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ONION AND ITS ACTIVE CONSTITUENTS AGAINST AGING

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ABSTRACT

Aging is the normal transition time after a flurry of activity. Although there are quite a few age-related changes that tax the body, disability is not necessarily a part of aging. Health and lifestyle factors together with the genetic makeup of the individual, determine the response to these changes. In this study, we try to ameliorate the abnormalities accompanying aging by natural products known by their highly antioxidant effect.

In this pilot study thirty male aged rats (1.5-2 years old) (measured according to length of tail and weight of rats) were divided into three groups (10/each group); a control aged group, onion oil treated group and dipropyl disulphide (DPDS) treated group. Another thirty male adult rats (≈ 6 months old), had the same division as the aged ones. Biochemical measurements were done at the end of the experiment (4 weeks) to study the treatments effect on liver function, kidney function, metabolic effect, monoamines and fertility.

All the liver function indicators; AST, ALT, GGT, LDH, ALP and total bilirubin were significantly high ($p<0.001$) in the aged group and decreased significantly after treatments, while the adult groups were non-significantly changed. The measured kidney function biomarkers showed also the change only with aged and aged treated groups with a significant ($p<0.001$) decrease in total protein and albumin and an increase in urea and creatinine concentration, these changes ameliorated in the same manner with either onion oil or DPDS. The

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lipid profile showed a high cholesterol, triglyceride concentration ($p<0.05$) and HDL and LDL concentration ($p<0.001$) but a non-significant increase in HDL concentration. The tested treatments succeed to improve ($p<0.001$) this lipid profile changes except HDL concentration. The monoamines level was decreased in the aged rats then increased significantly after treatments ($p<0.05$ with norepinephrine & serotonin and $p<0.001$ with dopamine). Taking testosterone as indicators for fertility, the treatments had a significant increment effect ($p<0.001$) on the decreased testosterone level of the aged rats.

the present pilot study demonstrate that onion oil and its active constituent are good fighters for aging while it do change on liver function, Kidney function, Protein, Lipid profile, monoamines and fertility hormones and need more study to prevent earlier aging.

Keywords:Onion oil, liver function, Kidney function, Protein, Lipid profile, monoamines.(dopamine, nor epinephrine, serotonin), testosterone, DPDS

INTRODUCTION

Aging is a syndrome of changes that are deleterious, progressive and universal. Aging damage occurs to molecules (DNA, proteins, lipids), to cells and to organs. Diseases of old age (diseases which increase in frequency with age, such as arthritis, osteoporosis, heart disease, cancer, Alzheimer's Disease, etc.) are often distinguished from aging (**Chen, *et al.,* 2007**).

The use of herbs and medicinal plants as the first medicines is a universal phenomenon. The onion is one of the oldest cultivated vegetables in history. Sulphur compounds in onions have been shown to be anti-inflammatory both by inhibiting formation of thromboxanes and by inhibiting the action of platelet-activating factor (PAF). Thiosulfinates condition anti-thrombotic benefits, including antioxidant activity reduced serum cholesterol and enhance in vitro platelet activity **(Briggs & Goldman ,2002)**. This later effect is important for cardiovascular health by reducing the probability that platelets aggregate in the blood, a major cause of heart attacks and strokes. Hence, thiosulphinates found in onion have been shown to inhibit in-vitro platelet aggregation (**Azu, & Onyeagba, 2006**).

Flavonoids are a second class of health enhancing compound produced by onions, they are chemical compounds active against microorganisms. They have been found in-vitro to be effective antimicrobial substance against a wide array of microorganisms such as the immune system, gene expression, blood flow and liver function. The beneficial health effects of the flavonoids are their antioxidative activity (**Harris, *et al*., 2001**). Antioxidants play a major role in defending cellular mitochondria against damage in the mitochondrial theory of aging (**Cheluvappa, *et al*., 2007)**.

