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Effect of Nigella Sativa Oil on Some Hematological Values in Aluminum-treated Rats.

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Abstract: Aluminum is a metal with no biological functions. Its organic accumulation can lead to toxic effects. The present study aimed to investigate the effect of NS oil (1ml/kg b.w.) on some hematological factors in presence and absence of Al toxicity and to study the oral administration of $ALcl_3$ (200mg/kg b.w.) on some body functions in rats for 4 weeks. Rats were allocated into 4 groups: normal rats, rats received a daily oral dose of $ALcl_3$, animals received a daily oral dose of NS oil and animals received oral doses of both $ALcl_3$ and NS oil. To achieve this aim blood cells counting, serum iron, total iron binding capacity, transferrin and plasma protein electrophoresis were performed, in addition to liver and kidney function tests and some biophysical analyses for Hb. Oral administration of Al resulted in decreasing serum albumin level, WBCs count, MCH and MCHC, and elevation of serum ALT & AST and creatinin levels, in addition to significant elevation of some physical parameters of Hb. NS oil improved some of these toxic effects of Al on liver and Hb viscosity.

Key words: Aluminum, Nigella, Hematological values.

INTRODUCTION

Although aluminum (Al) is the third abundant element and the most common metal in the earth's crust, it has no known biological function (Farina *et al.*, 2002). Its accumulation in humans can occur via the diet, drinking water, vaccines, antacids, parenteral fluids and inhaled fumes (Sharma and Mishra, 2006). Presently, Al utensils are widely used in the world, especially in the developing countries (Lin *et al.*, 1997). this may increase the Al content, particularly in the food that are salty, acidic or alkaline (Sharma and Mishra, 2006). Once in the blood circulation, Al is mainly transported by plasma transferrin in its sites left vacant by iron, and to a much lesser extent by albumin (Farina *et al.*, 2002). Aluminum accumulation has been associated with a variety of human pathologies such as anemia, osteodystrophy, joint diseases, muscular weakness and Alzheimer's diseases(Missel *et al.*, 2005).

Nigella sativa (NS), a spicy plant, known also as black cumin, is commonly used in the Middle East, North Africa and India. The effect of NS has been evaluated in animal studies. There are many reports on its biological activities including antihypertensive, anti-diabetic, anti-bacterial (Kökdil *et al.*, 2005), anti-tumour and immunomodulator (Kanter *et al.*, 2005). The pharmacological investigations of the effect of NS on heamatological factors are few, so the present study aimed to investigate the effect of NS on some hematological factors in presence and absence of Al toxication. Also, in most previous studies regarding the effects of Al on erythrocyte parameters, the chosen routes of Al administration (i.e. intraperitoneal or parenteral) do not simulate the main rout by which human population is exposed to Al (oral exposure) (Farina *et. al.*, 2005), consequently this work aimed to study the oral administration of Al on some body functions.

MATERIALS AND METHODS

A total number of 28 adult male Swiss albino rats weighing 150-200g were used throughout this study. The animals were housed in steel mesh cages (4/cage) and maintained for a week–acclimatization period on a commercial pellet diet.

A weight of 4g aluminum chloride ($ALcl_3$, 98% pure) was dissolved in 100ml distilled water to prepare a stock solution (40mg/ml). The solution was prepared weekly and stored in a well-stoppered bottle at 4°c. Al was daily administered to rats by a stainless-steel gavage needle at a sub-lethal dose level of 200mg/kg body weight. The LD_{50} value of $ALcl_3$ was previously reported to be 380-400mg/kg B.W. on oral administration in rats (Krasovskii *et al.*, 1979).

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Nigella sativa oil (crude oil obtained by squeeze) was orally administered to animals at a dose level of 1ml/kg (Zaoui *et al.,* 2002).

Rats were allocated into 4 groups (7 animals in each) as follows

Group 1 (C): normal rats as a control group received 0.5ml distilled water.

Group 2 (Al): rats received a daily oral dose of Al Cl3.

Group 3 (NS): animals received a daily oral dose of NS oil.

