Assessment of Antihyperglycaemic Activity of *Calotropis Procera* Leaves Lxtract on Alloxan-Induced Diabetes Male Rats

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Abstract

The present study aims studying the influences of treatment with methanol watery leaf extracts of *Calotropis procera* on Fasting blood glucose and Insulin level in alloxan induced diabetic male rats. phytochemical analysis of leaves extract reveals presence of Glycosides, Tannines, phenols compound, Flavonoids, Coumarins, Resins and terpenes. Also methanol watery leaf extracts of *Calotropis procera* dose 250 and 500mg/kg body weight showed significant lowering in the blood glucose from 348.16 ± 36.67 to 169.33 ±21.80 after 45 day of treatment. While showed non-significant enhancement in fasting serum Insulin level.

Key words: *Calotropis procera*,diabetes, fasting blood glucose **,** serum Insulin**.**

الخلاصة

الدراسة تهدف الى دراسة تاثير العلاج بالمستخلص الميثانولي المائي لاوراق نبات الدييباج على خفض مستوى الكلوكوز بالدم وزيادة مستوى الانسولين في ذكور الجرذان المستحث فيها السكري بالالوكسان. التحليل الكيميائي النباتي لاوراق نبات الدييباج يكشف عن وجود جليكوسيدات، تانينات، مركبات فينولية، الفلافونويدات، الكومارين، الراتنجات وتربينات. كذلك اظهر العلاج بالمستخلص تركيز ٢٥٠ و ٥٠٠ ملغم/كغم انخفاض معنوي واضح في مستوى الكلوكوز من ٣٤٨،١٦ ±٣٦،٦٧ الى ١٦٩،٣٣ ± ٢١،٨٠ بعد ٤٥ يوم بينما زيادة غير معنوية في مستوى الانسولين في المصل.

الكلمات المفتاحية: الأمصال والسكري، والسكر في الدم الصيام، والانسولين المصل

Introduction.

Diabetes mellitus DM is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both.(ADA,2013). Type-2 diabetes is also known as non-insulin-dependent diabetes mellitus or adult onset diabetes.It accounts for 85–90% of all cases of diabetes (Wong and Toh, 2009).

Some medicinal plants have been reported to be useful in treatment of diabetes worldwide and have been used empirically in antidiabetic and antihyperlipidemic remedies. Antihyperglycemic activity of the plants is mainly due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibiting the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes. (Patel *et al.,*2012) .

Calotropis procera (*C.procera*) belongs to the family Asclepiadaceae is a desert plant known as Ushar or madar in greeko-arab medicine, This plant is generally distributed in tropical and subtropical Africa and Asia.(Sharma *et al*.,2011) A wide range of chemical compounds was identified in *C.procera* including cardiac glycosides, flavonoids, phenolic compounds, terpenoids alkaloids, resins, anthocyanins, tannins, saponins, sterol, and proteolytic enzymes

(Shaker *et al*., 2010). The plant has been widely used in the Ayurvedic, Unani, Arabic and Sudanese-Indian traditional system of medicine for the treatment of various ailments. Almost all the parts of *c.procera* leaf, latex, stem, root, bark, flower and seed have been documanted to possess medicinal features. (Sudesh *et al*.,2012).

Material and methods

Preparation of methanol watery leaves extract of *Calotropis procera*

The Fresh leaves of *Calotropis procera* (Aiton) W. T., were collected and dried then milled into a fine powder with the help of a suitable grinder . According to Sato *et al*. (1990) the plants extracts were prepared .

Phytochemical tests of *Calotropis procera* leaves extract

Detection of some phytochemical compounds:

The methods described by Smolensk *et al*.,(1972) to detect alkaloids, Geisman (1962) to detect coumarins, Jaffer *et al*., (1983) to detect flavones , Shihata (1951) to detect glycosides, Saponins and resins,Harborne (1973) to detect Phenolic compounds, Al-Shami (1982) to detect tanins and Al-Maisary (1999) to detect terpens and steroids.

