Scientific Studies on Citrullus Colocynthis with Special Reference to Anti-hyperglycemic Activity

Mohammad Kamil, F. Ahmad and El. T. Abdallah
TCAM Research, ZCHRTM, DHLME, DOH 29300, Abu Dhabi, UAE

Abstract: *Citrullus colocynthis* is a plant used as a traditional medicine for the treatment of diabetes mellitus, one of the most prevalent endocrine disorder throughout the world with special reference in the Maghreb region and in the Middle East. The present study is based on laboratory experiments for pharmacognostic, physicochemical, phytochemical, elemental analysis, TLC (thin layer chromatography) fingerprinting, pharmacological & toxicological studies with special reference to anti-diabetic activity, along with an exhaustive scientific review on *Citrullus colocynthis* phytochemical and pharmacological activities described in literature. We investigated the anti-hyperglycaemic effect of aqueous extract of *Citrullus colocynthis* seeds administered intraperitoneally in normal Wistar rats and streptozotocin diabetic rats. The toxicity of this extract was also evaluated using lethal dose 50 methods (LD50).

Key words: *Citrullus colocynthis*, pharmacognostic, physicochemical, phytochemical, elemental analysis, TLC fingerprinting, pharmacological & toxicological studies, anti-hyperglycemic activity.

1. Introduction

The plant is very common in sandy places. *C. colocynthis* presents a curious look with patches here and there in the extensive blazing deserts of all the Arab countries. The plants are suffering from winter and recovering again in summer. They have wonderful adaptation ability to grow upon the hot sandy soil of summer months where scarcely any plant can survive. The tap root is succulent and very deep [1]. Throughout Arabia [2], the plant is wide spreading in UAE (United Arab Emirates) except in the western and southern part of the country.

Scientific name: *Citrullus colocynthis* (L.) Schrad.

Synonymy: *Colocynthis vulgaris* Schrad, *Cucumis colocynthis* L.

Local name: Handal, Murrah, Shary, Srew, Hanzal, Suri, Hedge.

Arabic name: Handalm Shary, Srew, Hanzal, Suri, Hedge.

Common name: Colocynth, Bitter apple, Bitter gourd, Desert squash.

2. Traditional and Medicinal Uses

The leaves are diuretic and used in treatment of jaundice and asthma. The root is useful in inflammation of breasts, amenorrhea, rheumatism, joint pains and is used externally in ophthalmia and uterine pains. The fruit is pungent, cooling purgative, anthelmintic, antipyretic and carminative. It cures, tumors, leukodema, ulcers, asthma, bronchitis, urinary discharge, enlargement of spleen, tuberculosis glands of the neck, dyspepsia, constipation, anemias, and throat diseases. The fruit pulp is purgative, diuretic, anti-epileptic, and is used against gonorrhea [1].

The leaves, seeds, roots and dried fruit are used to treat dog, insect and snake bites, as a laxative, to relieve pain in joints and as a hair colour. Powdered leaves mixed with water are taken as a laxative; crushed roots are mixed with goat’s milk and used as a purgative and to treat colic. In Yemen the seeds which are also used as a purgative, are mixed with other food to reduce its strong laxative action. Seeds crushed with water are used as shampoo to darken hair colour and crushed fruit mixed with oil is rubbed to
relieve pain in joints [2]. Very powerful purgative or
hydragogue cathartic, emetic and gastrointestinal
irritant. It is usually given with hyoscyamus to prevent
gripping. It is not given in case of pregnancy. It
stimulates hair growth [3].

3. Pharmacognosy & Phytochemistry

A transverse section of the seed shows that the seed
coat (testa) consists of a thick palisade-like epidermal
layer of long thickened cells of anticlinal walls having
brown rod-like structures giving a characteristic
flame-like appearance. The rod-like structures do not
extend to the outer part of the testa. The inner side of
this layer is adjacent to a grayish yellow layer of small
sclerenchymatous cells with thick pitted walls.

This is underlain by a number of other
sclerenchymatous layers which gradually become
large starting from the layer below the epidermis towards
the inner part of the seed. Larger sclerenchymatous
cells have yellow colors and they are strongly packed
together giving characteristic block-like structures.
They are heavily thickened with narrow longitudinally
branching lumens. The yellow endospermic cells are
polygonal and they contain oily droplets.