Group 4 (Al & NS): animals in this group received oral doses of both $ALcl_3$ and NS oil. Administration of the tested compounds started at the same day for the different groups and lasted for 4 weeks. Body weight of the animals in all groups was recorded weekly. At the end of the experimental period, blood samples were taken from the retro-orbital venous plexus under light ether anesthesia after a fast of 12h. Each sample was divided into three tubes, the first containing EDTA for hematological analyses, protein electrophoresis and iron and total iron binding capacity. The second tube containing heparin for the biophysical analyses and the last tube used for separation of serum which stored at -20°c for the biochemical analyses. Rats were then sacrificed by cervical dislocation. Liver was excised, rinsed from blood in isotonic sterile saline and used for histological investigation according to (Ilhan, 2004).

Serum albumin was determined according to (Doumas *et al.,* 1971). Kinetic determination of serum aminotransferases [(alanin (ALT) and aspartate (AST)] and creatinine were performed according to the methods of (Young, 1990), (Bowers and Wong, 1980). respectively, while serum urea was determined by enzymatic colorimetric method (Patton and Crouch, 1977). Plasma iron and total iron binding capacity (TIBC) were measured by the method of (Ramsay, 1957). While transferrin was calculated according to the equation (= 0.007 X TIBC (ug/dl) (Burtis and Ashwood, 2001). Iron saturation was calculated using the equation (serum iron: serum transferrin) X 70% (Korzets *et al.,* 2000). Haematological parameters were determined automatically by ABX coulter counter. Erythrocyte morphology was observed under a light microscope after staining with hematoxyline-eosin dye.

Hemoglobin (Hb) viscosity was assessed by using Ostwald's viscometer. Measurements were performed for native Hb at 37C, absorption = 1 at wavelength 578 nm (Lewis and Koepke, 1995). Relative, and reduced viscosities were calculated (red. = sp /C, sp = red. – 1). Radius of Hb molecule was calculated [(o – 1)/C = (N0 / M) r3 where r = (M (o –1) /$(CN0)^{1/3}$, where M is the molecular weight of Hb (64500 g/mol), and N0 Avogadro's number (6.023 x 10^{23} molecules/mol), C is the concentration of Hb, and o is the relative viscosity] (Raymond, 1990). Electrical conductivity was measured by using Hanna Hi 8633 instrument at 37C (Crow *et al.,* 1994). The pH of Hb was measured by using pH meter, pH level 1 at 37C. Hb was diluted until absorption at 578 nm = 1 according to (Cleg J.B.1966). Finally, plasma from control and treated groups were analyzed by SDS-PAGE according to (Stegeman *et al.,* 1988).

Statistical analysis:

Data were expressed as mean ±SD. Comparisons between groups were performed by one-way ANOVA, followed by LSD test. The F-test was significant at (P< 0.05) (Munro, 1997).

RESULTS AND DISCUSSIONS

Table. 1: Statistical significance of the liver and kidney function tests of sera of the different treated groups, compared to normal control.

Groups	Liver function test		Kidney function test		
	Albumin (g/dl)	ALT (U/L)	AST (U/L)	Urea (mg/dl)	Creatinine (mg/dl)
C Mean±SD	4.12±0.42[a]	32.43±4.35[a]	64.43±10.05[a]	24.14±2.86[a]	0.59±0.10[a]
Range	(3.6-4.6)	(28-37)	(50-79)	(18.9-27.6)	(0.50-0.80)
Al Mean±SD	2.85±0.41[b]	90.14±4.74[b]	110.57±17.94[b]	26.08±1.84[a]	0.78±0.06[b]
Range	(2.5-3.6)	(87-100)	(99-143)	(23.5-27.6)	(0.70-0.86)
NS Mean±SD	3.66±0.26[ac]	35.42±5.03[a]	60.93±10.81[a]	27.82±1.94[b]	0.77±0.13 b
Range	(3.3-4.1)	(28-42)	(50-77)	(24.5-30.0)	(0.50-0.88)
Al&NS Mean±SD	3.1 8±0.62[cb]	37.43±8.09[a]	89.79±21.54[c]	28.64±4.1 9b	0.78±0.25[b]
Range	(2.6-4.1)	(27-48)	(72-123)	(24.5-33.7)	(0.60-1.30)

Any two similar letters are non significant. LSD at P< 0.05.