Thin layer chromatography (TLC)

Thin layer chromatography (TLC) is widely used for the rapid analysis of phytochemical preparations. TLC was carried out on silica gel plates. The silica gel plates were activated in an oven at 105^{o}C for one hour. The TLC was carried out by spotting drops of each plant extract on the base of each plates and put it in a tank containing mobile phase (developing solvents) just to wet the lower edge of the plate but it was not adequate to wet the part of plate where spot were applied (origin) the solvent front then it migrated up the plate by capillary action .Afraction in quantifying migration of a compound on solvent system is the R_f value(Gibbons, 2005).

$$R_f = \frac{\text{Compound distance from origin(mid point)(cm)}}{\text{solvent travels(front distance from origin)(cm)}}$$

The mobile phases used were distilled water: ethyle acetate: methanol (20:60:20v/v)

Experimental design of study

Male white adult rats obtained from the Babylon University aged 2-3 months were used in this study. Water and industrialized dry food were supplied. The overall number of animals used was 36 rats after adaptation rats were divided into 2 groups 18 rats in each group, the first group were used for the induction of type2 DM. Diabetes was induced using multiple dose (3 doses) of alloxan 120 mg /kg body weight dose each 24h, caused sustained hyperglycemia (Al-Joubori,2013), the second group included 18 rats that were treated with distilled water. Then after the induction of DM, each group was subdivided into 3 groups that included 6 animals in each one.

Group 1 (NC:Normal control)which were not given any dose with any of the study materials (neither diabetogen; alloxan; nor the treatment), but they were given normal saline intraperitonialy (i.p.) and administrated distilled water orally by orogastric tube.

Group 2 (DC: diabetic control) Included animals which had been given alloxan i.p. and administrated distilled water orally but had not been treated with plants extracts.

Groups 3and 4 They were received alloxan i.p. and after the induction of DM (45 days), diabetic rats treated with methanolic extract of the leaves of *C. Procera* 250 and 500 mg/kg/day b.w., respectively administrated orally by orogastric tube for 45 days.

Group 5 and 6 non-diabetic rats treated with methanolic extract of the leaves of *C. Procera* 250 and 500 mg/kg/day b.w., respectively administrated orally by orogastric tube for 45 days.

Fastig blood glucose level (FBG) of fasting rats were measured weekly by using the glucometer and the rats with blood glucose level above 200 mg/dl were considered to be hyperglycemic and were selected for extract treatment.(Bhaskar and Ajay,2009; Neto *et al*.,2013).Blood samples were collected from the caudal vein tail for glucose estimation by a sterile needle, then a blood drop was put in contact with the strip of glucometer to measure the blood glucose level. At the end of treatment and sacrificing the animals, blood was collected directly by heart puncture. The clotted blood was centrifuged and serum was collected for measuring hormone insulin.

Serum Insulin level was measured according to the Rat Insulin ELISA Kit (Elabscience) {Catalog No:E-EL-R2466} .

Results :

Detection of Some Phytochemical Compounds in the *Calotropis Procera* leaves Extract

The result of Phytochemical detection of methanol watery leaf extracts of *Calotropis procera* revealed the presence of Glycosides, Tannines, phenols compound, Flavonoids, Coumarins, Resins and terpenes as shown in Tabl(1).

Table (1): Detection of some phytochemical compounds in the *Calotropis procera* leaves extract .

Phytochemical compound	Detection method		Methanol leaf extract of C.procera
	Reagent	Posative result	
Alkaloids	Mayer	White color	-
	Wagner	Brown color	-
Glycosides	Benedict	Red pellet	+
Tannines	Lead acetate 1%	White gelatinous pellet	+
Saponins	Shaken	For motion of foam	-
	Hgcl %	White color	-
Phenols compound	Ferric chloride 1%	Green bluish color	+
Flavonoids	Ethanol 50% + KOH 50%	Yellow color	+
Coumarins	Uv light	Yellow greenish color	+
Resins	HCL 4%	Turbidity	+
Volatile oil	Uv light	Bright pink color	-
Terpenes	Chloroform + acetic acid+ $_{H2SO4}$	Blue color	+

+ positive detection/ - negasitive detection

4.1.2: Thin layer chromatography (TLC) technique

Figure (1) explains the detection of phytochemical compounds by TLC on glass plates silica gel 20x20 cm when examined by visible and UV lights. Table 2 explains the number , properties and R_f value of each band for leaves extract.

