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CARDIOPROTECTIVE EFFECT OF *NIGELLA SATIVA* SEED AND OIL ON ISOPROTERENOL INDUCED MYOCARDIAL INFARCTION IN RATS

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ABSTRACT
The cardio protective effect of black cumin crude and ethanolic extract was studied on some biochemical parameters in male albino rats. The values were compared with known cardio protective agent verapamil. The rats were divided into five groups. Group I serve as a healthy control, group II was induced with Isoproterenol (2mg/kg/body wt) intravenously after 48h the rats were sacrificed, group III was treated with standard Verapamil (5μmoL/kg/body wt) for 2 days, followed by Isoproterenol after 2nd day of administration intravenously. After 48h the rats were sacrificed. The *Nigella sativa* seed extract was given in the dosage of (1000mg/kg/body wt) and oil extract was (2mL/kg/body wt) given orally for 21 days daily to the rats of group IV and V followed by Isoproterenol as in group II. The observation clearly indicates the level of LDH, CPK, AST, ALT, lipid profile, etc. were altered in myocardial infarction condition when compare to normal. The altered levels of all the parameters were maintained at near normal on pretreatment with *Nigella sativa* extracts. The above study clearly indicates the benefits of raw black cumin.

INTRODUCTION

Cardiovascular diseases have become the number one killer disease in many parts of the world. An increased risk of coronary heart disease is associated with a high serum cholesterol concentration and low density lipoprotein (LDL) and decreased high density lipoprotein (HDL) (Sheela Sasikumar and Shyamala Devi, 2001).

Myocardial infarction is commonly known as a heart attack. This is most commonly due to occlusion (blockage) of a coronary artery following the rupture of a vulnerable atherosclerotic plaque, which is an unstable collection of lipids (fatty acids) and white blood cells (especially macrophage) in the wall of an artery (Mallinson, 2010).

Although modern drugs are effective in preventing the disorders their use in often limited because of their side effects and adverse reactions. A wide assay of plants an alternative therapy for ischemic heart disease. Moreover, the plant kingdom represents a largely unexplored reservoir of biologically active compounds.

Nigella sativa commonly known as black seed have been used for nutritional and medicinal purposes in many Middle Eastern countries, and other parts of the world (El-Dakhakhani *et al.,* 2000; Ghosheh *et al.,* 1990). They have been traditionally used in the treatment of a number of aliments including respiratory health, stomach and intestinal health, kidney hypertension, bladder and liver function, circulatory and immune system supported and for general overall well being (Baser *et al.,* 1986; Handa, 1998; Deliorman *et al.,* 2002; Malhotra, 2006) *N.sativa* has been shown to produce multi-systemic beneficial actions (Ali and Blunden, 2003), including hypoglycemic (Bamosa *et al.,* 1997), hypocholestermic (Bamosa *et al.,* 2002) and antioxidant effects.

In the present investigation on cardio protective activity was conducted on isoproterenol induced myocardial infarcted rats were given pretreatment with or without crude and ethanolic extract of *Nigella sativa* effect and protection was studied mainly on the enzymes and lipid metabolism.

MATERIALS AND METHODS

Plant material

The *Nigella sativa* is collected from Adhiparasakthi Agricultural college medicinal park, in kalavai- 632506. Tamil Nadu-India. The *Nigella sativa* was dried and crushed in to fine powder formed.

Animals

Healthy adult male Swiss albino rats (weighing 160-250g) were used throughout the experiment. Animals were maintained at 22$\pm$2ºC with 45-55% relative humidity, 12h light and dark cycle. They were housed in well-ventilated polyurethane cages and have free access of tap water and laboratory pellet feed.

Preparation of crude extract

100g of *N.sativa* seed were grinded and mixed with water

and given to the rats. The crude powder extract was also given at the dose of 1000 mg/kg body weight.

Preparation of ethanolic extract

100g of *N.sativa* seed were grinded and extract with 200 mL of ethanol by the method of continuous hot extraction at 60ºC for 6h and evaporated. The extract yielded was viscous oil. This residual extract was dissolved in DMSO (dimethyl sulphoxide) 2 mL/kg of dose was given orally to the rats.