Data presented in Table (1) demonstrate a significant reduction in the serum albumin level in both Al-treated groups (Al and Al&NS), compared to control group, while Al-treated group only shows a significant reduction in serum albumin level, compared to NS-treated group. A significant elevation in the serum levels of ALT and AST was observed in Al-treated group, compared to all groups. On the other hand, the serum level of AST in Al & NS group was significantly higher than the NS group. Serum levels of urea and creatinine showed a significant elevation in NS and Al & NS groups, in addition to, a significant elevation in serum creatinine level in Al group, compared to control.

Table. 2: Statistical significance of the RBC_S and WBC_S counting, Hb Concentration and RBCS indices of the different treated groups, compared to control group.

Groups	RBCS ($x10^6$/ul)	WBCS ($x10^3$/ul)	HB (g/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)
C Mean±SD	5.93±0.30^a	5.16±0.84^a	12.2±0.49^a	33.09±1.7^a	56.43±1.4^a	20.53±0.9^a	36.71±0.99^a
Range	(5.4-6.3)	(4.0-6.5)	(11.8-13.0)	(31.1-35.4)	(55-58)	(19.4-22.1)	(35.5-38.2)
Al Mean±SD	6.19±0.34^{ab}	4.14±0.59^b	11.64±0.5^a	33.77±1.8^a	54.71±2.69^{ab}	19.36±0.9^b	34.59±1.26^{cb}
Range	(5.8-6.8)	(3.4-4.9)	(10.8-12.5)	(30.8-35.3)	(51-58)	(17.9-20.4)	(32.9-36.3)
NS Mean±SD	6.67±0.62^b	6.10±0.80^a	12.39±1.06^a	36.23±3.18^b	53.71±1.38^b	18.36±0.87^c	34.43±0.73^c
Range	(5.8-7.5)	(5.1-7.2)	(11.2-13.4)	(32.2-39.9)	(52-55)	(17.4-19.6)	(33.3-35.1)
Al&NS Mean±SD	6.1±0.49^a	5.80±1.5^a	11.79±0.46^a	33.21±1.78^a	54.71±2.06^{ab}	19.51±0.85^b	35.51±0.68^b
Range	(5.4-6.8)	(4.4-7.5)	(11.1-12.5)	(31.2-35.7)	(52-58)	(18.4-20.6)	(34.9-36.7)

Any two similar letters are non significant. LSD at $P < 0.05$.

Table (2) demonstrates a significant elevation in RBCs count and PCV in NS group, while for WBCs count Al group showed a significant reduction, compared to control group. Non significant results were seen for Hb concentration. For RBCs indices, significant reductions were observed in Al, NS and Al & NS groups in MCH and MCHC, compared to normal control. While for MCV, NS group showed a significant reduction, compared to control group.

Table. 3: Statistical significance of the plasma levels of iron and TIBC, in addition to the calculated iron saturation and transferrin.