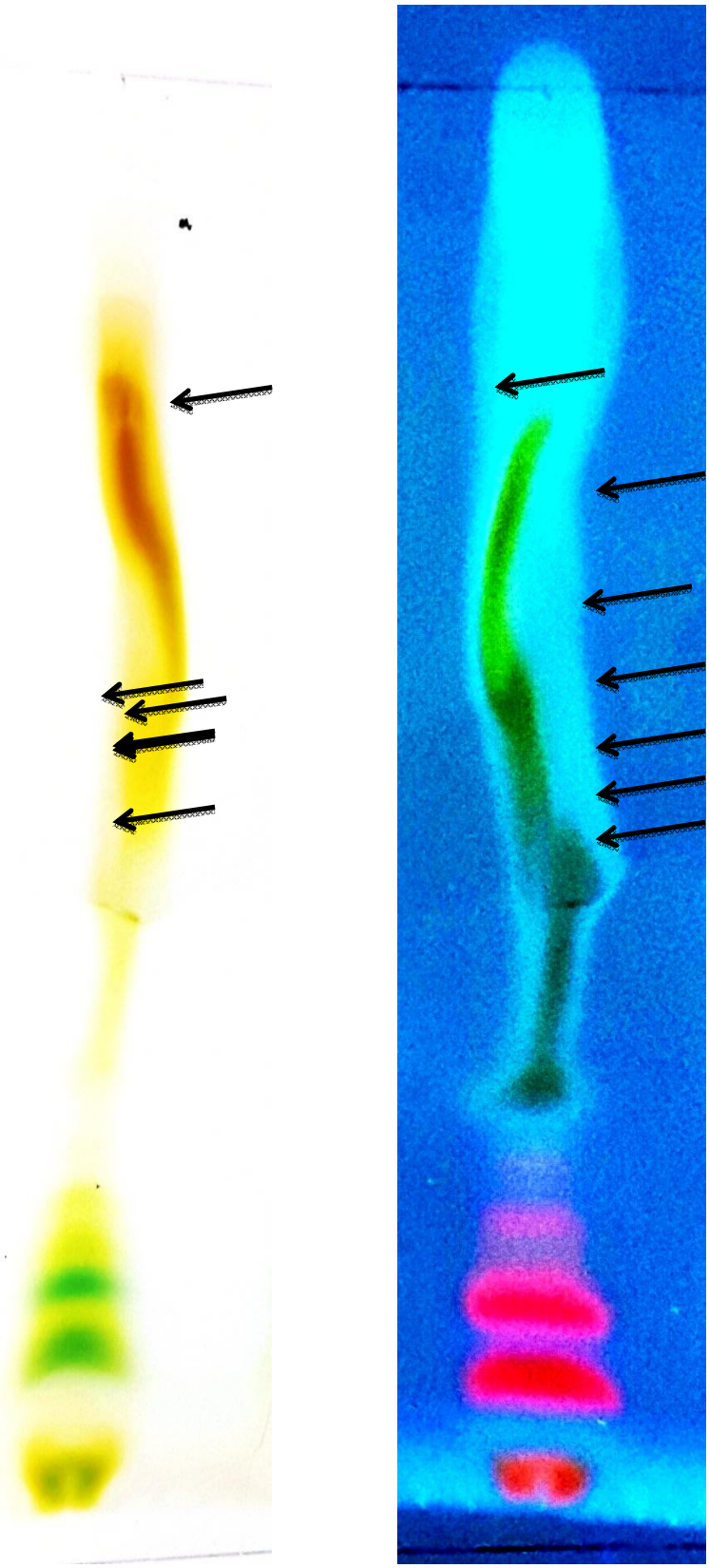
The result of using developing solvent system methanol: ethyl acetate: distal water D.W (20:60:20 v/v/v) was six bands in visible and 7 bands under UV light, R_F value for each bands was measured under visible and UV light .

Table (2)TLC of methanol – watery extract of Calotropis procera leaves using developing solvent system (methanol: ethyl acetate: D.W)

Detection method	Band properties		Number of bands
	Color	Rf Valu	
Visible light	Pale green	0.07	6
	Green	0.14	
	Yellow	0.16	
	Green	0.18	
	Yellow	0.45	
	Yellow	0.62	
UV – light	Purple	0.07	7
	Purple	0.14	
	Purple	0.16	
	Purple	0.45	
	Dark green	0.57	
	Dark green	0.62	
	Dark green	0.71	

Fasting blood glucose

The results of fasting blood glucose (FBG) levels ,fasting blood gloucose change in normal control and all experimental groups were explained in Table (3).Alloxan injection caused significant increase ($p\leq0.05$) in mean of FBG levels 333 ± 27.98 mg/dl as compared to normal control group 89.500 ± 6.26 mg/dl. There was significant decrease ($p\leq0.05$) in mean of FBG levels after treatment in diabetic with leaves extract dose 250 and 500 mg/kg group from 348.16±36.67 and 304±44.7 mg/dl to 169.33± 21.80and 183.66 ± 29.40 mg/dl respectively. When compared to diabetic control group and shows non-significant difference when compared to normal control group. Normal with leaves extract dose 250 and 500mg/kg groups show non-significant difference of FBG after treatment as compared with normal control .



