

 <p>ISSN NO. 2320-5407</p>	<p>Journal Homepage: - www.journalijar.com</p> <h2 style="text-align: center;">INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)</h2> <p style="text-align: center;">Article DOI: 10.21474/IJAR01/10037 DOI URL: http://dx.doi.org/10.21474/IJAR01/10037</p>	 <p>INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR) ISSN 2320-5407 Journal Homepage: http://www.journalijar.com Journal DOI: 10.21474/IJAR01</p>
---	---	--

RESEARCH ARTICLE

ANTIDIABETIC ACTIVITY OF THE FRUITS POWDER OF CISSUS ROTUNDIFOLIA ON ALLOXAN-INDUCED DIABETIC RABBITS.

Dr.Wael Mustafa Ali Mohammed.

Department of Biology, Faculty of Sciences, Aden university ,Yemen.

Manuscript Info

Manuscript History

Received: 10 September 2019

Final Accepted: 12 October 2019

Published: November 2019

Key words:-

Cissus rotundifolia, fruits powder
,Alloxan, diabetic rabbit's.

Abstract

Halas Cissus Rotundifolia(vitaceae). has been widely used as an traditional medicine in Yemen. This study was designed to assess the efficacy and safety of three doses of Cissus Rotundifolia fruits powder (CRFP) compared with glibenclamide. Hypoglycemic effects of CRFP were also investigated on alloxan-induced diabetic rabbits. CRFP and the standard drug glibenclamide were orally administered daily to diabetic rabbits for four weeks. Blood glucose levels were determined. The oral administration of CRFP didn't cause any signs of clinical abnormalities in the treated rabbits. Thus the given dose of drug appears to be safe. CRFP (1, 2 and 3 g/kg.p.o) showed a significant reduction ($P < 0.05$) in blood glucose level in 30 days. A maximum reduction of blood glucose level was occurred at the dose of 3 g/kg. phytochemical analysis indicated that Cissus rotundifolia fruits contains Fibers,protein,lipids,hydrolyzable carbohydrates,soluble sugars, free aminoacids,phenolic,tannin,anthocyanin,carotenoid,VitaminC,Vitamin A ,Macroelements, Microelements, Essential Amino Acids and Non-Essential Amino Acids.

Copy Right, IJAR, 2019,. All rights reserved.

Introduction:-

Diabetes is a serious, chronic disease that occurs either when the pancreas does not produce enough insulin (a hormone that regulates blood sugar, or glucose), Globally, an estimated 422 million adults were living with diabetes in 2014, compared to 108 million in 1980¹. The use of natural products with therapeutic properties is as ancient as human civilization and for a long time, mineral, plant and animal products were the main sources of drugs². A proximately 38 million US adults use herbal and dietary supplements³. Natural products and their derivatives represent more than 50% of all drugs in clinical use in the world. Higher plants contribute no less than 25% of the total during the last 40 years⁴.

The family Vitaceae is a tropical and subtropical family with 12 genera and about 700 species⁵. The genus Cissus L. (Vitaceae) comprises **350** species distributed in all tropical areas with a few species reaching temperate climates⁶. Many researchers investigation indicated that therapeutics Cissus species could treatment many disease^{7, 8, 9, 10}.

Cissus rotundifolia (CR)belongs to family vitaceae genus cissus¹¹. CR is found throughout East Africa, Zimbabwe, Mozambique, South Africa, parts of Central Africa, Egypt and the Arabian Peninsula¹². CR commonly called in Yemen alhals, alfaq; used as herbal product¹³. In Yemen the boiled leaves are eaten with meals.[11] In the

Corresponding Author:-Dr.Wael Mustafa Ali Mohammed.