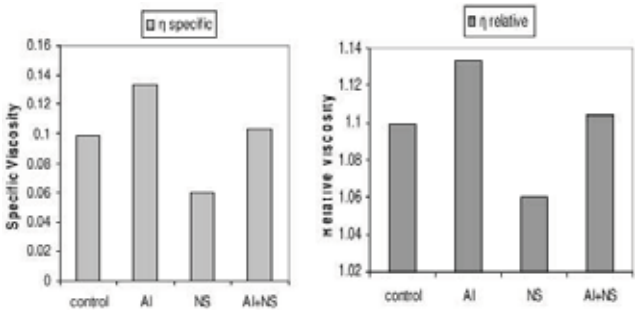
Groups	Iron (ug/dl)	TIBC (ug/dl)	Iron saturation (%)	Transferin (ug/dl)
C Mean±SD	151.71±32.14^a	241.0±21.36^a	64.33±18.9^a	1.69±0.15^a
Range	(110-195)	(215-260)	(42.3-90.7)	(1.50-1.82)
Al Mean±SD	144.43±1 8.56^a	249.00±27.0^a	58.82±12.89^a	1.75±0. 19a
Range	(118-161)	(190-268)	(46.3-84.2)	(1.33-1.87)
NS Mean±SD	82.7 1±17.22^b	283. 14±26.9^b	29.72±7.89^b	1.98±0. 19b
Range	(64-110)	(260-320)	(20-42.3)	(1.82-2.24)
Al&NS Mean±SD	81.29±4.61^b	290.57±24.4^b	28.11±2.34^b	2.03±0.17^b
Range	(75-86)	(260-320)	(25-31.4)	(1.82-2.24)

Any two similar letters are non significant. LSD at $P < 0.05$.

Data presented in table (3) illustrate a significant reduction in both plasma iron level and iron saturation in NS and Al & NS groups, in the contrary, both TIBC and transferrin showed a significant elevation in these groups, compared to control group.

Tab. 4: Statistical comparison of biophysical parameters and different groups of rats.

	relative	specific	conductivity	radius of Hb	PH Value
control	1.099 ± 0.012	0.099 ± 0.001	0.3 ± 0.023	23.57 ± 0.31	6.57 ± 0.03
Al	1.133 ± 0.011	0.133 ± 0.002	0.675 ± 0.013	24.59 ± 0.34	6.605 ± 0.031
NS	1.06 ± 0.012	0.06 ± 0.001	0.55 ± 0.011	20.21 ± 0.21	6.52 ± 0.023
Al+NS	1.104 ± 0.011	0.104 ± 0.001	0.6 ± 0.021	24.44 ± 0.032	6.59 ± 0.04



(a) (b)

Fig. I: Statistical comparison of mean values of Hb viscosities in the different treated groups, compared to control group.

Fig. (I) and table(4) illustrates a significant reduction in Hb viscosity in rats treated with NS, while those treated with Al showed a significant elevation, compared to normal rats. Animals in group Al&NS showed a non significant result when compared to control, in spite of the significant elevation observed compared to NS group.

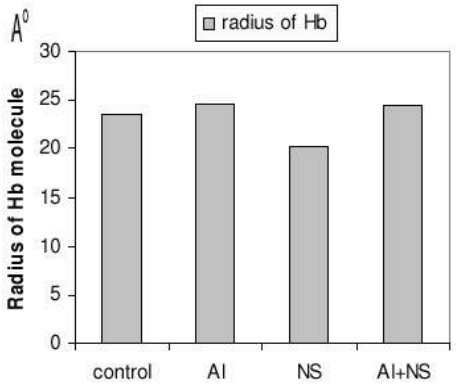


Fig. II: Statistical comparison of mean values of the radius of Hb molecules in the different treated groups, compared to control group.

Fig. (II) illustrates a significant increase in radius of Hb in rats treated with Al and AL&NS, while those treated with NS showed a significant reduction, compared to normal rats.

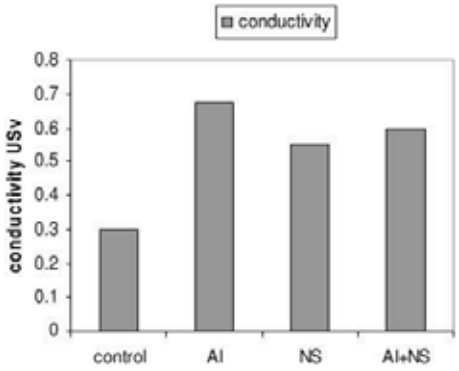


Fig. III: Statistical comparison of mean values of Hb conductivity in the different treated groups, compared to control group.

Fig. (III) demonstrates a significant elevation in Hb conductivity in different treated groups compared to control one. Conductivity in Al group showed more significant elevation than the other treated groups.

