy male albino rats.

The haematological results of this study provide clear evidence that the intraperitoneal injection of tigernut oil to normal rats at different doses caused an increase in RBCs, WBCs, and PLTs counts as well as Hb and HCT values. The observed increase in RBCs count, as well as Hb and HCT values may be attributed to the synchromous increase in serum iron absorption in the different treated groups. This may be due to the high content of iron in tigernut tubers (Addy and Eteshola, 1984 ; Jeong *et al.,* 2000).

However, the mild increase in WBCs count may occur due to the treatment with the natural plant as an indication of defence mechanism and immune response (Ghazanfar, 1994). Moreover, the obtained positive change in haematological parameters in rats treated with tigernut oil may be attributed to its high contents of unsaturated fatty acids where they can improve certain haematological parameters. These findings are consistent with previous study (Brown and Roberts, 1991) that shows the beneficial effect of fish oil supplemented diet on haematological variables related to cardiovascular disease. Similarly, a series of studies have reported that the diets enriched with monounsaturated fatty acids especially oleic acid influence the developing haematological indices as indicated by the improvement in the red blood cells characteristics where its count and HCT value were significantly increased in piglets fed from birth to 18 day with formulas containing canola oil with high oleic acid (Sheila *et al.,* 1999).

The present data of the biochemical studies suggested also the desirable effects of tigernut oil on most the estimated parameters including glucose, lipid fractions, transaminases, and electrolytes. Concerning the effect of tigernut oil on glucose level, there was a good correlation of dose effect relationship, where the occurred maximum significant decrease was recorded at higher treated dose of tigernut oil treatment. Such effect however may be attributed to the antidiabetic action of tigernut (Raut and Gaikwad, 2006). The hypoglycemic effect of tigernut oil may be related to the ability of the unsaturated fatty acids of the tigernut to increase the number of insulin receptors and to decrease hepatic gluconeogenesis (Raut and Gaikwad, 2006), therefore increasing insulin activity and metabolic improvements (Das, 1995 ; Merzouk and Khan, 2003; Rivellese and Lilli, 2003).

Furthermore, the effect of tigernut oil on serum total bilirubin level and transaminases (ALT and AST) activity which used as markers of liver functions was investigated in the present study.The current results confirmed by previous studies of Ghazanfar (1994), Mehta *et al*. (1999) as well as Johnson and Mullinix (2003). In the present study, a desirable change in the estimated liver function parameters was detected reflecting the role of the studied oil as a hepatoprotective agent. This findings, however is in agreement with Mehta *et al*. (1999). In this concern, a number of studies have also suggested the positive relation between the consumption of oil enriched with mono and poly unsaturated fatty acids and liver enzymes activity (Rustan *et al.,* 1993; Owu *et al.,* 1998; Edemm and Akpanabiatu, 2006).This could be attributed to the effect of unsaturated fatty acids on the membranes that keep the liver integrity and the permeability of the membranes constant (Owu *et al.,* 1998). The transaminases (ALT and AST) are of value as indices of possible liver damage, in detecting the presence of toxicity to the liver or alterations in membrane architecture of the cells of the liver. More important than the absolute ALT and AST values is the ALT/AST ratio where a high ALT/AST ratio indicates pathology involving the liver (Stroev and Makarova, 1984). ALT/AST value when greater than 1.00 indicates alterations involving the liver cells (Tietz, 1982). The present ALT/AST ratio did not indicate possible adverse pathological effects involving the livers of the test rats that treated with tigernut oil. It does appear that the consumption of tigernut oil supports normal enzyme activities.

Regarding the results of kidney functions (urea and creatinine), it was found that tigernut oil play a beneficial role in the maintenance of the normal renal functions. Positive effect of polyunsaturated fatty acids (PUFA) in retardation of the progression of chronic renal disease is known since last decades and is under evaluation. PUFA are beneficial on the lipid and immune abnormalities secondary to chronic renal failure (CRF) and may have a useful effect on progression of CRF (Reddy *et al.,* 2002 ; Tsipas and Morphake, 2003). As reported by Melhado *et al,* (1992), this effect may be attributed to the positive role of unsaturated fatty acids in preservation of glomerular filtration rate and effective renal plasma flow.

According to the obtained data the present study adds another potential benefit of tigernut oil to previously mentioned benefits, which appear to aid in the induction of a good lipid profiles resulting from inhibiting the biosynthesis of cholesterol concentration and elevating HDL-cholesterol concentration. In addition, the elevation in the HDL-C/ total cholesterol ratio was observed during treatment with tigernut oil and this is entirely acceptable due to changes in HDL-C and total cholesterol. Such results may be due to the presence of high amount of mono unsaturated fatty acids (MUFAs) such as oleic acid in tigernut oil where MUFAs increase HDL-c concentration in both animals and humans, primary by delaying the clearance of HDL apo A-I from the plasma compartment (Brousseau *et al.,* 1995). Also, poly unsaturated fatty acids (PUFAs) such as linoleic acid which is mainly present in tigernut oil was found to decrease LDL-C and VLDL as well as LDL-C /HDL-C ratio indicating the inhibitory effect of PUFAs on the hepatic synthesis and secretion of triglyceride-rich VLDL (Nenseter *et al.*, 1992 ; Rustan *et al.,* 1993). On its own, the major n-6 fatty acid in the diet is α-linoleic acid, which serves as a precursor for arachidonic acid (20:4n-6), which has important biological effects in the body (Siguel *et al*., 1987).