A B

Figure(1) 1)TLC of methanol –watery of *C.procera* leaves extract using developing solvent system methanol: ethyl acetate: distal water D.W (20:60:20 v/v/v)A : visible light 6 bands, B: UV light 7 bands,

Table (3) Mean and Standard error of fasting blood glucose befor and after treatment with the *Calotropis procera* leaves extract and Fasting blood Gloucose change in experimental rats

Group	Fasting blood Gloucose befor tratment mg/dl (mean ± S.E)	Fasting blood Gloucose after treatment mg/dl (mean ± S.E)	Fasting blood Gloucose change mg/dl (mean ± S.E)
Normal contral	89.500 ± 6.26	90.16 ± 3.60 a	0.66 ± 5.96
Diabetic control	333 ± 27.98	313.83 ± 44.35 b	19.16 ±45.21
Diabetic with leaves Extract 250 mg/kg	348.16 ± 36.67	169.33 ± 21.80 c	178.83 ±40.32
Normal with leaves Extract 250 mg/kg	85.500 ± 5.79	84.50 ± 8.28 ad	1.00 ± 7.33
Diabetic with leaves Extract 500 mg/kg	304 ± 44.7	183.66 ± 29.40 ec	120.33 ± 23.43
Normalwith leaves Extract 500 mg/kg	98.16 ± 3.04	96.16 ± 3.53 afd	2.00 ± 3.50

Different letters refer to significant difference between groups .
Similar letters refer to non significant difference n=6 each group .

Fasting serum insulin

Diabetic control group shows significant decrease ($p \leq 0.05$) in mean of fasting serum insulin 1.46±0.48 µu/ml as compared with normal control group which was 4.13±0.68 µu/ml, whereas diabetic with leaves extract dose 250 and 500 mg/kg group reveals non-significante increase in insulin level2.21±0.24 and 2.08±0.25 respectively as compared with diabetic control group while normal with leaves extract dose 250 and 500 mg/kg group reveal non-significante differences in Insulin level as compared with normal control group but significante differences as compared with the other groups as show in table(4).

Table(4) characterization of Insulin level in experimental rats(mean ±S.E)

Group	Fasting Serum Insulin (µu/ml) (mean ± S.E)
Normal contral	4.13 ± 0.68 a
Diabetic control	1.46 ± 0.48 b
Diabetic with leaves Extract 250 mg/kg	2.21 ± 0.24 cb
Normal with leaves Extract 250 mg/kg	3.22 ± 0.42 ad
Diabetic with leaves Extract 500 mg/kg	2.08 ± 0.25 ebc
Normalwith leaves Extract 500 mg/kg	5.07 ±0.54 af

Different letters refer to significant difference between groups
Similar letters refer to non significant difference n=6 each group

Discussion

Detection of some phytochemical compound in the *Calotropis procera* leaves extract

The preliminary qualitative chemical tests of plant extract in this study confirmed the presence of Glycosides, Tannines, phenols compound, Flavonoids,

Coumarins, Resins and terpenes by different detection methods. The existence of these components in this species is an indication that it may have some medicinal potential.

These results are consistent with other studies which showed the phytochemical analysis of crude extract of *Calotropis procera* leaf reported the presence of terpenoids, saponins, tannins, cardiac glycosides, phenol and tannin, protein, acidic compounds and carbohydrates. The flavonoids and tannins are phenolic compounds and plant phenolics are major group of compounds that act as primary antioxidants (Muzammal,2014).

Also methanol leaf extracts of *Calotropis procera* revealed the presence of Glycosides, Protein, Triterpenoids, Steroids, Flavonoids Tannin and Phenolic. Where Petroleum ether extract showed presence of Glycosides, Protein, Triterpenoids and Steroids only.(Tiwari *et al.*,2014)

Detection of some phytochemical compound by Thin layer chromatography (TLC) technique

The present results of TLC are consistent with other studies, In TLC profile of *Calotropis procera* leaves hexane extract a number of bands appeared at different Rf under different wave length of UV and 5 bands in white light were observed. (Dwivedia *et al*.,2014).

Fasting blood glucose (FBG)

Alloxan has been used to induce experimental diabetes model due to the selective destruction of the insulin-producing pancreatic beta-islets. Table (3) clearly demonstrated significant increase ($p \leq 0.05$) of FBG level in diabetic group as compared with normal control group and persistant hyperglycemia in diabetic animals .