Address:-Department of Biology, Faculty of Sciences, Aden university ,Yemen.

southern region of Saudi Arabia, their leaves are widely consumed after cooking by local people as leafy vegetables¹⁴

Biological studies reported on the species of *Cissus rotundifolia* used for treating malaria^{15,16}. Analgesic, anti-inflammatory, antiulcerative, antioxidant and hepatoprotective activity^{17,18,19}. Anti-diabetic activity^{20,21,22}. Phytochemical screening of *Cissus rotundifolia* leaves and stem extracts is steroids, flavonoids, proteins¹⁶. β -sitosterol, Magnificol, β -sitosterol-Dglucoside, Quercetrin, Linarin, Quercetin, Isoorientin and Vitamins C & E²². Nutritional evaluation of wild plant *Cissus rotundifolia* showed that it contains an appreciable amount of protein, fat, crude fiber and minerals. Protein fraction contains a relatively high level of essential amino acids; fat contains a high concentration of unsaturated fatty acids; Macroelements (Magnesium, Sodium, Potassium and Microelements (Iron, Zinc, Manganese, Copper, Chromium¹⁴.

Nutritional and phytochemical content of investigated wild fruit:

(Fibers, protein, lipids, hydrolyzable carbohydrates, soluble sugars, free amino acids, phenolic, tannin, anthocyanin, carotenoid, Vitamin C, Vitamin A²³. Macroelements : Sodium, Potassium, Calcium, Magnesium, Phosphorus. Microelements : Iron, Zinc, Copper. Amino acids: Essential Amino Acids (EAAs) : Threonine, Lysine, Leucine, Isoleucine, Valine, Phenylalanine, Histidine, Non-Essential Amino Acids (Non-EAAs) : Aspartic acid, Tyrosine, Alanine, Glycine, Serine, Glutamine, Arginine²³.

The objective of this study is to assess the fruits powder' of *Cissus rotundifolia* activity on diabetic rabbits.

Materials and methods:-

Chemicals :

Alloxan monohydrate was obtained from Sigma Co(St.Louis,MO. and glibenclamide from alharery pharmacy. Alloxan was dissolved in saline solution for intraperitoneal administration.

Plant material.

The fruits of *Cissus rotundifolia* was collected from alkhofej area, Tor ALbaha district, Lahj , Yemen. after collecting the fruits, it was washed and cut into small pieces, then it was dried in order to change it into powder ,The plant was identified and authenticated by taxonomist in Botany of Aden Univ.yemen.

Experimental animals:

The animals (locally rabbits) were purchased from the local market in Lahi governorate, Yemen. The rabbits were divided randomized into sex groups comprising of 10 animals weighed 1200-1350g in each group. They were allowed to acclimatize for two weeks and were maintained. All animals were given free access to standard chow and tap water. The fruits powder suspension was administered orally twice per day.

Induction of experimental diabetes

Diabetes mellitus was induced in rabbits by single intraperitoneal injection of freshly prepared solution of alloxan (120 mg/kg b.w) in physiological saline after overnight fasting for 12 h²⁴. marginal ear vein blood glucose levels were measured, 7 ,15 and 30 days after alloxan injection .The rabbits were fed by the preparations by gastric intubation.

Experimental design

1. Group I: Normal control rabbits received normal saline 10 ml /kg/day and free food for 30 days
2. Group II: Diabetic control rabbits received alloxan in single dose (120 mg/kg, i.p.).did not any treat.
3. Group III: Treated diabetic rabbits received CRFP (1g /kg/body weight, in 2 ml distilled water daily, for 30 days.
4. Group IV: Treated diabetic rabbits received CRFP (2g/kg/ body weight, in 2 ml distilled water daily, for 30 days.
5. Group V: Treated diabetic rabbits received CRFP (3g /kg/ body weight, in 2 ml distilled water daily, for 30 days.
6. Group VI: Diabetic rabbits treated with glibenclamide (5 mg/kg/ body weight, in 2 ml distilled water daily, for 30 days).

Statistical analysis:-

The results are expressed by the means and standard deviations(SD). The statistical analysis was carried out using paired t-test and One-way analysis (ANOVA). Statistical P. value at the level of <0.05 was considered to be significant.

Results:-

The oral administration of CRFP of *Cissus rotundifolia* leaves didn't cause any signs of clinical abnormalities in the treated rabbits. Thus the drug dose given appears to be safe. Continuous treatment with CRFP (1, 2 and 3g/kg) for a period of 30 days showed a significant decrease in the blood glucose level in diabetic rabbits. A maximum reduction of blood glucose level was occurred at the dose of 3 g/kg. p. o. of CRFP .Table NO (1).