Experimental design

In the experimental a total of 30 rats were used. They were divided in to five groups in each group six rats were selected. Group I is a healthy control, group II was induced with Isoproterenol (2mg/kg/body wt) intravenously, After 48 hours the rat were sacrificed. Group III was treated with standard Verapamil (5μmol/kg/body wt) for 2 days, followed by Isoproterenol after 2nd day of administration intravenously. After 48h the rats were sacrificed. The Nigella sativa seed extract was given in the dosage of (1000mg/kg/body wt) and oil extract was (2mL/kg/body wt) given orally for 21 days daily to the rats of group IV and V followed by Isoproterenol as in group II.

Sample collection

After 48h of Isoproterenol administration the animals were sacrificed by decapitation. Blood sample were collected and serum is separated for all biochemical analysis.

Heart tissue was dissected out and washed in ice cold saline immediately.

Histopathological studies

Histopathological evaluation was performed on lower portion of the heart tissue. Fresh heart tissue were excised and then fixed in 10% formalin for 24h. The fixative was removed by washing through running tap water for overnight. After dehydration through a graded series of alcohols, the tissues were cleaned in methyl benzoate, embedded in paraffin wax. Section were cut into 5μm thickness and stained with hematoxylin and cosin. After repeated dehydration and cleaning, the sections were mounted and observed under light microscope with magnification of 10 xs for histological changes.

Biochemical studies

Lactate dehydrogenase and creatinine phosphokinase was studied by the method of king (1965) and Okinaka *et al.,* (1961). The activities of Aspartate transaminase and alanine transaminase were estimated by the method of Reitmann and Frankel (1957). The levels of creatine and protein were determined by the method of Owen *et al.* (1954) and Lowry *et al.* (1951). The level of cholesterol and triglycerides were estimated by the method of Parekh and Jung (1970) and Foster and Dunn (1973). High density lipo protein, low density lipo protein and very low density lipoprotein levels were evaluated by Zlatkis *et al.* (1953) and Friedward *et al.* (1972).

Biochemical determination was carried out using shimadzu spectrophotometer.

Statistical analysis

ANOVA, statistical treatment applied under one way classification, changes were considered significant of the p values was <0.01, <0.05. The values expressed as mean $\pm$ SD.

RESULTS AND DISCUSSION

The aim of the present investigation was to evaluate the protective effect of pretreatment of *Nigella sativa* medicinal plant in Isoproterenol induced rats. Our results clearly indicates that the consumption of *Nigella sativa* seed (1000mg/kg/body wt) and oil (2mL/kg/body wt) maintain the cholesterol, enzymes and the other biochemical parameters in the level of significance <0.01, <0.05 in myocardial infracted rats, when compare to normal rats.

Table 1 depicts the levels of serum enzymes creatinine phosphokinase (CPK), lactate dehydrogenase, Aspartate transaminase, alanine transaminase in normal and experimental rats. Injection of single high dose of Isoproterenol induced the necrotic damage to the myocardial membrane (Manjula *et al.,* 1992).

Isoproterenol is well known cardio toxic agent due to its ability will destruct myocardial cells. As a result of these cytosolic enzymes Aspartate transaminase and alanine transaminase were released in to blood stream and serve as the diagnostic markers of myocardial damage (Gurgun *et al.,* 2008).

The prior administration of *Nigella sativa* seed crude powder and ethanolic oil extract showed a significantly ($<0.01$and<0.05) maintain the levels of serum marker enzymes. Because the reduced extent of myocardial damage and therefore restricted the leakage of these enzymes from the myocardium (Jorge *et al.,* 1998).

Table 2 depicts the levels of cholesterol, triglycerides, HDL, LDL, and VLDL in normal and experimental rats. The level of cholesterol, triglycerides, LDL, VLDL is increased and HDL is decreased in myocardial infarcted group II rats. The pretreatment on *Nigella sativa* seed and oil showed significantly <0.01 and <0.05 maintain the level in group IV and V, when compare to group I normal rats.