Onions are indigestible plant components that fermented in the body and help maintain the health of the gut and colon; **(Suru, 2008).**

Also, onions are useful in lowering cholesterol; it is converted into bile acids. They are thought to protect against some cancers by breaking down the cholesterol rich membranes of cancer cells. Saponins have been anti- inflammatory, antifungal, antiyeast, anti-parasitic, antibacterial, antimicrobial and antiviral activity (**Augusti, 1996**). Other studies are being undertaken the potential relationships between onions consumption and diabetic management, antiasthmatic roles and anti-inflammatory roles, the positive role on brain health and memory. In addition, onions may have therapeutic effects, including an antidote for heavy metal poisoning and liver protective properties, and monoamines (**El-Shenawy, *et al*., 2008)**.

The present study aimed to investigate the in vivo effects of onions on aging by their highly antioxidant effect.

MATERIALS AND METHODS

1- Experimental Animals

Thirty white male albino rats (*Rattus norvegicus*) aging about 3-4 months and weighting about 100 – 120 gm. were used as adult animals. Another thirty of the same genus aging about (1.5 – 2 years) and weighting about 250 – 400 gm. are used as senile animals. The rats were obtained from the animal house of Viscera Research Institute, Giza, Egypt. They were kept under observation for 7 days before onset of the experiment to exclude any intercurrent infection. The animals were housed in plastic good aerated cages at normal atmospheric temperature (25 ± 5ºC) as well as normal 12 hours light / dark cycle. They were given daily access of water and standard diet.

2-Treatment agents:

Onion oil was purchased from (Cap–pharm) El-Obour city, Cairo, Egypt**.**1ml corn oil/kg b.wt. was given daily for 4 weeks. Dipropyl disulphide with its chemical formula is C_3H_7-S-S- C_3H_7 was purchased from Aldrich Co., U.S.A., in a dose of 100 mg in 0.8 ml distilled water was given daily for 4 weeks.

3– The experimental design:

The rats were divided into six groups (10 rats/one):

1- The first group was regarded as adult control group, and given 1ml corn oil/kg b.wt. during the experimental time. (4 weeks). The second group was orally given onion oil.

2- The third group was orally given dipropyl disulphide (DPDS).

3- The fourth group was regarded as aged control group, and given 1ml corn oil/kg b.wt. during the experimental time (4weeks).

4- The fifth group was orally given onion oil.

5- The sixth group was orally given dipropyl disulphide (DPDS).

By the end of the experiment at the day before scarifying, animals were deprived of food overnight (10-12) hours. The trunk blood samples were collected and left to coagulate and were centrifuged at 3000 r.p.m. for 15 minutes. Serums obtained were used to measure all the parameters needed.

Biochemical measurements:

Transaminases and lactate dehydrogenase activities in the liver was determined according to **Schumann and Klauke (2003).** Alkaline phosphatase (ALP) activity was determined**.** Total Bilirubin concentration was determined according to **Doumas *et al*. (1971).** Kits were purchased from Human Diagnostics Chemical Company (Germany).

Total protein was determined according to **Henry and Row (1964).** Albumin concentrations were determined according to **Doumas *et al*. (1971).** Urea concentration was determined according to **Patton and Crouch (1977).** Creatinine concentration was determined according to Henry **(1984).**

Lipid profile was measured using reagent kits purchased from Reactivos Spinreact Company (Spain). Total lipids concentration was determined according to the method of **Frings *et al*. (1972).** Triglycerides concentration was determined according to the method of **Fossati and Prencipe (1982).** Total cholesterol and HDL-

cholesterol concentration, after precipitation of LDL and vLDL-cholesterol, were measured according to **Allain *et al.* (1974).** LDL-cholesterol concentration was determined according to **Friendewald *et al*. (1972)** formula:

LDL –Cholesterol = total cholesterol – $\frac{\text{triglyceride}}{5}$ – HDL – cholesterol

Serum vLDL-cholesterol was calculated according to formula: vLDL-cholesterol concentration = triglycerides / 5, (**Gruntysm, et al, 2004**).
Monoamines concentration was detected in tissue (in which monoamine activity is determined according to thiamine deficient – rats) by high performance liquid chromatography (HPLC) according to **Hendrickson and Meredith (1984).** Testosterone was measured according to **Ruchhoft *et al*. (1996)** using reagent kits purchased from Dia Sorin (Italy).

