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ALUMINIUM INDUCED HEAMATOLOGICAL CHANGES AND INFLUCENCE OF *SALCIA OBLONGA* EXTRACT IN WISTAR ALBINO RATS

J. Mary Vimalakumari Kalaiarasi, M. Raja and A. Anitha Joice

P.G & Department of Advanced Zoology and Biotechnology, Loyola College, Chennai-34

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ABSTRACT

The evaluation of powder extract of *Salacia oblanga* for hypolipidemic activity, biochemical changes in normal and Aluminium toxicity induced White Albino Wistar Female Rats. In conclusion, exposure to Aluminium has resulted in a decrease in erythrocyte, peripheral erythrocyte count and blood hemoglobin during short term exposure for 7 days. But this seems to be back to normal serum when exposure was continued for a further period of 7 days. The initial effect of decrease in red blood cells count and hemoglobin was found to be counteracted by co – administration of *S.oblonga*. The fluctuations seen in the Esonophil, Basophil and Lymphocyte count during exposure to *S. oblonga* seems to be is off on exposure for further period of 7 days. Thus, *S.oblonga* seems to have a beneficial effect is Aluminium effect in Aluminium induced hypoxia, seen during early phase of exposure to Aluminium.

INTRODUCTION

The greatly increased use of metals has introduced a new noxious hazard to animals and humans. Therefore, the persistence of metals in the environment has led to growing concern about the direct and indirect exposures of pregnant females and their unborn babies. Placental transfer of most metals places the health of unborn at the highest risk. Also, the newborn can be affected through breast feeding or direct exposure (Sastray, 1995 and WHO, 1997). Therefore, Prolonged exposure to some of these contaminants may induce teratogenic effects, abortion, reproductive failure and/ or immunotoxicity (Piramanayagam *et al.,* 1996;Carson, 2000 and Sharma and Mishra, 2006) both by their direct cellular toxic action and by interfering indirectly with the ypothalamo-hypophyseal function (Flora *et al.,* 2003). Nowadays, aluminum (Al) is widely used in treatment of drinking water, drugs (e.g., Antacids), deodorants and antiperspirants preparations, preservation of wood; the disinfection of stables and slaughter houses and in manufacture of alloys (WHO, 1997 and ATSDR, 2006). Al is present also in many food products, vegetables, cereals and beverages (Filipek *et al.,* 1987; U. S. Public Health Service, 1992; Beliles, 1994 and ATSDR, 2006). A wide array of medicinal plants and their active constituents play a role in antioxidant activity. *Salacia oblanga* (Family: Celastraceae/Hippocrateaceae) is an important source of chemicals of immense medicinal and pharmaceutical importance such as salacinol, mangiferin and kotanalol which are effective as antidiabetic, antiobese, hepatoprotective, hypolipidemic and antioxidant agent. Hence, this review consider the importance of the genus *Salacia* and n attempt is made to present macroscopical, phytochemical and pharmacological activities of the genus *Salacia* (Padmaa, Leena and Angelin, 2008). So the present study to investigate the serum glucose, Hemoglobin, Erythorocytes, Leucocytes, protein and differential count Aluminium chloride induced White Albino Wistar female rats.

*Corresponding author: loyo_raja2003@yahoo.co.in;
dr.kalaiarasi@yahoo.co.in

MATERIALS AND METHODS

The present study was carried out jointly at Loyola College, Chennai and in the Departments of Pharmacology and Environmental Toxicology of Dr. A.L.M. Post Graduate Institute of Basic Medical Sciences (Sekkizhar Campus), Taramani, Chennai. The study has been designed for the evaluation of Hydroalcholic powder extract of *Salacia oblonga* for biochemical changes and heamatological studies in normal and aluminum toxicity induced White Albino Wistar Female Rats.