High level of circulating cholesterol and its accumulation in heart tissue are well associated with cardiovascular damage

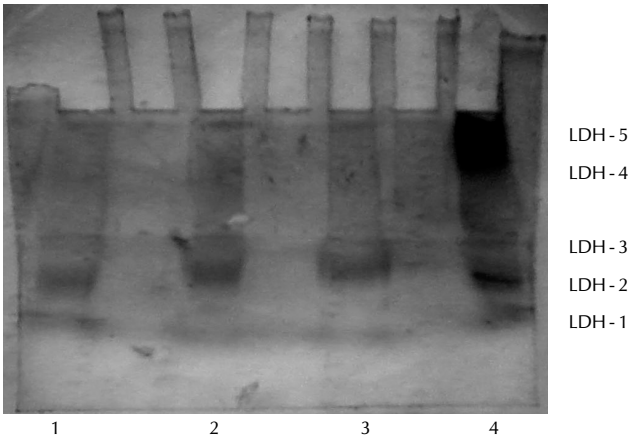

Figure 1: Electerophoretic pattern of LDH isoenzymes in serum of Normal and experimental animals

Lane 1 = Normal; Lane 2 = Isoproterenol induced; Lane 3 = *Nigella sativa* crude powder extract treated; Lane 4 = *Nigella sativa* oil ethanolic extract treated

Table 1: Activities of serum CPK, LDH, AST, ALT in normal, induced, standard, *Nigella sativa* seed and oil treated rats. Values are expressed as Mean ± SD

S.no	Parameters	Normal	Inducer Isoproterenol	Verapamil + Isoproterenol	*Nigella sativa* seed + Isoproterenol	*Nigella sativa* oil + Isoproterenol
1.	CPK	80.54 ± 0.875	145.57 ± 0.916	75.38 ± 0.702	82.34 ± 0.535*	84.116 ± 2.874**
2.	LDH	145.79 ± 1.167	219.42 ± 0.50	148.69 ± 1.022	148.69 ± 1.022*	149.23 ± 2.810**
3.	AST	55.53 ± O.561	95.70 ± 0.873	53.601 ± 0.510	60.21 ± 2.775*	61.13 ± 5.391**
4.	ALT	19.41 ± 0.718	25.60 ± 0.802	19.48 ± 6.65	23.64 ± 2.309*	22.73 ± 2.320**

Level of significance $p < 0.01$*, $p < 0.05$**, CPK, LDH, AST, ALT- IU/L

Table 2: Activities of serum cholesterol, triglycerides, HDL, LDL and VLDL in normal, induced, standard, *Nigella sativa* seed and oil treated rats. Values are expressed as Mean ± SD

S.no	Parameters	Normal	Inducer Isoproterenol	Verapamil + Isoproterenol	*Nigella sativa* seed + Isoproterenol	*Nigella sativa* oil + Isoproterenol
1.	Cholesterol	73.95 ± 1.15	161.15 ± 0.72	79.70 ± 0.72	77.88 ± 3.30*	79.81 ± 10.72**
2.	Triglycerides	7.29 ± 0.39	16.93 ± 0.49	7.79 ± 0.45	10.68 ± 2.51*	10.31 ± 2.50**
3.	HDL	45.52 ± 0.65	31.51 ± 0.907	49.36 ± 7.80	45.94 ± 1.90*	44.80 ± 1.95**
4.	LDL	31.54 ± 0.51	45.12 ± 0.306	29.84 ± 0.56	30.72 ± 0.668*	30.94 ± 1.37**
5.	VLDL	29.41 ± 0.59	35.48 ± 0.99	29.84 ± 0.56	30.72 ± 0.668*	30.94 ± 1.37**

Level of significance $p < 0.01$*, $p < 0.05$** Cholesterol, triglycerides, HDL, LDL, VLDL- mg/dl

Table 3: Activities of serum troponin T and protein in normal, standard, *Nigella sativa* seed and oil treated myocardial infracted rats. Values are expressed as Mean ± SD

S.no	Parameters	Normal	Inducer Isoproterenol	Verapamil + Isoproterenol	*Nigella sativa* seed + Isoproterenol	*Nigella sativa* oil + Isoproterenol
1.	TroponinT	0.385 ± 0.10	1.27 ± 0.57	0.383 ± 0.081	0.65 ± 0.131*	0.628 ± 0.172**
2.	Protein	6.28 ± 0.228	7.91 ± 0.68	6.28 ± 0.327	6.79 ± 0.356*	6.92 ± 0.669**