Table 1:-Mean values \pm S.D. of glucose (mg /dL) of control and different treated rabbits.

Groups	DAY: 7	DAY:15	DAY:30
Normal control	75.38 \pm 0.89	73.40 \pm 1.5	81.80 \pm 0.87
Diabetic control	208.8 \pm 2.93	265.9 \pm 1.378	336.3 \pm 4.06
Glibenclamide	222.5 \pm 0.48	172.8 \pm 7.19	132.5 \pm 1.96
CRFP(1g/kg)	221.3 \pm 2.95	190.6 \pm 3.64	163.3 \pm 4.14
CRFP (2g/kg)	230.6 \pm 5.25	167.8 \pm 3.48	118.6 \pm 3.52
CRFP (3g/kg)	225.7 \pm 1.96	141.3 \pm 1.56	98.4 \pm 3.14

Discussion:-

NO lethality or any toxic reactions were found at any of the selected CRFP doses until the end of the study period. The oral administration of CRFP of *Cissus rotundifolia* fruits didn't cause any signs of clinical abnormalities in the treated rabbits. Thus the CRFP dose given appears to be safe. Toxicity Studies did not show any side effects of cissus genus²⁵. Alloxan has been observed to cause a massive reduction of the β -cells of the islets of Langerhans and induce hyperglycemic^{26, 27}. The mechanism behind alloxan diabetogenic effect is not very clear. However, it has been suggested that it damages pancreatic beta cells through formation of superoxide anions and hydrogen peroxide but not hydroxyl radicals^{28, 29}. This causes DNA strand-breaks (30) and depletes NAD⁺ stores^{31, 32}. One study suggested that the generation of free radicals through redox cycling with glutathione was not enough to cause diabetes by alloxan ,but it seems that hydrophilicity of this compound is a necessary condition for its diabetogenicity³³. Some reports indicate that the formation of superoxide and hydroxyl radicals is responsible for the alloxan-induced cytotoxicity^{34, 35, 36}. Pancreas from alloxan-induced diabetic animals showed a 70% reduction of beta cells areas. At the same time, there was an increase in the areas occupied by delta cells, and no alteration in glucagon-producing cells³⁷.

Mechanism of action of the standard drug glibenclamide:

Binding of glibenclamide with its receptor leads to the closure of the potassium channels which opens calcium channels for influx of Ca²⁺ ions into the cytoplasm and release of insulin from the pancreatic islets. These K⁺ channels are responsive to ATP/ADP ratio and close when the ratio increases because of an increase in glucose metabolism^{38, 39}. Sulphonylureas such as glibenclamide stimulate insulin secretion from pancreatic β cells principally by inhibiting ATP-sensitive K⁺ channels⁴⁰.

Possible Mechanism of action of the phenolic compounds found in *Cissus rotundifolia*: This study showed that the CRFP of *Cissus Rotundifolia* cause a significant hypoglycemic effect in alloxan diabetic rabbits. The maximum reduction in fasting blood glucose (FBG) was observed at a dose of 3 g /kg b.w. Thus, g /kg/.w. was found to be the optimum dose of CRFP on FBG of diabetic animals. These findings are supported by those of^{21,22,23}. There were a lot of studies that support the anti- hyperglycemic or anti-diabetic effect of several medicinal plants worldwide^{41, 42, 43}. A number of researchers have reported that genus cissus have antidiabetic properties in various studies^{44,45,46,47,48,49,50}. The cissus genus (vitaceae) extracts are characterized by a high content of flavonoids. An important source of flavonoids in the diet and the flavonoids found in cissus genus are known to be strong antioxidants⁵¹.

Conclusion: The antihyperglycaemic activity may be due to the presence of several bioactive anti-diabetic principals.