Powder extract: The powder extract of *S. oblonga* was got from Department of Pharmacology and Environmental Toxicology, Dr. A.L.M. Post Graduate Institute of Basic Medical Sciences (Sekkizhar Campus), Taramani, Chennai. The nature of it was dark brown powder. All the extracts were stored in refrigerator at 4°C.

Experimental animals: The present study was conducted after obtaining the approval of the experiment protocol by the Institutional Animal Ethical Committee and CPCSEA (PROPOSAL NO: 01/ 039/ 2010, dated 1st June). This protocol met the OECD Guidelines for testing of chemicals. Number of animals approved for the study was 24 rats weighing between 240-290g, nulliparous and non-pregnant were used in this test. All animals used in this study were procured from Central Animal House Facility, Dr. A.L.M.

Post Graduate Institute of Basic Medical Sciences, (Sekkizhar Campus), Taramani, Chennai (Reg No : 205/ CPCSEA).

Housing: The animals were housed in well ventilated air conditioned Animal House at a constant temperature of 23±2°C, with the relative humidity of 55-60%. Lighting was artificial with the sequence of 12 hours light and 12 hours dark. The animals were housed on spacious polypropylene cages with paddy husk as bedding material. The animals were maintained on standard pellet diet and purified (Aquaguard). The animals were provided with food and water except during fasting. Each animal in the cage was marked on its tail with marker for appropriate identification.

Experimental setup: The Aluminium chloride and *S. oblonga extract* was subjected to biochemical studies at two dose levels: 200 mg/kg and 400 mg/kg (by mouth) for 21 days. Animals were grouped into 4 groups (n=6): Group 1 served as normal control, received Distilled water = 1 ml;, Group 2 served as treatment, and received Aluminium Chloride (300mg / kg; Group 3 served as Treatment, and received plant extract of *S. oblonga* (400mg / kg. Group 4 Treated with both Aluminium chloride and plant extract (250mg/ml, 100 mg/ml). The treatments were given for 21 days. The blood samples were withdrawn from retro orbital plexus at 1^{st} , 7^{th} , 14^{th} day and the biochemical studies were conducted.

Acute Toxicity Studies: The acute oral toxicity study was carried out according to the guidelines set by Organization for Economic co-operation and development (OECD) guidelines. Healthy Wistar rats (150-180 g) were used for this study. The two doses of 2000 mg/kg (by mouth) and 5000 mg/kg (by mouth) of the test samples were given to two groups containing 5 animals in each group. The treated groups were monitored for 14 days, for mortality and general behavior.

***Powder Extract of S. oblonga and Aluminium chloride treated*:** Animals received 200 mg/kg body weight of the powder extract of *S. oblonga*. Animals received 300 mg/kg body weight of aluminium chloride and 400 mg/kg of powder extract of *S. oblonga*. The powder extract of *S. oblonga* and aluminium chloride in the form of solution with double distilled water using 16 gauge oral feeding tube continuously for 21 days.

Isolation of serum: On the 0^{th}, 7^{th} and 14^{th} day, the rats were anesthetized using anesthetic ether and blood was collected from retro – orbital plexus. After collection, the blood was kept at 37°C for 30 min. Later it was centrifuged at 2000 rpm for 15 min to separate serum, which was used for biochemical estimations (Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), Urea, Bilirubin and Creatinine) and values were tabulated. The tabulated values were analysed with suitable statistical method.

Experimental procedure

Estimation of glucose: The ascensia Entrust blood glucose test is based on measurement of electrical current caused by the reaction of glucose with the reagents on the electrode of the test strip. The blood sample drawn into the reaction zone of the test strip through capillary action. The sample reacts with glucose oxidase (*A. niger*) triggering the oxidation of glucose in the blood. Electrons are generated, producing a current that is proportional to the to the glucose in the sample. After the reactioin time, the glucose concentration in the sample is displayed. The test results will either be displayed in milligrams of glucose per deciliter (mg/dl) or millimoles of glucose per litre (mmol/L). Blood glucose values will vary depending on food intake, medication dosage, health, stress or exercise, consult your physician or healthcare professional for the target value appropriate for ypu, standard medical practice goals for a typical non –pregnant individual with diabetes are. Before meals= 80 to 120 mg/dl (4.4to 6.7mmol/L); bedtime glucose: 100 to 140 mg/dl (5.6 to 7.8 mmol/L).