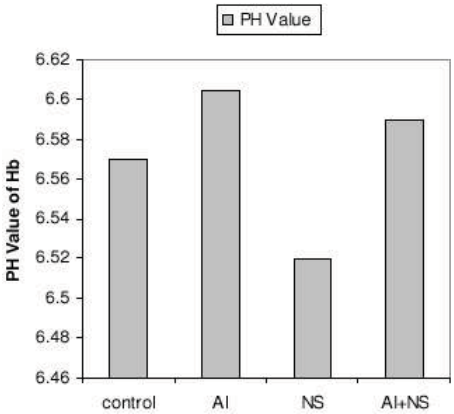


Fig. IV: Statistical comparison of mean values of the pH of Hb in the different treated groups, compared to control group.

Fig. (IV) demonstrates a significant elevation in the pH of Hb molecules in Al-treated group, while in NS-treated group there was a significant reduction, compared to all other groups.

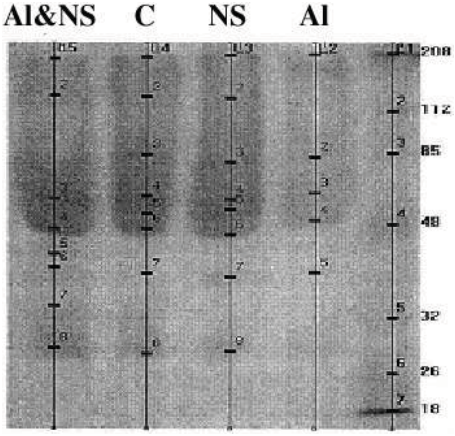


Fig. V: Plasma protein electrophoresis from normal and treated rats.

Fig. (V) shows the plasma protein electrophoresis from the different studied groups. Results showed that the three treated groups are similar in its separation with control. Also, the amount of protein separated in Al group is smaller. Fig(VI) shows presence of poikilocytosis (change in cell shape) in Al –treated group (b). Fig. (VII) shows presence of severe degeneration in livers of rats treated with $ALcl_3$ (b) and a slight degeneration in rats treated with both Al and NS oil (d).

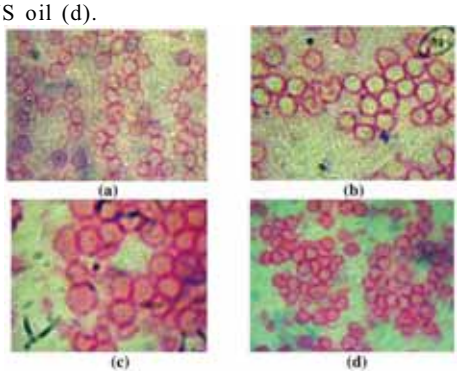


Fig. VI: Photomicrographs of erythrocyte films observed by light microscope (40xmagnification): (a) normal morphology of erythrocytes from control rats, (b) abnormal erythrocytes from Al-treated group, (c) general view of red cells from NS group and (d) general view of red cells from Al&NS group.

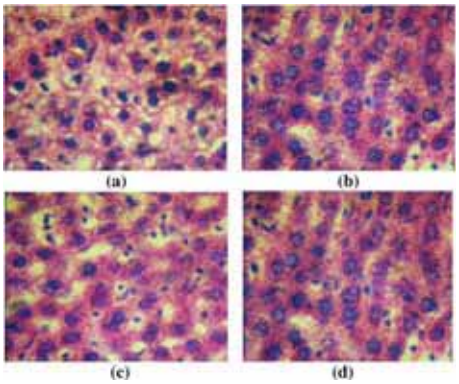


Fig. VII: Photomicrographs of rat liver observed by light microscope (40x magnification): (a) liver cells of normal control, (b) liver cells from Al-treated rats, (c) liver cells from NS-treated rats and (d) liver cells from Al&NS- treated rats.