4. Powdered Plant Material

The material consists of the crushed seeds of the
plant. It is a greasy yellow brown coarse gritty powder
that has a slight characteristic odour and a very bitter
persisting taste. Microscopically, the powder shows
light brown thick sheet-like fragments of seed testa
layers. It shows the testa palisade-like epidermal layer
with brown rod-like structures having flame-like
appearance in addition to isolated groups of light
yellow small sclerenchyma cells of the testa; occasional
individual cells are also observed. It also shows larger
yellow-colored sclerenchyma cells strongly packed
and heavily thickened with narrow longitudinally
branching lumens. Yellow endospermic cells are
polygonal and they contain oily droplets (Fig. 2).

Fig. 1 Whole plant and fruit.

Fig. 2 (a) fragment of the seed showing the seed testa with the characteristic epidermis composing of yellowish to brown palisade cell thickened with rods giving a flame-like appearance (flame cells); the other dark part of the fragment is composed of layers of the sclerenchyma of the testa with thick pitted walls. (b) A fragment of the seed endosperm showing its yellow-coloured polygonal cells that contain oil droplets. (c) Fragments of the seed showing different tissues, different types of cells and, cell contents and oil droplets.
5. Chemical Constituents

Constituents of seeds: alkaloid, steroid glycosides and flavonoids [4]. Sterols, citibitol, Glucose and α-spinasterol from fruits and seeds.

(α-elaterin-2D-glucopyranoside) and α-elaterin (cucurbitacin E) and citrullol from pulp. Glycosides in fruit: elaterin, elatericin B (cucurbitacin I) and dihydroelatericin B (cucurbitacin L). Myristic, palmitic, stearic, myristoleic, palmitoleic, oleic, linoleic acids and citrullonol from seed oil. Hentriacontane, n-octacosanol and 1,26-hexacosanediol. Linoleic (51.3%), oleic (24.1%), palmitic (12.8%) and stearic acids (9.8%) in seed oil.

Cucurbitacins B, E, I, cucurbitacin E-2 glucoside, Quercetin and kaempferol in leaves, fruits and flowers. hentriacontane, elateridine, hexanorcucurbitacin I and its 16-O-acetyl derivative and Cucurbitacin T from fruits. glycocide as 2-Oβ-D-glucopyranosyl-22,23,24,25,26,27-hexanorcucurbitacin I and 2-Oβ-D-glucopyranosylcucurbitacin I, 2-Oβ-D-glucopyranosylcucurbitacin E and 2-Oβ-D-glucopyranosylcucurbitacin L [5-9]. glycosides: elaterin (cucurbitacin E), elatericin B (cucurbitacin I) and dihydroelatericin B (cucurbitacin L) [10].

Cucurbitacin glycosides:

2-Oβ-D-glucopyranosyl-cucurbitacin I,
2-Oβ-D-glucopyranosyl-cucurbitacin I,
2-Oβ-D-glucopyranosyl-cucurbitacin L, and the novel glycoside, 2-Oβ-D-glucopyranosyl-cucurbitacin L and the novel glycoside, 2-Oβ-D-glucopyranosyl-[5-9] hexanorcucurbitacin I [11]. Flavones C-glycosides in the fruits which contained: isovitexin, iso-orientin, iso-orientin 3'-methyl ether [12]. Flavone glucosides in the fruits: isosapararin, isovitexin and isoorientin 3'-O-methyl ether. Two cucurbitacin glucosides: 2-Oβ-D-glucopyranosylcucurbitacin I and 2-Oβ-D-glucopyranosylcucurbitacin L [13] in fruits: 2-Oβ-D-glucopyranosylcucurbitacin E [14].

The following chemical studies have been carried out at ZCHRTM on the seeds of Citrullus colocynthis.

(1) Physicochemical constants (%)

- Loss of weight in drying at 105°C: 6.20
- Absolute alcohol solubility: 18.80
- Water solubility: 6.85

(2) Successive extractives (%)

- Petroleum ether (60-800): 1.96
- Chloroform: 1.70
- Absolute alcohol: 2.85

(3) Ash values (%)

- Total ash: 2.49
- Water soluble ash: 1.17
- Acid insoluble ash (10% HCl): Nil

(4) pH values (aqueous solution)

- pH of 1% solution: 5.846
- pH of 10% solution: 5.697

(5) Elemental analyses (Table 1)

Table 1  Elemental analyses.