References:-

1. World Health Organization 2016 <https://www.who.int/diabetes/global-report/en/>
2. S.M.K. Rates. Plants as source of drugs. *Toxicon*, 2001, 39: 603–613.
3. Kennedy J, Wang CC, Wu CH. Patient disclosure about herb and supplement use among adults in the US. *Evid Based Complement Alternat Med*. 2008; 5:451-456.
4. Ameenah Gurib-Fakim. Medicinal plants: Traditions of yesterday and drugs of tomorrow .*Molecular Aspects of Medicine*.2006; 27:1–93.
5. Heywood, V.H. Flowering Plants of the World. B.T. Batsford, London, 1993; 336 pp.
6. Wen, J., Nie, Z.-L., Soejima, A. & Meng, Y. Phylogeny of Vitaceae based on the nuclear GAI1 gene sequences. *Canadian Journal of Botany*.2007; 85: 731–745.
7. Glauce SB Viana, Ana Carolina C Medeiros¹, Ana Michelle R Lacerda, L Kalyne AM Leal, Tiago G Vale¹ and F José de Abreu Matos. Hypoglycemic and anti-lipemic effects of the aqueous extract from *Cissus sicyoides* .*BMC Pharmacology* 2004, 4:9.
8. Salgado JM, Mansi DN, Gagliardi A. *Cissus sicyoides*: analysis of glycemic control in diabetic rats through biomarkers. *J Med Food*. 2009; Aug; 12(4):722-7.
9. Garima Mishra, Saurabh Srivastava, B.P.Nagori. Pharmacological and Therapeutic Activity of *Cissus quadrangularis*: An Overview. *International Journal of PharmTech Research*. Vol.2, No.2, pp 1298-1310, April-June 2010.
10. Priyanka Vijay and Rekha Vijayvergia .Analgesic, anti-inflammatory and antipyretic activity of *Cissus quadrangularis*. *Journal of Pharmaceutical Science and Technology* Vol. 2 (1), 2010, 111-118.
11. Dubaie, A. & Al-Khulaidi, A. (2005): Medicinal and Aromatic Plants of Yemen. Obadi Center for Scientific Publications, Sana'a, Yemen. 311pp. (In Arabic).
12. Food Agricultural Organization (FAO) (1988). Traditional food plants A source book for promoting the exploitation and consumption of plant foods in Arid semi-Arid and semi-humid lands of Eastern Africa. Rome: FAO. pp. 234-245.
13. Al-Mamary M. A. (2002). "Antioxidant activity of commonly consumed vegetables in Yemen." *Malaysian Journal of Nutrition*, 8(2): 179-189.
14. Mohamed korish 2015. nutritional evaluation of wild plant *Cissus rotundifolia*. *Ital. J. Food Sci.*, 2016; 28: 43-49.
15. Ali, A.; Al-rahwi K. & Lindequist, U. (2004): Some medicinal plants used in Yemeni herbal medicine to treat Malaria. *African journal of Traditional, Complementary and Alternative Medicines*, 1: 72-76.
16. Alshawsh, M.; Mothana R. & Al-shamahy H. (2009): Assessment of antimalarial activity against *Plasmodium falciparum* and phytochemical screening of some Yemeni medicinal plants. *eCAM*, 6(4): 453–456.
17. Raslan M. A. (2015). "Phytochemical and Bioactivity Evaluation of *Cissus rotundifolia* and *Sansevieria cylindrica* Growing in Egypt." PHD. Faculty of Pharmacy, Cairo University.
18. Ataa A. Said, Elsayed Ali Aboutabl, Sally A. El Awdan, Mona A. Raslan (2015). Proximate analysis, phytochemical screening, and bioactivities evaluation of *Cissus rotundifolia* (Forssk.) Vahl. (Fam. Vitaceae) and *Sansevieria cylindrica* Bojer ex Hook. (Fam. Dracaenaceae) growing in Egypt. *Egyptian Pharmaceutical Journal*. [Downloaded free from <http://www.epj.eg.net> on Saturday, January 09, 2016, IP: 41.45.40.65.
19. Al-Fatimi, M.; Wurster, M.; Schroder, G. & Lindequist, U. (2007): Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. *Journal of Ethnopharmacology*, 111: 657–666.
20. Wael Mustafa Ali Mohammed.(2012) Effect of feeding by *Cissus rotundifolia* leaves on some physiological changes resulting from diabetes mellitus in Rats. PHD. Faculty of sciences, Damascus University.
21. Ali A. Al-Mehdar, Adel M. Albattah(2016). Evaluation of Hypoglycemic Activity of *Boswellia carterii* and *Cissus rotundifolia* in Streptozotocin/Nicotinamide-Induced Diabetic Rats. *Yemeni J Med Sci* 2016 (in press) <http://dx.doi.org/10.20428/YJMS.10.1.A4>.
22. Akram Ali Mohamed Shalabi(2017). Chemical and Biological Assessment of *Cissus rotundifolia* (Forssk.) Vahl Growing in Yemen. PHD. Faculty of Pharmacy, Cairo University.
23. Ahmad K. Hegazy, Amal A. Mohamed, Sami I. Ali, Nasser M. Alghamdi, Amal M. Abdel-Rahman, Sanad Al-Sobeai (2019) Chemical ingredients and antioxidant activities of underutilized wild fruits . *Heliyon* 5 (2019) e01874
24. Akthar AK. Effect of *Momordica Charantia* on blood sugar level of Normal and Alloxan rabbits. *Planta Medica*. 1981;42:205-212
25. Aimmanas Attawish, Pranee Chavalittumrong, Songpol Chivapat, Anchalee Chuthaputti, Sadudee Rattanajarasroj and Somkiat Punyamong. Subchronic toxicity of *Cissus quadrangularis* Linn. *Songklanakarin J. Sci. Technol*. Vol. 24 No. 1 Jan.-Mar. 2002.