Estimation of Protein: Serum sample (Bovine serum albumin (BSA) Sigma chemicals, 5 mg in 10 ml of 1 N NaOH) were obtained by the allowing the whole blood left undistributed at room temperature for 2 hrs the clot formed was carefully separated from the wall of the tube with an applicator stick. This was stored in refrigerator for 24 hrs to permit clot contraction. After decantation and cool centrifugation (4°C) at 1000 x g for 30 minutes, the serum was aspirated and processed for estimation of protein. To 0.5 ml of the serum sample, 5ml of reagent [50 ml of reagent Sodium carbonates (2 g in 100 ml of 0.1 N NaOH)] mixed with 1 ml of reagent Cupric sulphate (500 mg in sodium tartar ate) was added and mix well after 10 minutes 0.5 ml of 1 N Folins phenol reagent was added. Reagent blank with 0.5 ml of saline and standard with 0.5 ml of BSA Solution were processed similar to the sample. 30 minutes after adding folins phenol reagent, the optical density measured at 500nm in brush and lam spectronic- 20. Spectrophotometer .the protein concentration of the samples was calculated by comparing I with the optical density of the standard concentration and expressed as grams per millimeters.

Erythrocyte counts: Haemocytometer was placed on the microscope on the microscopy stage and the counting area located. A coverglass was placed over the chamber portion so that its edge was in line with that of the slant edge of hemocyometer. Blood was drawn upto 0.5 marks in the thoma type blood-diluting pipette for red cells by suction. This was diluted with gower's fluid (sodium sulfate (Na2 So4) 12.2g and glacial acetic acid 33.3 ml made upto 200ml with distilled water) to the 101 mark effecting 1:200 dilution, after removing the rubber tubing and sealing both ends with thumb and fore finger, the pipette was shaken with a rapid figure – eight motion for 1 minutes, controlling the upper opening of the pipette with the fore finger , a drop of fluid was discharged on to a filter paper and the fluid adhering the stem wiped away . Holding the pipette at about 45°C, the tip of the pipette was placed along the edge of the cover glass and the hemocytometer, the fluid was slowly released and allowed to seep into the counting are without any air bubbles or overflow into the moats. The cells were allowed to settle for about 3 minutes; the diaphram and condenser were adjusted for good contrast and resolution. The cells in the four corner large squares and the central large square of the central 1mm2 area were counted. The formula used to determine the number of cell per millimeter using large squares in the central 1 mm^2 area was

$$\frac{\text{Total cells counted x 25 x dilution x 104}}{\text{Number large squares counted}}$$

Leukocyte counts: Blood was drawn into a thoma –type pipette (for white) to the level of 0.5 marks and diluted with WBC diluting fluid (2ml of 1% gentian violet in 100 ml distilled water containing 1.0 ml glacial acetic acid) to the 11 marks effecting a 1:20 dilution. The remaining procedure was similar to erythrocyte counting except that, the cells in the sixteen large squares in each of the four corner 1-mm2 areas were counted. The following formula was used for the determination of number of cells per millimeter.

$$\frac{\text{Total cells counted x dilution x 104}}{\text{Number of 1-mm2 areas counted.}}$$

Determination of Hemoglobin: Hemoglobin was determined by the method of hawk et al. (1966). Using Sali's hemometer, blood was drawn upto 20 µl mark of the hemometer tube pipette and discharged into the hemoglobinometer tube containing 0.1N hydrochloric acid upto 10 mark on the % side, after complete lysis the contents were diluted with distilled water to match the reference color and reading were recorded.