Discussions:

Aluminum (Al) is a metal with no biological function. Its organic accumulation can lead to toxic effects (Bazzoni *et al.,* 2005). The present study was designed to investigate the effect of Nigella sativa (NS) oil (1ml/kg) on serum iron level as well as some hematological values in Al-treated rats (200mg/kg) for 4 weeks. Aluminum exposure can result in Al accumulation in the liver and the metal can be toxic to the hepatic tissue at high concentration (El-Demerdash, 2004). As reported before, NS was used to treat liver diseases and resulted in significant protection against drug induced hepatotoxicitic effects (El-Kadi and Kandil, 1987). Therefore, evaluation of some of the liver functions was conducted in order to determine the effects of NS on both the liver synthetic activity indicated by albumin level and hepatotoxicity indicated by the activity of ALT and AST in Al-treated rats. The significant reduction in serum albumin level in Al-treated animals, either single (Al group) or combined with NS (Al & NS) (Table 1), confirm the hepatic toxicity since albumin is synthesized in liver. Our results are in line with that of (El-Demerdash, 2004), who reported that albumin level was significantly reduced in rats treated with $ALcl_3$ (34mg/kg) for 30 days.

Serum AST activity is more useful to asses the histological severity of the liver disease, probably this cytosolic and mitochondrial enzyme is present in higher quantities in the liver compared to cytosolic ALT and thus more released when the tissue damage is more severe (Michel, 1998). On the other hand, ALT is more specific in the liver than AST (Marshall, 2000). In the present study, serum ALT and AST activities progressively increased in Al-treated rats during the period of the experiment, as compared with control (Table 1). In Al&NS group, Nigella oil improves elevation of serum ALT and return it to normal and reduced the serum level of AST significantly than in Al group, although still significantly higher than control, suggesting that Nigella has hepatoprotictive effect against hepatotoxicity of Al. This suggestion is confirmed by results obtained by the microscopic examination of livers of rats treated with Al, where degeneration among the hepatic tissue was observed, while in presence of NS this degeneration was improved to some extent (Fig. VII).

Although plasma urea concentration is often used as an index of renal glomerular function, measurement of plasma creatinine provides a more accurate assessment. Treatment with $ALcl_3$ caused a significant elevation in serum creatinine level while serum urea is not changed, compared to control group. In addition, NS groups (either single or combined with Al) illustrated a significant elevation in both urea and creatinine levels, compared to control, although the parameters were within the normal ranges (Table 1). Changes in plasma urea are a feature of renal impairment but it is important to consider possible extra-renal influences on urea concentrations before ascribing any changes to an alteration in renal function . So the elevation of serum urea level in NS group may be revealed to the high content of amino acids present in the NS oil. This suggestion is confirmed by the reduction in serum urea to creatinine ratio in Al group, compared to NS-treated groups and control (33.44, 36.72, 36.13 and 40.92, respectively). This abnormal reduced ratio is caused by liver disease (Marshall, 2000).

Our results are in line with those of (Vittori and co-workers 1999), who stated that kidney function of the Al-treated animals was unaltered, as the blood urea concentration was 8.3mmol/l in control group and 8mmol/l in rats receiving Al. On the contrary, (El-Demerdash 2004), reported that elevation in plasma urea and creatinine levels in Al-treated rats is considered as a significant marker of renal dysfunction.

Intragastrical administration of 200mg $ALcl_3$/kg body weight (alone) causes statistically significant reductions in WBCs count MCH and MCHC (Table 2). Where as, NS group showed significant reductions in MCV, MCH, MCHC and plasma iron and transferrin saturation, in addition to pronounced elevation in RBCs count, PCV, TIBC and transferrin (Tables 2 & 3). These results illustrate that NS, perhaps, stimulates erythropoiesis or accelerates the maturation of erythrocytes leading to withdrawal of plasma iron and giving rise to disturbances in iron measurements. This increase in RBCs number leads to elevation in PCV. Since Al influences negatively on erythropoiesis (Nasiadek *et al.,* 1996), so Al&NS group showed no significant change in RBCs count and consequently PCV. MCV is closely related to an adequate supply of iron to Hb . Both iron deficiency and the reduction in transferrin saturation, in both NS and Al&NS groups, allow the production of erythrocytes with lower MCV. Decreased Hb and PCV were described by other authors under different conditions of toxicity, which refer mainly to a different Al species, doses, times of chronic administration and different ages of the animals (Chmielnicka *et al.,* 1996. Turgut *et al.,* 2004 and Bazzoni *et al.,* 2005).