26. Lenzen, S., and Panten, U. Alloxan: history and mechanism of action. *Diabetologia* .1988; 31: 337-342.
27. Sharma, S.B.; Nasir, A.; Prabhu, K.M.; Murthy, P.S.; Dev, G. Hypoglycaemic and hypolipidemic effect of ethanolic extract of seeds *Eugenia jambolana* in alloxan-induced diabetic rats. *J. Ethnopharmacol.* 2003, 85:201-206.
28. Jorns, A., Tiedge, M., Lenzen, S., and Munday, R .Effect of superoxide dismutase, catalase, chelating agents, and free radical scavengers on the toxicity of alloxan to isolated pancreatic islets in vitro. *Free radicals, Biology and Medicine*, 1999; 26: 1300-1304.
29. Sentman, M. L., Jonsson, I. M., and Marklund, S. IEnhanced alloxan-induced beta cell damage and delayed recovery from hyperglycaemia in mice lacking extracellular superoxide dismutase. *Free Radical, Biology and Medicine*. 1999; 27: 790- 796.
30. Yamamoto, H., Uchigata, Y., and Okamoto, H. Streptozotocin and alloxan induce DNA strand-breaks and poly (ADP-Ribose) syntheses in pancreatic islets. *Nature*.1981; 294: 284-286.
31. Askar, M. A., and Baquer, N. Z. Changes in the activity of NADH-oxidase in rat tissues during experimental diabetes. *Biochemistry and Molecular Biology International*, 1994; 34: 909-914.
32. Le Doux, S. P., Hall, C. R., Forbes, P. M., Patton, N. J., and Wilson, G. L. Mechanisms of nicotinamide and thymidine protection from alloxan and streptozotocin toxicity. *Diabetes*.1988; 37:1015-1019.
33. Munday, R., Ludwig, K., and Lenzen, S. The relationship between the physicochemical properties and the biological effects of alloxan and several Nalkyl substituted alloxan derivatives. *Journal of Endocrinology*, 1993; 139: 153-163.
34. Waguri, M., K. Yamamoto, J. Miyagawa, Y. Tochino, K. Yamamori, Y. Kajimoto, H. Nakajima, H. Watada, Y. Yamasaki, T. Hanafusa and Y. Matsuzawa, 1997. Demonstration of two different processes of β cells regeneration in a new diabetic mouse model induced by selective perfusion of alloxan. *Diabetes*, 46: 1281-1290.
35. Ahrén, B. and G. Sundkvist, 1995. Long-term effects of alloxan in mice. *Int. J. Pancreatol.* 7: 197-201.
36. Zhang, H., J.M. Zdzienicka and U.T. Brunk, 1992. Alloxan cytotoxicity involves lysosomal damage. *Acta Pathol. Microbiol. Immunol. Scand.*, 100: 309-316.
37. Lima, M.A., L.M.B. Lima, D.P.C. Rita, F.C. Navarro, R.S. Tatsukawa, G.A. Pereira, L.C. Reis, M.E.A. Abreu and M.F. Borges, 2001. Quantitative analysis of cells of the pancreatic islets in rats under effect of alloxan. *Medicinal, Ribeirao Preto*, 34: 308-314.
38. Panten, U. Schwanstecher, M.,Schwanstecher, C. Sulfonylurea receptors and mechanism of sulfonylurea action. *Exp. Clin. Endocrinol. Diabetes* 1996; 104: 1-9.