Differential Leukocyte counts: A drop of blood was placed near one edge of a clean, dry grease free, thin glass slide the edge of another slide was placed near the blood drop. After the blood spread to the width of the slide, a thin uniform smear was made by moving the spreader slide at angle of 45° over the other slide by a single smooth stroke, the smear was allowed to dry in slide. The smear was completely converted by Leisman's stain (1.5 Leishman powder in 1000ml of methyl alcohol). After 2 minutes an equal quantity of Sorensen's buffer (p H = 6.8) was added, this was washed after 10 minutes with the buffer, the smear was allowed to dry at room temperature. Differential counting of the smear was done under oil immersion objective, general morphology and distribution of the leukocytes and erythrocyte were observed. The present of different types of leukocytes in an evenly distributed area was recorded.

Statistical analysis: The observation of the study was noted and analyzed with suitable statistical methods: One – way ANOVA is done to evaluate whether there is any evidence that the means of the population differ. If the ANOVA leads to a conclusion that there is evidence that the group means differ, then Tukey multiple comparison test is used. The value of $P < 0.05$ was considered to be significant. The statistical analysis was performed using SPSS for Windows version 10 Software.

RESULTS AND DISSCUSSION

In the present study, its influence on the biochemical changes induced by aluminium chloride has been studied from 7th and 14th day. Oral administration of *Salacia* extract and Aluminium chloride for two weeks significantly lowered the serum at 14th day. The entire test was shown increased in 7th day and decreased in 14th day. When compared to the control group ERYTHROCYTES (RBC) (mill/cumm) found to be increased in Group II treated with Aluminium Chloride ($p \leq 0.05$) and no significant change was seen in Group II treated with Aluminium, Group III treated with *S. oblonga* and Group IV treated with both aluminum and *S. oblonga* after 14 days of exposure. When compared to the control group found to be increased in on LEUKOCYTES (WBC) (cumm) Group IV treated with both Aluminium Chloride and *S. oblonga* ($p \leq 0.05$) after seven days and no significant change was seen in Group II treated with Aluminium, Group III treated with *S. oblonga* and Group IV treated with both aluminum and *S. oblonga* after 14 days of exposure. When compared to the control group found to be increased in HEAMOGLOBIN Group II treated with Aluminium Chloride ($p \leq 0.05$) and no significant change was seen in Group II treated with Aluminium, Group III treated with *S. oblonga* and Group IV treated with both aluminum and *S. oblonga* after 14 days of exposure.

When compared to the control group NEUTROPHILS no significant change was seen in Group II treated with Aluminium, Group III treated with *S. oblonga* and Group IV treated with both aluminum and *S. oblonga.* When compared to the control group ESONOPHILS found to be increased in group III treated with *S oblonga* ($p \leq 0.05$) and in Group IV treated with both Aluminium Chloride and *S. oblonga* ($p < 0.05$) after seven days and no significant change was seen in Group II treated with Aluminium, Group III treated with *S. oblonga* and Group IV treated with both aluminum and *S. oblonga* after 14 days of exposure. When compared to the control group found BASOPHILS to be increased in in group III treated with *S. oblonga* ($p \leq 0.05$) and in Group IV treated with both Aluminium Chloride and *S. oblonga* ($p \leq 0.05$) after seven days and no significant change was seen in Group II treated with Aluminium, Group III treated with *S. oblonga* and Group IV treated with both aluminum and *S. oblonga* after 14 days of exposure. When compared to the control group LYMPHOCYTES found to be increased in Group II treated with Aluminium Chloride ($p \leq 0.05$), in group III treated with *S. oblonga* ($p \leq 0.05$) after seven days and no significant change was seen in Group II treated with Aluminium, Group III treated with *S.oblonga* and Group IV treated with both aluminum and *S. oblonga* after 14 days of exposure.When compared to the control group MONOCYTES no significant change was seen in Group II treated with Aluminium, Group III treated with *S. oblonga* and Group IV treated with both aluminum and *S.oblonga.*