| Element | Std. conc. µg/mL (ppm) | Sample conc. mg/mL | Samples absorbance | Actual conc. mg/mL | Actual conc. (%) |
|---------|-------------------------|---------------------|--------------------|-------------------|-----------------|
| Cr      | 1, 2, 4                 | 20.056              | 0.0000             | 0.00000           | 0.00000         |
| Zn      | 0.5, 1, 2               | 20.056              | 0.1055             | 0.050555          | 0.0050555       |
| Cu      | 0.5, 1, 2               | 20.056              | 0.0198             | 0.0094450         | 0.009445        |
| Fe      | 1, 2, 4                 | 20.056              | 0.0104             | 0.0199350         | 0.0019935       |
| K       | 1, 2, 4                 | 20.056              | 1.5608             | 0.883215          | 0.0883215       |
| Pb      | 1, 2, 4                 | 20.056              | 0.0024             | 0.01265           | 0.001265        |
| Cd      | 0.25, 0.5, 1            | 20.056              | 0.0000             | 0.00000           | 0.00000         |

1 ppm conc. = 1 µg/mL; Actual conc. (%) = Actual conc. (ppm) × 0.0001 [1 ppm = 0.0001%].
(6) UV spectral studies (Table 2)

Table 2  UV spectral studies.

| Ultraviolet Spectrum (USP reference) | Sample conc. (mg/mL) | Solvent | Intestinal fluid simulated without pancreatic pH = 7.5 ± 0.1 (Fig. 3) | Gastric fluid simulated without pepsin pH = 1.2 ±0.1 |
|-------------------------------------|----------------------|---------|---------------------------------------------------------------|--------------------------------------------------|
| @max (nm) | @min (nm) | Abs. (λmax-λmin) |
| 4.6 | Intestinal fluid simulated without pancreatic pH = 7.5 ± 0.1 (Fig. 3) | 274 | 259 | 1.008-0.957 |
| - | Gastric fluid simulated without pepsin pH = 1.2 ±0.1 | No shift | No shift | - |

Fig. 3  Intestinal fluid simulated without pancreatic pH = 7.5 ± 0.1

(7) TLC (thin layer chromatography) (Fig. 4)

Fig. 4  TLC fingerprint of Pet. ether (60-800) extract (track 1) and MeOH extract (track 2).

Mobile phase Fig. A, C & D: Ethyl acetate, methanol, water (100:13.5:10), B: Toluene, ethyl acetate (93:7), Detection A: UV 254 nm C: UV 365 nm, Derivatization B & D: Vanillin-Sulphuric acid-vis.

6. Pharmacological and Toxicological Studies

The important pharmacological and toxicological activities of the plant Citrullus colocynthis reported in various scientific journals have been presented in the present brief review:

The methanolic extract from the fruit of C. colocynthis showed an inhibitory effect on ear passive cutaneous anaphylaxis reactions as a type I allergic model in mice [15]. C. colocynthis pulp extract is a long-serving laxative [16]. In UAE many traditional plants such as the Citrullus colocynthis (Handal) are used as antidiabetic remedies. The plasma level of ALT (alanine aminotransferase), ALP (alkaline phosphatase), AST (aspartate aminotransferase), GGT (gamma-glutamyl transferase), and LDH (lactic dehydrogenase) increased significantly after the onset of diabetes. Oral administration of the plant extract reduced the plasma level of AST and LDH significantly. However, the plant extract failed to reduce the increased blood level of GGT and ALP in diabetic rats. BUN (blood urea nitrogen) increased significantly after the onset of diabetes. No significant difference was observed in the blood creatinine, K⁺, Na⁺, Ca²⁺ and P levels of normal and diabetic rats. The plant extract did not have any effect on BUN level; however, it caused an increase in the level of K⁺, Na⁺ in diabetic rats. In conclusion, oral administration of the aqueous extract of the C. colocynthis can ameliorate some of the toxic effects of streptozotocin [17].

A crude 50% ethanol extract of C. colocynthis was administered orally to male albino rats for evaluation of antifertility effects. A 50% ethanol extract of C. Colocynthis showed an anti-androgenic nature, thereby reduced reversible infertility in male albino rats [18].
Table 3  Pharmacological and safety evaluation studies on the Citrullus colocynthis seeds (aqueous extract).

| Activity                        | Results |
|--------------------------------|---------|
|                                | Strong  | Moderate | Mild | Negative |
| Anti-diabetic                  | √       |          |      |          |
| Analgesic                      | √       |          |      |          |
| Antidepressant                 |         |          |      | √        |
| Effect on rabbit jejunum       | √       |          |      |          |
| Effect on rat fundus           | √       |          |      |          |
| Effect on Guinea pig ileum    | √       |          |      |          |
| Effect on right rat atria      |         |          | √    |          |
| Locomotor ↓                    | √       |          |      |          |
| Motor co-ordination & grip strength ↓ | √   |          |      |          |
| Rectal temperature ↓           | √       |          |      |          |
| Lethality ↑                    |         |          | √    |          |

Feeding the mixture of *C. colocynthis* caused more pronounced effects and death of rats. Vital organ lesions accompanied by anemia and leucopenia were correlated with changes in serum ALP, AST and ALT activities with alterations in concentrations of total protein, albumin, urea and other serum constituents. Serum bilirubin concentration did not change [19]. *Citrullus colocynthis* extract was found to be free of hepatotoxic effects in concentrations up to 100 mg/mL incubation mixture when liver slices were incubated in William’s medium E for 22 h. Carbon tetrachloride induced hepatotoxicity could not be prevented or alleviated. Moreover, the damage was sometimes enhanced by higher extract concentrations [20].