From the present results, it is of interest to note that hypoglycemic effect is not related directly to insulin action and may be mediated by another mechanism. In recent times, Meddah *et al.,*(2009) showed that chronic oral administration of *Nigella. sativa* seeds to rats inhibited intestinal glucose absorption, which may contribute to the hypoglycemic effect ,Or it may caused an increased sensetivity of insulin receptors on cell membrane to the insulin. So the decrease in glucose may be due to the decreased hepatic gluconeogenesis after treatment(Fararh *et al*.,2005) .

These results are consistent with (Neto *et al.,*2013)who found that *C. procera* leaves extract induced a decrease in blood glucose that was similar to the standard anti-diabetic drug metformin and this effect was also reflected by the decrease of daily water and food intake. In addition, the oral glucose tolerance test performed at the end of treatment that showed clearly the animals treated daily with *C. procera* not only had lower fasting glucose levels than the diabetic control group, but also improved their metabolic state through increased glucose tolerance in a manner similar to the metformin-treated group, Etuk and Mohammed (2009) also showed that administration of 200 mg/kg of aqueous extracts *C. procera,* produced a significant reduction ($P \leq 0.05$) in blood glucose levels in the alloxan induced diabetic rats.

Fasting serum insulin

The results shows significant decrease ($p \leq 0.05$) in fasting serum insulin level in diabetic control group as compared to normal control group. This reduction in insulin may be due to the alloxan and its reduced product dialuric acid, establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide with a simultaneous massive increase in cytosolic calcium concentration, which causes rapid

destruction of pancreatic β-cells (Frode,2008). wherease it reveals increase in insulin level in tratment group (diabetic with leaves extract dose 250 and dose 500mg/kg) but non-significant increase as compared with diabetic control group. Insulin levels in treatment group increase as a result to daily oral administration of leaves extract for 45 days, this result reflects that the hypoglycemic effect may be because of improvement pancreatic secretion of insulin from β-cell of islets, or it may be due to various classes of phytochemicals present in the *C.procera* leaves extract that have included alkaloids,Flavonoids and tannins.

Alkaloids have been linked to the regeneration of the β cells of the pancreas (Singh and Gupta,2007). Flavonoids and tannins are known for their antioxidant properties and anti diabetic activities Flavonoids are known to regenerate the damaged beta cells in the alloxan induced diabetic rats and acts as insulin secreter (Balamurugan *et al.,* 2013) .

In Study of Yadav *et al*.(2014) several parts of *C.procera* supporting our finding that ethyl acetate extract of leaf of *Calotropis procera* 250 exhibited amarked anti-hyperglycemic activity in streptozotocin induced diabetic rats by lowering the HbA1C% and improving the insulin levels. While regarding normal with leaves extract dose 250 and 500mg/kg group insulin level did not affected as compared with normal control group this shows no side effect of this extract.

References

Al-Joubori, M. A., 2013, Histological and Cytological Effects of Some Plants Extracts on Hyperglycemic Male Rats. A Thesis Submitted to the Council of the College of Science University of Babylon.

AL-Maisary, M., 1999, Effect of oil and alcoholic extract of Azdirachta indica on some pathogenic fungi of plant. M. Sc. thesis Al- Mustansria University. Iraq.

Al-Shami, S., 1982, Study of some pharmacological and toxilogical properties of*Achillea* flowers. M.Sc. thesis, Baghdad University.

American Diabetes Association, 2013,: Diagnosis and Classification of Diabetes Mellitus ., Diabetes Care journals. Volume 36, Supplement 1.

Balamurugan, K., Nishanthini A. And Mohan V. R., 2013, Antidiabetic Antihyperlipidamic Activity of Ethanol Extract of Melastoma malabathricuml Leafe in Alloxan Induced Diabetic Rats. Internaional Journal of Pharmaceutical Reserch and Bio-Science.; Volume 2(5):223-236.

Bhaskar V. H and Ajay,S. S., 2009, Antihyperglycemic and antihyperlipidaemic activities of root extracts of Calotropis procera (Ait.) R. Br on streptozotocin induced diabetic rats. Jordan Journal of Biological Sciences.,Volume2, p:177-180.

Dwivedi, B., Singh, A., Mishra, S., Singh, R., Pant, P., Thakur, L.K. *et al*.,2014, Evaluation of Phytochemical Constituents By Gas Chromatography-Mass Spectroscopy & Hptlc OF Calotropis procera. World Journal of Pharmaceutical Research.,Volume 3, Issue 7, 708-715. Research Article ISSN 2277 – 7105.