When compared to the control group GLUCOSE (mg/dl) no significant change was seen in Group II treated with Aluminium, Group III treated with *S. oblonga* and Group IV treated with both aluminum and *S. oblonga.* When compared to the control group PROTEIN (g/dl) no significant change was seen in Group II treated with Aluminium, Group III treated with *S. oblonga* and Group IV treated with both aluminum and *S. oblonga.* Aluminium has potent to have many biological effects. In the present study its effect on selected hematological and Biochemical parameters were screened in Wistar Albino Rats during a short term exposure period of 14 days. *S. oblonga* has been quoted to have a specimen of medicinal properties and in this study the hydroalcoholic extract of the leaves of *S. oblonga* was screened for its property on the influence of aluminium. Exposure to aluminium for 7 days decrease the erythrocyte number; decreased the peripheral erythrocyte count

Table 1: Effect of Aluminium chloride and *Salacia oblonga* on erythrocytes (RBC) (mill/cumm), Leukocytes (WBC) (cumm) and Heamoglobin in Wistar albino rats

Groups	Erythrocytes (RBC) (mill/cumm)		Leukocytes (WBC) (cumm)		Heamoglobin	
	7th day	14th day	7TH day	14th day	7TH day	14th day
Group I	4.40	4.40	7133.33	7133.33	11.17	11.17
	± 0.37	± 0.36	± 0992.30	± 0992.30	± 01.04	± 01.04
Group II	2.54 *	3.38	6866.67	6258.33	9.47 *	10.47
	± 0.79	± 0.79	± 1772.76	± 1534.08	± 0.70	± 00.88
Group III	3.19	4.43	6408.00	7783.33	10.03	11.57
	± 0.79	± 0.79	± 1442.71	± 0971.94	± 01.64	± 00.43
Group IV	4.67	4.24	11700.00*	7858.33	10.33	10.87
	± 0.49	± 0.49	± 01648.03	± 1855.91	± 00.41	± 00.58

Group I : Control (Distilled water = 1 ml); Group II : Aluminium Chloride (300mg / kg); Group III : Plant extract of *Salacia oblonga* (400mg / kg); Group IV : Aluminium Chloride and plant extract. Values are mean± S.D.: n=6 in each group; $p \leq 0.05$; * Experimental group compared with control group; where the significance performed by one- way ANOVA followed by Post Hoc Dunnett's test.

Table 2: Effect of Aluminium chloride and *Salacia oblonga* on Neutrophils, Esonophils and Basophils in Wistar albino rats

Groups	Neutrophils		Esonophils		Basophils	
	7th day	14th day	7TH day	14th day	7TH day	14th day
Group I	38.33	38.33	2.50	2.50	6.00	6.00
	± 01.37	± 01.37	± 0.84	± 0.84	± 2.37	± 2.37
Group II	39.83	31.83	2.50	1.50	2.83	7.33
	± 03.54	± 06.21	± 1.05	± 0.84	± 1.72	± 2.14
Group III	36.33	33.00	5.17*	2.17	1.83*	5.67
	± 02.94	± 06.07	± 0.75	± 0.98	± 1.17	± 1.63
Group IV	38.33	37.33	4.67*	1.50	1.83*	5.50
	± 2.58	± 05.57	± 1.03	± 1.05	± 1.17	± 1.97

Group I : Control (Distilled water = 1 ml); Group II : Aluminium Chloride (300mg / kg); Group III : Plant extract of *Salacia oblonga* (400mg / kg); Group IV : Aluminium Chloride and plant extract. Values are mean± S.D.: n=6 in each group; $p \leq 0.05$; * Experimental group compared with control group; where the significance performed by one- way ANOVA followed by Post Hoc Dunnett's test.