Statistical analysis:
Data analysis was made using SPSS version 15.0 for windows.

RESULTS

All the liver function indicators; AST, ALT, GGT, LDH, ALP and total bilirubin were significantly high ($p<0.001$) in the aged group and decreased significantly after treatments, while the adult groups were non-significantly changed. The measured kidney function biomarkers showed also the change only with aged and aged treated groups with a significant ($p<0.001$) decrease in total protein and albumin and an increase in urea and creatinine concentration, these changes ameliorated in the same manner with either onion oil or DPDS. The lipid profile showed a high cholesterol, triglyceride concentration ($p<0.05$) and HDL and LDL concentration ($p<0.001$) but a non-significant increase in HDL concentration. The tested treatments succeed to improve ($p<0.001$) this lipid profile changes except HDL concentration. The monoamines level was decreased in the aged rats then increased significantly after treatments ($p<0.05$ with norepinephrine & serotonin and $p<0.001$ with dopamine). Taking testosterone as indicators for fertility, the treatments had a significant increment effect ($p<0.001$) on the decreased testosterone level of the aged rats.

Table 1: Changes in liver function of adult and aged rats treated with onion oil or DPDS.

Parameter / Group	AST (U/L)	ALT (U/L)	GGT (U/L)	LDH (U/L)	ALP (U/L)	Total bilirubin (md/dl)
Adult	0.257 ± 0.061^{c}	179.50 ± 15.672^{d}	282.500 ± 23.399^{bc}	12.333 ± 0.764^{cd}	31.333 ± 1.229^{cd}	36.00 ± 1.713^{bc}
Adult + onion oïl	0.213 ± 0.029^{c}	167.833 ± 23.999^{d}	237.00 ± 18.590^{c}	11.166 ± 1.352^{de}	30.167 ± 1.815^{d}	36.333 ± 1.764^{bc}
Adult + DPDS	0.170 ± 0.020^{c}	155.667 ± 16.511^{d}	297.30 ± 58.126^{bc}	9.666 ± 0.882^{e}	32.667 ± 0.882^{cd}	33.167 ± 1.887^{c}
Aged	0.577 ± 0.069^{a}	684.333 ± 26.669^{a}	402.167 ± 30.714^{a}	38.833 ± 1.424^{a}	57.333 ± 4.753^{a}	49.833 ± 3.945^{a}
Aged + onion oïl	0.333 ± 0.036^{bc}	272.666 ± 24.528^{c}	359.500 ± 27.948^{ab}	16.166 ± 1.014^{b}	37.50 ± 1.911^{c}	37.833 ± 1.352^{bc}
Aged + DPDS	0.312 ± 0.034^{bc}	407.166 ± 23.736^{b}	321.50 ± 16.714^{abc}	15.50 ± 1.608^{bc}	48.50 ± 1.996^{b}	41.667 ± 1.453^{b}
F – probabilityy	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001
LSD at. the 5% level	0.173	86.574	88.552	3.312	6.786	7.142

*Data are expressed as Mean ± SE. *Number of animals in each group is ten.

*Means which are not significantly different are followed by the same superscript symbol (s).

Table 2: **Changes in proteins and kidney function of adult and aged rats treated with onion oil or DPDS.**

Parameter / Group	Total protein (g/L)	Albumin (g/L)	Urea (mg/dl)	Creatinine (mg/dl)
Adult	**12.53 ± 0.536^{a}**	**4.597 ± 0.120^{a}**	**18.666 ± 0.882^{c}**	**0.594 ± 0.036**
Adult + onion oïl	**14.958 ± 0.210^{a}**	**4.532 ± 0.106^{a}**	**12.667 ± 2.124^{c}**	**0.584 ± 0.091**
Adult + DPDS	**14.039 ± 0.331^{a}**	**4.428 ± 0.141^{a}**	**14.560 ± 2.028^{c}**	**0.563 ± 0.066**
Aged	**5.772 ± 0.210^{c}**	**3.217 ± 0.209^{c}**	**36.500 ± 3.749^{a}**	**0.789 ± 0.020**
Aged + onion oïl	**6.385 ± 0.089^{bc}**	**3.965 ± 0.134^{b}**	**27.333 ± 2.642^{b}**	**0. 744 ± 0.008**
Aged + DPDS	**7.220 ± 0.212^{b}**	**3.618 ± 0.111^{bc}**	**27.166 ± 2.272^{b}**	**0.585 ± 0.020**
F – probabilityy	**p<0.001**	**p<0.001**	**p<0.001**	**P>0.05**
LSD at. the 5% level	**1.274**	**0.402**	**6.8676**	**-**
LSD at. the 1% level	**1.716**	**0.541**	**9.248**	**-**