(Turgut and co-workers 2007), found that i.p. injection of young rats with 5mg Al2(SO4)3/kg b.w. for 2 weeks leads to significant reduction in MCV and RBCs deformability and significant increments in whole blood viscosity. On the other hand, (Mahieu and colleagues 2000). studied the sequential effects of intoxication with Al(OH)3 (80mg/kg b.w., i.p.) on rats from weaning and up to 6 months. During the 1st month, Al affected only the RBCs count and PCV. Microcytosis, noted from the 2nd month, was accompanied by a decrease in MCH, where as during the 3rd and 4th months PCV and Hb levels were lowered. The persistent decrease in MCH could indicate that Al actually affects the heme group synthesis.

The effect of Al on erythrocyte morphology was studied by the optic microscope which illustrated the presence of abnormal erythrocytes that lost the typical biconcave shape, in samples from Al-treated rats. Our results are in line with (Vittori and co-workers 2002). who reported that Al induce alterations of erythroid cells. The viscosity of Hb solution can vary greatly depending on the solute (Hb) molecules which possess the native helical conformation or shaped in coils. Often kinetics of denaturation from helix to random coil can conveniently followed by measuring changes in the solution's viscosity over a period of time relative (Raymond, 1990). Our results showed a pronounced increase in the Hb viscosities (rel, sp and red.)in both Al and Al&NS groups and significant reduction in NS group, compared to control group (Fig. I). (Robson and Pain 1976). proved that viscosity enhancement means a globular protein (Hb) unfolds. Any partial unfolding of the structure destabilizes the remaining structure which simultaneously collapse to random coil (Bettelheim and March, 1998 and Pallister, 1999). This is accompanied by an increase in the fractional volume of molecule, so that the specific viscosity $_{(sp)}$ increases.

The attraction between solvent and solute molecules is measured by reduced viscosity (red.) which has been explained for the volume occupied by solvated macromolecules . So, the elevation in red. in Al group indicates partial unfolding in Hb, which can also explain the increase in the radius of the Hb molecule (Fig. II). On the other hand, NS group showed significant reduction in both Hb viscosity and radius (Fig. I & II).

All functional biological molecules (such as Hb) exist in ionized states in an aqueous environment. Thus measurements of conductivity at various concentrations have led to an understanding of the extent to which substances are ionized in water, the association of ions with the surrounding water molecules and the way by which ions move in water (Blythe, 1979).

Our results illustrated that Al-treatment increased both Hb conductivity and pH, while NS group showed significant increase in conductivity and reduction in pH, compared to control group (Fig. III & IV). These pH variations alter ionization states of amino acid side chains which change protein charged distributions and hydrogen bonding requirements. This causes changes in both stability and rate of many biochemical parameters. (El-Antri and colleagues, 1990). demonstrated that pH depends in the tertiary structural changes and quaternary organization of the and interface of oxyhemoglobin in solution. Also, Hb induced by pH variation was associated with the constrained chains exhibiting and a looser of -interface association.

Electrophoresis shows that the plasma protein of the three treated groups (Al, NS and Al&NS) are separated in similar ways as in control but the amount of protein in Al group was smaller (Fig. V).

Conclusion:

Aluminum has adverse effects on human health. Oral exposure is the main route by which human population is exposed to Al, which leading to hepatotoxicity, kidney dysfunction and changes in erythrocyte shape and their Hb conformation. The present study demonstrated that NS oil administered in combination with aluminum minimized its hazards. Consequently, using NS as spicy could be beneficial in alleviating aluminum toxicity. But more studies should be paid to the effect of NS on erythropoiesis.

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