Table 3: Effect of Aluminium chloride and *Salacia oblonga* on Lymphocytes and Monocytes in Wistar albino rats

Groups	Lymphocytes		Monocytes	
	7th day	14th day	7TH day	14th day
Group I	44.67	44.67	8.83	8.83
	± 02.80	± 02.80	± 3.60	± 3.60
Group II	49.00	43.50	5.83	12.67
	± 01.55	± 02.95	± 1.72	± 03.78
Group III	49.33*	45.50	7.50	13.83
	± 02.73	± 03.94	± 3.15	± 03.19
Group IV	46.33	45.50	9.00	10.17
	± 03.72	± 01.38	± 2.28	± 04.62

Group I : Control (Distilled water = 1 ml); Group II : Aluminium Chloride (300mg / kg); Group III : Plant extract of *Salacia oblonga* (400mg / kg); Group IV : Aluminium Chloride and plant extract. Values are mean± S.D.: n=6 in each group; $p \leq 0.05$; * Experimental group compared with control group; where the significance performed by one- way ANOVA followed by Post Hoc Dunnett's test

Table 4: Effect of Aluminium chloride and *Salacia oblonga* on glucose (mg/dl) and protein (g/dl) in Wistar albino rats

Groups	Glucose (mg/dl)		Protein (g/dl)	
	7TH day	7TH day	7TH day	14th day
Group I	111.83	111.83	8.30	8.30
	± 042.74	± 042.74	± 0.53	± 0.53
Group II	113.17	113.17	9.40	6.67
	± 033.50	± 033.50	± 0.79	± 2.96
Group III	101.00	101.00	11.12	8.01
	± 012.85	± 012.85	± 03.06	± 1.53
Group IV	129.00	129.00	8.95	7.40
	± 036.91	± 036.91	± 0.78	± 3.04

Group I : Control (Distilled water = 1 ml); Group II : Aluminium Chloride (300mg / kg); Group III : Plant extract of *Salacia oblonga* (400mg / kg); Group IV : Aluminium Chloride and plant extract. Values are mean± S.D.: n=6 in each group; $p \leq 0.05$; * Experimental group compared with control group; where the significance performed by one- way ANOVA followed by Post Hoc Dunnett's test.

significantly. This effect seems to wear off during continuous of the treatment as no such significant suppression in the count was seen after 14 days of exposure. This observation indicates the possibility of hypoxic state during short term exposure rate. By this the recovery of the erythrocyte count during continuation of the exposure has been explained by the fact that hypoxia during exposure for 7 days could have been the triggering factor for increased erythrocyte production so that oxygen supply to the tissues can be maintained. This effect of aluminium is found to have been suppressed by *S. oblonga* as suppression is erythrocyte count was not seen after 7 days exposure in the group treated with both the extract and the aluminium. Decrease in erythrocyte the blood hemoglobin content has also been found to be decreased, during exposure for 7 days. This decrease in hemoglobin can also contribute for hypoxia. Exposure for 7 – 14 days with aluminium has not resulted in any significant charge in the peripheral leucocytes count. *S. oblonga* exposure also did not cause any significant change.

However, the exposure to both aluminium as well as *S. oblonga* has resulted in an increased leucocytes count after 7 days of treatment. This effect seems to be temporary as no such increase was seen at the end of 14th day during combined exposure. Differential cell count has revealed that there was no significant change in the neutrophil count during exposure to aluminium or *S. oblonga* or a combination of both after 7 – 14 days of exposure. An increase is esonophil count was seen after 7 days of exposure to *S. oblonga.* This seems to be a temporary effect as no such increase was seen after 14 days of exposure. The increase seen after 7 days of exposure to *S. oblonga* is also reflected during treatment with both Salacia oblonga as well as aluminium. Though a basophil decrease has been observed after 7 days of treatment with *S. oblonga* or combination of *S. oblonga* and aluminium. The effect was not seen after 14 days of treatment. A mild increase in lymphocyte count seen after 7 days of treatment with *S. oblonga* was not seen after 14 days of treatment. There was no significant change in monocyte count exposure with *S. oblonga* or Aluminium or both *S. oblonga* and Aluminium. Study of serum protein and blood glucose is revealed that there was no significant change in the serum content of these two during exposure to Aluminium or *S. oblonga* or a combination of both aluminium and *S. oblonga* for a period of 7 – 14 days. Serum protein glucose levels were found to be within normal levels and much fluctuation was seen.