Infusions of *C. colocynthis* Schrad (Cucurbitaceae) fruits are traditionally used as antidiabetic medication in Mediterranean countries. The present study showed that different *C. colocynthis* seed extracts have an insulin tropic effect which could at least partially account for the antidiabetic activities of these fruits. Aqueous extract of the rind of *C. colocynthis* possesses a hypoglycemic effect and its hypoglycemic action could be attributed for more extent to the presence of saponin [21]. Plant has been reported to possess anti-diabetic; diuretic, anti-epileptic, anti-bilennorrhea, and effects against gout and ascites [22].

Acute toxicity and histopathological effects of saponin (extracted from the plant *C. colocynthis*) on mice was assessed. The median lethal dose (LD₅₀) of the saponin was 200 mg/kg. The histological changes were confined to the small intestine, liver and kidney, whereas the stomach, large intestine and heart appeared normal [23].

Oral administration of 0.25 g/kg/day dose of *C. colocynthis* fruits or 0.25 g/kg/day of *R. stricta* leaves for 42 days proved to be not fatal but, that the mixture of the two plants (0.25 g + 0.25 g/kg/day) proved fatal within 26 days with profuse diarrhea, dehydration, loss in condition, ataxia and decumbency, prior to death [24]. Feeding the mixture of *C. colocynthis* and *N. oleander* caused more marked effects and death of rats [25].

The following pharmacological and safety evaluation studies were carried out on the *Citrullus colocynthis* seeds (aqueous extract) in our lab (Table 3).

7. Summary of the Results

The extract showed significant anti-nociceptive activity in the hot-plate test. The plant extract caused a strong spasmogenic activity which provides a scientific basis for its use in regulation of bowel function through rapid transit and relieving constipation (laxative activity). Overdose may produce gastrointestinal disturbance (Stomach upset; Diarrhea). The effect on isolated rat fundus showed the purgative nature of the plant extract. The plant extract revealed positive inotropic agent increasing myocardial contractility are used to support cardiac function. The plant extract
failed to show the anti-depression activity. Oral administration of the plant extract 200 and 250 mg/kg proved not fatal but showed profuse diarrhea, gross lack of coordination of body movement and showed state of leaning, resting, or reclining. Significant anti-hyperglycemic activity was observed as shown in conclusion (Fig. 5).

8. Conclusions

The results obtained show an anti-hyperglycaemic effect of *Citrullus colocynthis* seed extracts on diabetic rats, significant hypoglycemic effect in mice and a stability of the blood glucose level to normal values on the normal rats.

References

[1] Batanouny, K. H., et al. 1999. *Wild Medicinal Plants in Egypt—An Inventory to Support Conservation and Sustainable Use.* Egypt: The Palm Press, p. 42.

[2] Ghazanfar, S. A. 1994. “The Flora of United Arab Emirates. An introduction—Al Ain.” In *Handbook of Arabian Medicinal Plants*, edited by Western, A. R. CRC Press.

[3] Fawzi, M. K. 1995. *Weeds in the United Arab Emirates.* University of U.A.E.

[4] Ambi1, A. A., Abdurrahman, E. M. M., Sule, I., Patel, U. U., Abdurrahman, Y. R., and Ibrahim, N. D. G. 2007. “Phytochemical Screening and Histopathological Studies on the Seeds of *Colocynthis citrullus* in Albino Rats.” *Nig. Journ. Pharm. Sci.* 6 (2): 7-13.

[5] Rastogi, and Mehrotra. 1990. *Compendium of Indian Medicinal Plants*, Vol. 1, PID, New Delhi, p. 105.

[6] Rastogi and Mehrotra. 1991. *Compendium of Indian Medicinal Plants*, Vol. 2, PID, New Delhi, p. 185.

[7] Rastogi and Mehrrota. 1993. *Compendium of Indian Medicinal Plants*, Vol. 3, PID, New Delhi, p. 174.

[8] Rastogi and Mehrotra. 1995. *Compendium of Indian Medicinal Plants*, Vol. 4, PID, New Delhi, p. 188.