Additionally, this study denoted a normal serum electrolytes (Na^+, K^+, Ca^{+2}, and Mg^{+2}) concentration after treatment with tigernut oil. It has been suggested that this oil is more save for the physiological activity of the muscle and cell membrane permeability resulting electrolytes balance. While the observed significant change in serum irons concentration may be due to increased iron absorption in rats treated with tigernut oil.

Another explanation for the positive effect of tigernut oil on the selected tested parameters may be attributed to another volatile constituent including α-copaene, cyperene, β-selinene, β-cyperone and α-cyperone which are found in the oil of tigernut tubers and makes up about 0.5-1.0 % of the dried tubers as previously mentioned by Tam *et al.* (2007). However this mechanism needs further investigations. Along this line, the obtained desirable action of tigernut oil on the estimated parameters may be related also to its antioxidant activity through its inhibitory effects on nitric oxide and its ability to scavenge the oxidative-initiating agents in addition to superoxide productions resulting in the maintenance of the cellular functions (Seo *et al,* 2001; Pal and Dutta, 2006).

In conclusion, the present results provide evidence that tigernut oil has a beneficial effects on both haematological and biochemical blood indices and can be used for maintenance and improvement the physiological status.

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فاعلية تأثير زيت حب العزيزعلى بعض دلائل الدم الهيماتولوجية والبيوكيميائية في ذكور الجرذانِ البيضاءِ

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فى كثيرمن مجالات الطب والغذاء يثارالحديث حول الأحماض الدهنية غير المشبعة واثرها في الحفاظ على صحة الإنسان ، وقد تبين أن الزيوت النباتية من أكثر المواد الغنية بهذه الأحماض ، ومن ثم اجريت هذه الدراسة لإلقاء الضوء على أحد هذه الزيوت وهو زيت حب العزيز لندرة التعامل معه ، وقد تم إختبار تأثيره على بعض المعايير الهيماتولوجية والبيوكيميائية في ذكور الجرذانِ البيضاءِ لتقييم مدى امكانية استخدامه فى الوقاية من بعض المشاكل الصحية.

بعد استخلاص الزيت من درنات حب العزيز أو ما يعرف بنبات السعد تم حقنه للجرذان بجرعتين مختلفتين (0.1مللى و 0.5 مللى / كجم من وزن الجسم) لمدة ستة اسابيع متتالية وقورنت التغيرات فى هاتان المجموعاتان بالمجموعة الضابطة. وقد تم قياس بعض دلائل الدم الهيماتولوجية والبيوكيميائية لمعرفة التأثير الحادث على الجرذان بعد تعاطى زيت حب العزيزلمدة ستة أسابيع. وقد أظهرت النتائج زيادة ملحوظة فى كل من عدد كرات الدم الحمراء ومستوى الهيموجلوبين والهيماتوكريت وكانت الزيادة مضطردة بزيادة الجرعة، كما سجلت الدراسة زيادة ولكن ذو دلالة غير احصائية فى عدد كريات الدم البيضاء والصفائح الدموية. أما بالنسبة لباقى معايير صورة الدم الكيمائية (MCV – MCH - MCHC) كان التأثير ذومغزى احصائي عند كلا الجرعتين. كما سجلت النتائج البيوكيميائية نقص فى مستوى الجلوكوزفى المصل وهذا الإنخفاض كان له تأثير ذو دلالة احصائية مرتفعة عند الجرعة العالية، بالإضافة للتغيرات الإيجابية فى وظائف الكبد والكلى فى الجرذان المعاملة بزيت حب العزيزوقد استدل على ذلك من الإنخفاض الحادث فى مستوى البيليروبين الكلى والنقص الطفيف فىنشاط انزيمات الكبد والبولينااا والكرياتينين فى مصل الدم. أيضا تم تسجيل نقص ملحوظ فى مستوى الكوليسترول الكلى وزيادة فى الليبوبروتين ذو الكثافة المرتفعة فى مصل الجرذان المعاملة مقارنة بالجرذان الضابطة. ومن ناحية أخرى فقد لوحظ دور زيت حب العزيز فى الحفاظ على الإتزان الملحى حيث احتفظ مستوى كل من الصوديوم والبوتاسيوم والكالسيوم بالتركيز الطبيعى بينما زاد تركيز الحديد زيادة معنوية بزيادة الجرعة.

وبناء على ما سبق : فإن البحث يشير الى الدور الإيجابى لزيت حب العزيز فى الحفاظ على المحتويات القياسية للدم ويعزى ذلك لإحتوائه على كميات كبيرة من الأحماض الدهنية غير المشبعة الأحادية الممثلة فى حمض اوليك ذى التأثير الفعال فى حماية الجسم من الأمراض، لذا نوصى بالمزيد من الدراسات المستقبلية لإثبات إمكانية استخدامه في الحد من بعض المشاكل الصحية.

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