*Data are expressed as Mean ± SE. *Number of animals in each group is ten.

*Means which are not significantly different are followed by the same superscript symbol (s).

Table 3: **Changes in lipid profile of adult and aged rats treated with onion oil or DPDS.**

Parameter / Group	Triglycerides (mg/dl)	Total cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Adult	56.00 ± 4.663^{c}	56.167 ± 5.771^{ab}	23.833 ± 1.64^{b}	31.667 ± 1.685	10.833 ± 0.601^{d}
Adult + onion oïl	51.833 ± 3.962^{c}	55.333 ± 4.849^{ab}	21.333 ± 1.452^{b}	34.833 ± 1.557	11.667 ± 0.881^{cd}
Adult + DPDS	60.333 ± 5.449^{c}	53.167 ± 2.837^{b}	21.667 ± 1.173^{b}	32.167 ± 0.945	10.500 ± 0.763^{d}
Aged	110.833 ± 9.482^{a}	74.667 ± 5.921^{a}	41.833 ± 2.089^{a}	31.333 ± 1.116	22.333 ± 1.054^{a}
Aged + onion oïl	74.00 ± 9.954^{bc}	58.333 ± 5.983^{ab}	38.667 ± 1.838^{a}	35.667 ± 0.715	17.167 ± 0.872^{b}
Aged + DPDS	64.166 ± 5.449^{bc}	57.333 ± 7.136^{ab}	38.500 ± 1.669^{a}	34.00 ± 1.155	13.500 ± 0.764^{c}
F – probabilityy	p<0.05	p<0.05	p<0.001	P>0.05	p<0.001
LSD at. the 5% level	32.869	20.181	4.818	--	2.409

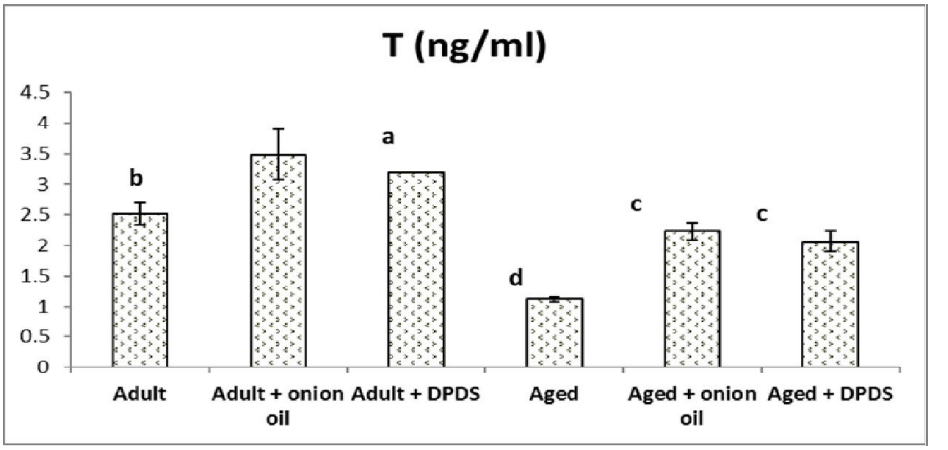
*Data are expressed as Mean ± SE. *Number of animals in each group is ten. *Means which are not significantly different are followed by the same superscript symbol (s).