[9] Rastogi and Mehrotra. 1998. *Compendium of Indian Medicinal Plants*, Vol. 5, PID, New Delhi, p. 210.

[10] Lavie, D., Willner, D., and Merenlender, Z. 1964. “Constituents of *Citrullus colocynthis* (L.) Schrad.” *Phytochemistry* 3 (1): 51-6.

[11] Natiq, A. R., Hatam, D., Whiting, A., and Nahia, J. Y. 1989. “Cucurbitacin glycosides from *Citrullus colocynthis*.” *Phytochemistry* 28 (4): 1268-1271.

[12] Galal, T. M., Saleh, H. E.-S., Afifi, M. S., and Rosazza, J. P. N. 1997. “C-p-Hydroxybenzoylglycoflavones from *Citrullus colocynthis*.” *Phytochemistry* 44 (1): 187-90.

[13] Delazar, A., Gibbons, S., Kosari, A. R., Nazemi, H., Modarresi, M., Nahar, L., and Sarker, S. D. 2006. “Flavone C-Glycosides and Cucurbitacin Glycosides from *Citrullus colocynthis*.” *Tehran University of Medical Sciences* 14 (3): 109-14.

[14] Chaturvedi, M., Mali, P. C., and Ansari, A. S. 2003. “Induction of Reversible Antifertility with a Crude Ethanol Extract of *Citrullus colocynthis* Schrad Fruit in Male Rats.” *Pharmacology* 68: 38-48.

[15] Yoshikawa, M., Morikawa, T., Kobayashi, H., Nakamura, A., Matsuhira, K., Nakamura, S., and Matsuda, H. 2007. “Bioactive Saponins and Glycosides. XXVII. Structures of New Cucurbitane-Type Triterpene Glycosides and Antiallergic Constituents from *Citrullus colocynthis*.” *Chem Pharm Bull* 55: 428.

[16] Lorenz, P. R., Lippmann, F., Dürrling, K., Solf, M., and Geissler, J. 2005. “Pharmaco-Toxicological and Clinical Studies with Colocynthis Pulp Extracts.” *Arzneimittelforschung* 55: 621-63.

[17] Al-Ghaithi, F., El-Ridi, M. R., Adeghate, E., and Amiri, M. H. 2004. “Biochemical Effects of *Citrullus colocynthis* in Normal and Diabetic Rats.” *Mol Cell...*
Scientific Studies on Citrulus Colocynthis with Special Reference to Anti-hyperglycemic Activity

[18] Torkey, H. M., Abou-Yousef, H. M., Abdel Azeiz, A. Z., and Farid, H. E. A. 2009. “Insecticidal Effect of Cucurbitacin E Glycoside Isolated from Citrullus colocynthis against Aphis craccivora.” Australian Journal of Basic and Applied Sciences 3 (4): 4060-6.

[19] AL-Qarawi, A. A., and Adam, S. E. 2003. “Effect of Combination of Capsicum frutescens and Citrullus colocynthis on Growth, Haematological and Pathophysiological Parameters of Rats.” Phytother Res 1: 92-5.

[20] Barth, A., Müller, D., and Dürrling, K. 2002. “In Vitro Investigation of a Standardized Dried Extract of Citrullus colocynthis on Liver Toxicity in Adult Rats.” Exp Toxicol Pathol 54: 223-30.

[21] Abdel-Hassan, I. A., Abdel-Barry, J. A., and Tariq Mohammeda, S. 2000. “The Hypoglycemic and Antihyperglycaemic Effect of Citrullus colocynthis Fruit Aqueous Extract in Normal and Alloxan Diabetic Rabbits.” J Ethnopharmacol 71: 325-30.

[22] Guiso Gallisa, F. 2002. Information in: Sincich, F. Bedouin Traditional Medicine in the Syrian Steppe. Rome: FAO, 114-5.

[23] Diwan, F. H., Abdel-Hassan, I. A., and Mohammed, S. T. 2000. “Effect of Saponin on Mortality and Histopathological Changes in Mice.” East Mediterr Health J 6: 345-51.

[24] Adam, S. E. I., Al-Farha, A. H., and Al-Yahya, A. 2000. “Effect of Combined Citrullus colocynthis and Rhazya stricta Use in Najdi Sheep.” Am J Chin Med 28: 385-90.

[25] Al-Yahya, M. A., AL-Farhan, A. H., and Adam, S. E. 2000. “Preliminary Toxicity Study on the Individual and Combined Effects of Citrullus colocynthis and Nerium oleander in Rats.” Fitoterapia 71: 385-91.