CONCLUSION

In conclusion, exposure to Aluminium has resulted in a decrease in erythrocyte, peripheral erythrocyte count and blood hemoglobin during short term exposure for 7 days. But this seems to be come back to normal serum when exposure was continued for a further period of 7 days. The initial effect of decrease in red blood cells count and hemoglobin was found to be counteracted by co – administration of *S. oblonga*. The fluctuations seen in the esonophil, basophil and lymphocyte count during exposure to *S. oblonga* seems to be is off when exposure was continued for future period of 7 days. Thus, *S. oblonga* seems to have a beneficial effect is Aluminium effect in Aluminium induced hypoxia, seen during early phase of exposure to Aluminium chloride.

REFERENCES

American Diabetes Association; standards of Medical care for patients with Diabetes mellitus (position statement), diabetes care 25(suppl, 1) s37, 2002.

Atkin S, jaker MA, Chorost MI and reddy S, Fingerstik glucose determinatioin shock, *Annals of Internal Medicine,* 1991; 114; 1020-1024.

Collene AL, Hertzler SR, Williams JA et al. Effects of a nutritional supplement containing *S. oblonga* extract and insulinogenic amino acids on postprandial glycemia, insulinemia, and breath hydrogen responses in healthy adults. *Nutrition*, 2005;21:848-54.

Cheesbrough, M. and Mc Arthur, J, 1976, a laboratory manual for rural tropical hospitals p.40 Churchill Livingstone, London.

Garvey, J.S, Cremer, N.E. and Sussdorf, D.H .1977. Methods in immunology,3rd edn. Pp1-545. W.A Benjamin, Inc., USA.

Heacock PM, Hertzler SR, Williams JA et al., Effects of a medical food containing an herbal alpha-glucosidase inhibitor on postprandial glycemia and insulinemia in healthy adults. *J Am Diet Assoc*., 2005;105:65-71.

Hawk, P.B., Oser, B.L and Summerson, W.H 1966, Practical Physiological Chemistry, 14th edition Churchill, London.

NCCLS, Protection of laboratory workers from occupationally Acquired infection; approved guideline – second edition. NCCLS Document M29-A2 (isbn 1-56238 –453-8).

Lowry, O.H., Rosebrough, N.J., Farr, A.L and Randall, R.J. 1951. Practical measurement with the folin phenol reagent. *J. Biol, Chem.,* 193 .265-275.

Tietz fundamentals of clinical chemistry, 5th edition, edited by brutis CA and ashwood E.R, W.B. Saunders Co., Philadelpia, PA, 2001, p444.

Tatiya, A.U., A.S. Kulkarni, S.J. Surana and N.D. Bari, 2010. Antioxidant and hypolipidemic effect of *Caralluma adscendens* roxb. in alloxanized diabetic rats. *Int. J. Pharmacol.,* 6: 400-406.

Toxic effects of metals, Jie Liu, Robert A. Goyer, and Michael P. Waalkes

Matsuda H, Murakami T, Yashiro K *et al.* Antidiabetic principles of natural medicines. IV. Aldose reductase and qlpha-glucosidase inhibitors from the roots of *S. oblonga* Wall. (Celastraceae): structure of a new friedelane-type triterpene, kotalagenin 16-acetate. *Chem Pharm Bull., (Tokyo)*. 2000;47:1725-9.

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