Table 4: **Changes in monoamines (serotonin, (**Ser), Dopamine, (DOP**), Noradrrenaline(NE)) of adult and aged rats treated with onion oil or DPDS.**

Parameter / Group	Monoamines (µg/g tissue)		
	Ser	DOP	NE
Adult	0.063 ± 0.001^{a}	0.516 ±0.014^{a}	1.509 ± 0.025^{a}
Adult + onion oil	0.066 ± 0.002^{a}	0.513 ± 0.004^{a}	1.494 ± 0.017^{a}
Adult + DPDS	0.059 ± 0.001^{a}	0.534 ± 0.019^{a}	1.434 ± 0.043^{ab}
Aged	0.036 ± 0.010^{c}	0.355 ± 0.007^{c}	0.991 ± .028^{d}
Aged + onion oil	0.044 ± 0.001^{b}	0.403 ± 0.007^{b}	1.372 ± 0.013^{bc}
Aged + DPDS	0.049 ± 0.002^{b}	0.390 ± 0.011^{b}	1.313 ± 0.047^{c}
F – probabilityy	p<0.001	p<0.05	p<0.05
LSD at. the 5% level	0.006	0.034	0.091
LSD at. the 1% level	0.009	0.046	0.123

*Data are expressed as Mean ± SE.*Number of animals in each group is ten. *Means which are not significantly different are followed by the same *superscript symbol (s).

Figure 1: **Changes in fertility hormones of adult and aged rats treated with onion oil or DPDS.**



DISCUSSION

Aging is a universal process to humans. At present it is an international phenomenon that is occurring across the globe including the third world. Aging has economic, ethical, lifestyle, political and health care implications to society. In aging tissues the oxidative stress increases due to decreased activity of antioxidant enzymes and proteolysis increases due to decreased activity of aminotransferases (**Moorthy, et al., 2005).**

Ageing of the brain is an important factor in overall ageing and mortality, and new insights have the relationship between neuroregulation and ageing. (**Bernard, 2002).**

Onions are native to Asia and the Middle East and have been cultivated for over five thousand years. Onions were highly regarded by the Egyptians. Not only did they use them as currency to pay the workers who built the pyramids, but they also placed them in the tombs of kings, such as Tutankhamen, so that they could carry these gifts bestowed with spiritual significance with them to the afterlife **(George, 2014**).

Polyphenol content of onions including their rich concentration of flavonoid polyphenols, onions also provide a particularly large amount of quercetin. Onion's antioxidants—including its hallmark flavonoid antioxidant, quercetin also with anti-inflammatory benefits. These antioxidants help prevent the oxidation of fatty acids produces fewer pro-inflammatory messaging molecules **(Azuma, *et al.*, 2007**).

Serum liver enzyme activities and total bilirubin concentration were represented in (**Table, 1)**. Experimental data which obtained from the adult control group revealed the mean values of these enzyme activities. These all values weren't significantly changed either with onion or DPDS treatment. On the other hand, the experimental data recorded a significant ($P< 0.001$) elevation of these enzyme activities. In aged group. These elevations were significantly decreased with both the treatments except with LDH activity which showed non-significant increase. Bilirubin showed non-significant change in the adult groups while it increased significantly ($P< 0.001$) in the aged rats and ameliorated significantly after treatments. These results agreed with **Elnima, *et al*. (1983) and Griffiths, *et al.* (2002).**

Total protein and albumin detailed high levels in the adult control group and did not change significantly after treatments. (**Table 2**).The aged group recorded low levels of total protein and albumin these levels increased after treatment to record higher level of total protein with DPDS and higher level of albumin with onion oil. Urea exhibited no significant difference between the adult groups while it displayed a significant increase in the aged group compared to adult one. Creatinine no significance between the experimental groups at all. These findings detected by **Didry, *et al.* (1987) and Ali, *et al*. (2000).**