Level of significance $p < 0.01$*, $p < 0.05$**

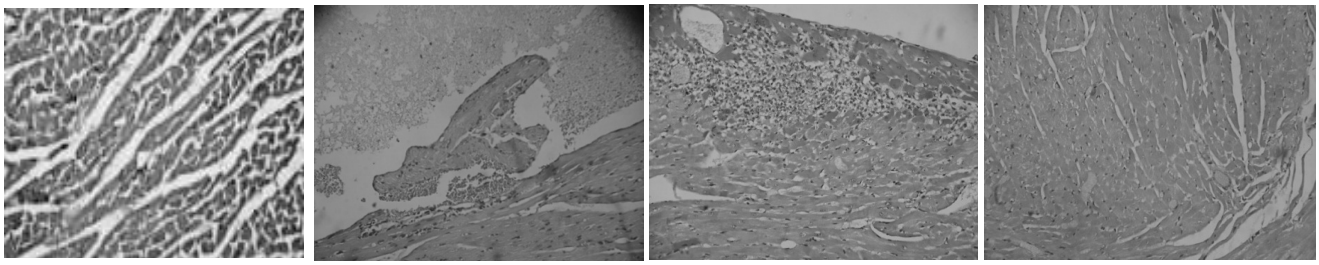

Figure 2, 3, 4 and 5: The histopathological study of different group of rats normal and experimental rats; Figure 2: A normal rat was showing normal architecture of the heart (HE× 10x); Figure 3: ISPH Myocardial infracted rat showing degenerative changes, hyalihization of muscle fibers and cellular infiltration (HE×10x); Figure 4 and 5: *Nigella sativa* seed and oil pretreated rat reveals less cellular infiltration, normal muscle fibers and the cardio protective effect are evident from reduced myocardial damage even after ISPH administration (HE× 10x)

(Sheela Sasikumar and Shyamala Devi, 2001)

Isoproterenol administration resulted in significant increase in the free fatty acid level. Hypertriglyceridemia seen in Isoproterenol treated rats, a condition observed in ischemic heart disease, is due to decrease in the activity of lipo protein lipase in the myocardium resulting in decrease uptake of triglycerides from circulation.

The *N.sativa* seed and oil is contains thymoquinone may be responsible for the hypocholestermic action in rats. The levels are maintained at near normal when compared to the normal in group IV and group V rats (Badary *et al.,* 2000; Zaoui *et al.,* 2002).

Administration of Isoproterenol mainly raised LDL cholesterol and decreased HDL cholesterol in group II experimental rats. High levels of LDL cholesterol have positive relation and decreased levels of HDL cholesterol have negative relation with myocardial infarction (Sheela Sasikumar and Shyamala Devi, 2001).

They have also reported HDL inhibits the uptake of LDL by arterial wall and also facilitated the transport of cholesterol from peripheral tissue to the liver where it is catabolised and excreted out of the body.

The pretreatment with *Nigella Sativa* seed and oil increases the HDL cholesterol. The extract mechanism of action of *Nigella Sativa* is not known, however it has been proved that volatile oil of *Nigella Sativa* has two main constituents (i.e.) Nigellone and Thymoquinone which play a key role in prevention of heart disease (Gad *et al.,* 1963; Babayan *et al.,* 1978; Abdel and Attia, 1993).

Tale 3 depicts the levels of protein, Troponin T in normal and experimental rats. The pretreatment on *Nigella sativa* seed and oil showed significantly < 0.01 and < 0.05 maintain the level in group IV and V, when compare to group I normal rats.

Troponin-T ng/dl

Protein g/dl

Troponin-T is a protein found in cardiac tissue. When the myocardial damage occurs by cytosolic troponin reach the blood stream quickly resulting in a rapid peak of serum troponin (Nigam, 2007).

In this study, significant increased level of troponin T in serum of Isoproterenol treated rats. Increased level of troponin was due to leakage from the damaged heart tissue into the blood stream as a result of necrosis induced by Isoproterenol in rats.

Pretreatment with *Nigella sativa* powder and oil to Isoproterenol treated group IV and V rats restored the level of troponin-T in serum indicates the protective action of *Nigella sativa* against peroxidative damage.

The increased level of protein was observed in the serum of Isoproterenol administered rat is, at least in part, due to leakage of enzymes and protein bound components from the damaged myocardium to the systemic circulation.

Thymoquinone (TQ), bioactive and the most abundant constituent of the volatile oil of the black seed, has been shown to possess an anti-inflammatory and antioxidant effects. These are also may be the protective action, of *Nigella sativa* seeds and oil reduces the leakage of enzymes protein bound components.