Etuk, E. U. and Mohammed, B. J., 2009, Informant consensus selection method: A reliability assessment on medicinal plants used in north western Nigeria for the treatment of diabetes mellitus. African Journal of Pharmacy and Pharmacology Vol. 3(10). pp. 496-500.

Fararh, K. M., Shimizu, Y., Shiina, T., Nikami, H., Ghanem, M. M., and Takewaki, T., 2005,Thymoquinone reduces hepatic glucose production in diabetic hamsters. Res. Vet. Sci., 79: 219–223.

Frode, T. S. and Medeiros, Y. S., 2008, Journal of Ethnopharmacology. 115, 173–183.

Geisman, T., 1962, Chemistry of Flavonoid Compounds. Macmillan Co. New York.

Gibbons, S., 2005, An Introduction to Planar Chromatography. From: Methods in Biotechnology, Vol. 20, Natural Products Isolation, 2nd ed.P77:116.

Harborne, J. B., 1973, Phytochemival Methods. Chapman and Hall. New York.

Jaffer, H. J., Mahmod, M. J., Jawad, A. M., Naji, A. and Al-Naib, A., 1983,: Phytochemical and biological screening of some Iraqi Plants.Fitoterpia, LIX. pp:229.

Meddah, B., Ducroc, R., El Abbes, F. M., *et al*.,2009,: *Nigella sativa* inhibits intestinal glucose absorption and improves glucose tolerance in rats. J. Ethnopharmacol., 121:419-24.

Muzammal, M., 2014, Study on Antibacterial Activity of Calotropis procera . Original Research Article. peerj.preprints.430v1 | CC-BY 4.

Neto, M. C. L., de Vasconcelos, C. F. B., Thijan, V. N Caldas, G. F. R, Araujo, A.V; Silva, J. H. C;*et al.*,2013, Evaluation of antihyperglycaemic activity of *Calotropis procera* leaves extract on streptozotocin-induced diabetes in Wistar rats. Revista Brasileira de Farmacognosia.23: p913-919

Patel DK, Kumar R, Laloo D, Hemalatha S., 2012, Diabetes mellitus: An overview on its pharmacological aspects and reported medicinal plants having antidiabetic activity. Asian Pacific Journal of Tropical Biomedicine.,; 2(5): 411-420.

Sato, T., Onse, Y., Nagase, H. and Kito, H.,1990, Mechanism of antimutagenicity of aquatic plant extracts against (benzo (a) yrene) in the Samonella assay . J. Mut. Res., 241:283-290 .

Shaker, K. H., Morsy, N., Zinecker, H., Imhoff, J. F., Schneider, B., 2010, Secondary metabolites from *Calotropis procera* (Aiton).Phytochemistry. 3, 212-216.

Sharma, A. K., Kharb, R. andKaur, R., 2011,pharmacognostical Aspect of Calotropis procera (Ait International Journal of Pharma and Bio Sciences. ISSN 0975-6299 VOL2/ ISSU3.

Shihata, I.,1951,A pharmacological study of *Anagallis arvensis*. M. D. Vet. Thesis, Cairo, Uni. Egypt.

Singh, N. and Gupta, M., 2007, : Regeneration of b cells in islets of Langerhans of pancreas of alloxan diabetic rats by aceton extract of Momordica charantia (Linn) (bitter gourd) fruits.Indian Journal of Experimental biology.Vol 45.pp 1055-10662.

Smolensk, S., Silnis, H. and Fransworth, N., 1972, Alkaloid screening. *Liydia.*, 35:31.

Sudesh, G., Bhawana, G., Karishama, K., Pooja, S., 2012, Ethnopharmaco-ogical potenitial of *Calotropis procera:*An overview.international research journal of pharmacy. issn 2230- 8407.page 19:22.

Tiwari, A., Singh, S. and Singh, S., 2014, Chemical Analysis of Leaf Extracts of Calotropis procera. International Journal of Scientific and Research Publications, Volume 4, Issue 1, ISSN 2250-3153.

Wong, L. Y., Toh, M. P., 2009, Understanding of Diabetes Mellitus and Health-preventive Behaviour Among Singaporeans . Ann Acad Med Singapore.,38:478-86.

Yadav, S. K., Nagori, B. P and Desai, P. K., 2014, Pharmacological characterization of different fractions of Calotropis procera (Asclepiadaceae) instreptozotocin induced experimental model of diabeticneuropathy. Journal of Ethno Pharma cology. 349:357.