The lipid profile was indicated in (**table, 3)**, triglyceride and LDL–cholesterol showed the same manner in their measurement, with the significance only between the adult groups and aged groups with no significant effect of treatments. On the other hand, cholesterol levels recorded no significant difference between all the groups except between the aged group and the adult DPDS- treated group. The effect of treatments was indicated significantly in the aged group. DPDS indicate more decreasing effect than onion oil for the aged group, for the aged group treated with onion oil and for the aged group treated with DPDS. These results observed by **Azuma, *et al* (2007)** and **Kook, *et al*. (2009).**

Monoamines (presented by norepinephrine, dopamine and serotonin) recorded the same behavioural way with significant decrease in the aged group compared to the adult. The adult groups showed no significant difference with the adult-treated groups. The aged treated groups presented a significant amelioration compared to the aged on but with no significant difference between the treatments **(table 4).** These results disagreed with findings by **El-Aasr et al. (2010).**

The fertility was presented here by measuring testosterone hormone (**figure 1**) with a high significant decrease in the aged group compared to the adult control group. Treatments either with onion oil or DPDS indicated a significant increase in adult and aged ones. These findings agreed with **Wang *et* al. (2009).**

In conclusion the present pilot study demonstrate that onion oil and its active constituent are good fighters for aging while it do change on liver function, Kidney function, Protein, Lipid profile, monoamines and fertility hormones and need more study to prevent earlier aging.

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الملخص العربى

تأثير البصل ومكوناته على الشيخوخة

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هناك بعض العقاقير المستخدمة التي تقلل من تأثير الشيخوخة ولكن هذه العقاقير لها آثار جانبية.

تناولت هذه الدراسة: تأثير البصل ومكوناته كمكون طبيعي على الشيخوخة، اشتملت هذه الدراسة على ٦٠ عينة من فئران التجارب (٣٠) صغيرة السن حوالي (٦ أشهر و(٣٠) آخرين كبار السن حوالي(١٠٥ – ٢).

وقد تم تقسيم كل مجموعة إلى ثلاثة مجموعات: تحتوي على (١٠ فئران)

المجموعة الأولى: المجموعة الضابطة من الأصحاء

المجموعة الثانية: تناولت زيت البصل

المجموعة الثالثة: تم علاجها باستخدام DPDS

وقد تم عمل القياسات الكيميائية الحيوية الآتية:

وظائف الكلى والكبد وبعض البروتينات والنقلات العصبية (مونو أمين) وتيستوستيرون، والدهون.

وأشارت النتائج إلى وجود ارتباط بين استخدام البصل وهذة القياسات

فكانت جميع مؤشرات وظيفة الكبد. AST، ALT، GGT، LDH، ALP ومجموع البيليروبين عالية معنويا ($P <0.001$) في المجموعة الذين تتراوح أعمارهم بين وانخفضت بشكل ملحوظ بعد العلاج، في حين كان من أبرز المجموعات الكبار غير بشكل ملحوظ تغييرها. كما أظهرت المؤشرات الحيوية وظيفة الكلى قياس التغيير فقط مع جماعات المسنين والذين تتراوح أعمارهم بين تعامل مع ($P <0.001$) انخفاض كبير في البروتين الكلي والألبومين وزيادة اليوريا والكرياتينين التركيز، وهذه التغييرات تحسينها بنفس الطريقة مع أي زيت البصل أو DPDS. أظهر مستوى الدهون في الدم وارتفاع الكولسترول، والدهون الثلاثية تركيز ($P <0.05$) و HDL وتركيز ($P <0.001$ LDL)، ولكن زيادة غير كبيرة في تركيز HDL. العلاجات اختبار تنجح لتحسين ($P <0.001$) التغييرات الملف الشخصي الدهون باستثناء تركيز HDL. وقد انخفض مستوى أحاديات الأمين في الفئران الذين تتراوح أعمارهم بين صغار السن ثم زادت بشكل ملحوظ بعد العلاج ($P <0.05$ مع بافراز والسيروتونين و $P <0.001$ مع الدوبامين). اتخاذ التستوستيرون كمؤشرات للخصوبة، كان العلاجات تأثير الاضافة معنوي ($p <0.001$) على مستوى هرمون التستوستيرون انخفضت من الفئران الذين تتراوح أعمارهم بين كبار السن.