So it may be the responsible for the protective action of *Nigella sativa* in experimental group IV and V rats (Houghton *et al.,* 1995 and Keuk *et al.,* 2000).

Electerophoretic pattern of LDH isoenzymes

The Myocardial activity of LDH is high enough to make it unlikely that it could be a controlling step for lactate metabolism by the heart. In acute myocardial infarction, an estimate of the infarct size may be formed by measuring the rate of appearance and disappearance of LDH_1 in the blood. The LDH isoenzymes pattern of control (Lane 1) shows faint bands which is an indication of intact myocardium. Isoproterenol treated rats show prominent band (Lane 2) of the various LDH fractions which could be due to necrosis of the myocardium. Administration of Isoproterenol resulted in sharp increase in heart specific LDH_1 and LDH_5.

The increased LDH_1 isoenzymes could be due to the molecular adaptation of the myocardium to the mechanical stress of ischaemia. (Ballo and Messer, 1968)

It may serve as a compensatory mechanism in the failing heart as LDH_5 favours anaerobic metabolism compared with LDH_1. (Dawson *et al.,* 1964)

Pretreatment with *Nigella sativa* seed and oil (Lane 3and 4) decreased the intensity of elevation. The less prominent bands could be due to the minimal damage to the myocardium which confirms the cardio protective effect of *Nigella sativa*. The findings of the present study suggest that the herbal formulation of *Nigella sativa* may offer protection to the myocardium by preventing the lipid peroxidation of membrane bound poly unsaturated fatty acids, thus ensuring myocardial membrane structural integrity and function.

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REFERENCES

Abdel-Aal, E. S. M. and Attia, R. S. 1993. Characterization of black cumin (*Nigella sativa*) seeds. *Alex. Sci. Exch.* **14:** 483-496.

Ali, B. H. and Blunden, G. 2003. Pharmacological and toxicological properties of *Nigella sativa*. *Phyt other. Res.* **17:** 299-305.

Babayan, V. K., Koottungal, D. and Halaby, G. A. 1978. Proximate analysis, fatty acid and amino acid composition of *Nigella sativa L.* seeds. *J. F. Sci.* **43:** 1315-1319.

Badary, O., Abdel-Naim, A., Abdel-Wahab, M. and Hamada, F. 2000. The influence of thymoquinone on doxorubicin-induced hyperlipidemicnephropathy in rats. *J. Toxicol.* **143:** 219–26.

Ballo, J. M. and Messer, Jr. 1968. Lactate dehydrogenase isoenzymes in human hearts having decreased oxygen supply. *Biochem. Biophys. Res. Commun.* **33:** 487-491.

Bamosa, A. O., Basil, A. A. and Zubaida, A. A. 2002. The effect of thymoquinone on blood lipids in rats. *In. J. Pharmacol.* **46:** 195-201.

Bamosa, A. O., Ali, B. A. and Sowayan, S. A. 1997. Effect of oral ingestion of Nigella sativa seed on some blood parameters. *Saudi Pharm.* **5:** 126-129.

Baser, K. H. C., Honda, G. and Miki, **W. 1986.** Herb drugs and herbalists in Turkey. Institute for the study of languages and cultures of Asia and Africa, Tokyo.

Dawson, D. M., Good friend, T. L. and Kaplan, N. O. 1964. Lactate dehydrogenases functions of the two types. *Science.* **143:** 929-933.

Deliorman, D., Calis, T., Ergun, F., Dogan, B. S., Buharalioghu, C. K. and Kanzik, K. 2002. Studies on the vascular effects of the fractions and phenolic compounds isolated from *Viscum album*. *J. Ethnopharmacol.* **72:** 323-339.

El-Dakhakhani, M., Barakat, M., El-Halim 2000. M. Effect of N. sativa oil on gastric secretion and ethanol-induced ulcer in rats. *J Ethnopharmacol.* **72:** 299–304.

Foster, L. B. and Dunn, R. T. 1973. Stable reagents for determination of serum triglycerides by a colorimetric Hantsch condensation method. *Clin Chem.* **19:** 338-40.

Friedward, W. T., Levy, R. I. and Fredickson, D. S. 1972. Estimation of concentration of low density lipoprotein cholesterol in plasma without use of preparative ultracentrifuge *Clin Chem.* **18:** 499-502.