39. Luzi, L. Pozza, G. Glibenclamide: an old drug with a novel mechanism of action. *Acta Diabetol.* 1997; 34: 239-244.
40. Watkins, P.J. Insulin treatment. In: *ABC of Diabetes*, fifth ed. BMJ Publishing Group Ltd. 2003, pp. 19-24.
41. Amala Soumyanath. *Traditional medicines for modern times: antidiabetic plants* © 2006 by Taylor & Francis Group, LLC CRC Press is an imprint of Taylor & Francis Gro.
42. T.S. Frode and Y.S. Medeiros .Animal models to test drugs with potential antidiabetic activity. *Journal of Ethnopharmacology*. 2008; 115: 173–183.
43. Mustafa Aslan, Nilufer Orhan, Didem Deliorman Orhan, Fatma Ergun Hypoglycemic activity and antioxidant potential of some medicinal plants traditionally used in Turkey for diabetes. *Journal of Ethnopharmacology* 2010; 128:384–389.
44. Sama K., Murugesan K. and Sivaraj R. (2012). *In vitro* alpha amylase and alpha glucosidase inhibition activity of crude ethanol extract of *Cissus arnottiana*. *Asian Journal of Plant Science and Research* 4: 550-553.
45. Miura T.,Shindou C., Zhang W.-p., Suzuki I. and Ishida T. (2006). Antidiabetic activity of *Cissus sicyoides* in KK-Ay mice. *Journal of Traditional Medicines* 23(3): 89-91.
46. Srivastava A. K., Srivastava P., Mishra J., Singh M. P., Sharma A. K. and Shrivastava A. (2011). Antidiabetic activity of the stem extracts of *Cissus quadrangularis* linn. *Journal of Pharmacy Research* 4(11): 3873-3874.
47. Pvi Jayakumari, K. S., K. B., J. k., G. M., M. S. and V.Thirumurugan (2012). Studies on the Physico-Phytochemical and Anti-diabetic Properties of *Cissus quadrangularis* l. and *Solanum torvum* swartz. *International Journal Of Drug Discovery And Herbal Research* 2: 323-328.
48. Jimoh A., Tanko Y. and Mohammed A. (2013). Anti-diabetic effect of methanolic leaf extract of *Cissus cornifolia* on alloxan-induced hyperglycemia in wistar rats. *Annals of Biological Research* 4(3): 46.
49. Lekshmi R., Divya B. and Mini S. (2014). *Cissus quadrangularis* extract attenuates hyperglycaemia-mediated oxidative stress in streptozotocin-induced diabetic rats. *Redox Report* 19(5): 214-220.
50. Wael Mustafa Ali Mohammed, Abdulaziz A. Y. Abbas, Hatem Mohammed, Mohammed Ali Qasem, Hanan Abdulkareem M. Saleem5 and Ebtisam Yassin Shikoo.(2019). antidiabetic activity of *cissus roudnifolia* leaves supplement. *World Journal of Pharmaceutical Research*.volume 8,issue 2,47-55

51. Viana, G.S.B., A.C.C. Medeiros, A.M.R. Lacerda, L.K.A.M. Leal, T.G. Vale and F.J.A. Matos. Hypoglycemic and anti-lipemic effects of the aqueous extract from *Cissus verticillata*. BMC Pharmacol. 2004; 4: 1-7.