Gad, A. M., M. El-Dakhakhny and Hassan, M. M. 1963. Studies on the chemical composition of Egyptian *Nigella sativa L.* oil. *Planta. Medica.* **11:** 134-138.

Ghosheh, O., Houd, A. and Crooks, P. 1990. High performance liquid chromatographic analysis of the pharmacologically active quinones and related compounds in the oil of the black seed (*N. sativa* L.). *J. Pharm. Biomed. Anal.* **5:** 757–62.

Gürgün, C., Ildizli, M., Yavuzgil, O., Sin, A., Apaydin, A., Cinar, C. and Kültürsay, H. 2008. The effects of short term statin treatment on left ventricular function and inflammatory markers in patients with chronic heart failure. *Int. J.Cardiol.*, **123:**102-107. DOI: 10.1016/ J.IJCARD.2006. 11. 152.

Handa, S. S. 1998. The integration of food and medicine in India. In: Prendergast, H.D.V., Etkin, N.L., Harris,D.R., Houghton, P.J. (Eds.), Plants for Food and Medicine. Royal Botanic Gardens, Kew, pp. 57-68.

Houghton, P. J., Zarka, R., Herasvand, B. and Hoult, R. S. 1995. Fixed oil of Nigella sativa and derived thymoquinone inhibit eicosanoid generation in leucocytes and membrane lipid peroxidation. *Planta medicine.* **61:** 23-29.

Jorge, R., Neural, C., Ozaki, R. and Almeida, E. 1998. Improvement in the endothelium-dependent relaxation in hypercholesterolemic rabbit treated with vitamin E. Atherosclerosis. **140:** 333–9.

Keuk, I., Michalska, T., Lichszteld, K., Kaldna, A. and Aboul-Enein,

H. Y. 2000. The effect of Thymol and its derivatives on reaction generating reactive oxygen species. *Chemosphere.* **41:** 1059-1064.

King, J. 1965C. The dehydrogenase (or) oxidoreductase lactate dehydrogenase, in practical clinical enzymology (No strand co Ltd) London, 83.

Lowry, O. H., Rosebrough, N. J., Farr, X. L. and Randall, P. J. 1951. *J. Biol.Chem*. **193:** 265-275.

Malhotra, S. K. 2006. *Nigella* In: Peter, K.V. (Ed.), Hand book of Herbs and Spices, vol. 2. Wood head Publishing Ltd., Cambridge, pp: 206-214.

Mallinson, T. **2010.** Myocardial Infarction. *Focus on First Aid* **(15):** 15.

http://www.focusonfirstaid.co.uk/Magazine/issue15/index.aspx. Retrieved 2010-06-08.

Manjula, T. S., Geetha, A. and Devi, C. S. 1992. Effect of aspirin on isoproterenol induced myocardial infarction – a pilot study. *Ind. J. Biochem Biophys.* **29:** 378-379.

Nigam, P. K. 2007. Biochemical markers of myocardial injury. *Indian J. Clin. Biochem.* **22:** 1017. doi:10.1007/BF02912874.

Okinaka, S., Kumagai, H., Ebashi, S. et al. 1961. Serum creatine phosphokinase. Activity in progressive muscular dystrophy and neuromuscular disease. *Arch Neurol.* **4:** 520**-**525.

Owen, J. A., Iggo, T., Scandrett, F. J. and Stemart, I. P. 1954. *Biochem J.* **58:** 426-437.

Parekh, A. C. and Jung, D. H. 1970. *Anal.Chem.* **42:** 1423.

Reitman and Frankel, S. 1965. colorimetric method of Aspertate transaminase in practical clinical enzymology edited by J. King (D van company Ltd.) p. 180.

Sheela Sasikumar, C. and Shyamala Devi, C. S. 2001. Effect of 'Abana' Pretreatment on Isoproterenol – induced Hyperlipidemia in Rats. *Indian J. Pharmaceutical Sciences.* **2(63):** 101-104.

Zaoui, A., Cherrah, Y., Aloui, K., Mahassine, N., Amarouch, H. and Hassar, M. 2002. Effect of *N. sativa* fixed oil on blood homeostasis in rat. *J. Ethnopharmacol.* **79:** 23–6.

Zlatkis, A., Zak, B. and Boyle, A. J. 1953. *J. Lab. Clin. Med.* **4:** 486-492.