Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(1): 1226-1233
Received: 04-11-2017
Accepted: 05-12-2017

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Hepato toxic or hepato protective: A review of hepatic effects of *Citrullus colocynthis*

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Abstract
Citrullus colocynthis (CCT) is a medicinal herb known for its multiple pharmaceutical actions. The trend of its use in therapeutics and research has been increased over the time. It is very important to know its toxicity potential as it is being marketed in many areas of the world. The objective of this review was to evaluate the hepatic effects of CCT. For review, original research papers were searched using primary sources available on selected data bases and using specific key terms followed by a manual search into the secondary sources like review articles and books. The bibliographies of all the selected articles or review papers were searched manually to find the relevant literature. Literature shows conflicting data regarding hepatic effect of *Citrullus colocynthis*. It can be hepatotoxic when used in higher doses or longer durations. It is safer when given in small (50-100mg/kg) doses and small duration. Seed extracts are better tolerated than pulp by the experimental animals. However, further research is needed to identify and evaluate the hepatotoxic constituents of CCT. Inspite of the growing evidence regarding its medicinal value, there is dearth of any review of the hepatic effects of Colocynth reported to date. This review will provide a comprehensive overview of the hepatic protection or toxicity offered by *Citrullus colocynthis*.

Keywords: *Citrullus colocynthis*, colocynth, Tumbah, phyto toxicity, Hepatic

Introduction
Citrullus colocynthis (L.) Schard (Fig. 1), one of the four species of the desert vine, related to genus Cucurbitacae, is a perennial herb with a tuberous taproot [1]. It is also known as colocynth or bitter apple in English, Kolokinthe in German, Colocinte in French [2, 3], al-Hanzal in Arabic and tumba in Urdu [4]. It is also named as thymbre or earth gall [4]. It is widely distributed in the arid areas extending from the west coast of northern Africa, eastwards touching the northward to Mediterranean Sea [5].

Colocynth seeds have a historical background for its nutritional use when African tribes in Sahara used to make food from its seeds [5]. Colocynth seeds contains linolenic acid, linoleic acid, oleic acid, palmitic acid, stearic acid, arachdonic acid [6]. It has also been considered as a potential oil harvest [7]. Among the various fatty acids, major proportion (66.6%) is constituted by Linoleic acid. It has also been considered as a potential oil harvest. It is known to have flavanoids, alkaloids, glycosides, terpenoids [8] colocynthoisdes and cucurbitacins [6], amino acids, carbohydrates, phenols, saponins, glycosides, tannins, triterpenoid [8].

In addition to the nutritional value of its seeds, different parts of the plant have been investigated since long to evaluate its medicinal potential. It is known to have anti diabetic [6, 18, 19], beta cell regenerative [9], alpha cell hyperplastic(14), insulinotropic [15, 19], anti-neuropathic pain [17], antioxidant [18], anti inflammatory [19-21], hypolipidemic [22, 23], anti cancer [24, 25], hepato protectivity [26, 27], antifungal [28-30], anti candidal [31, 32], antimicrobial [33], scorpion envenomation [34], hair tonic in Alopecia [35-36], insecticidal [37], anti-ulcer [38], and anti-fertility effects [39-41]. It is known to be a hydragogoue artic used in constipation, hepatic congestion. It's pulp for constipation, fever and worms; root for cough and asthma; seed oil for snake bites, bowel complaints, and hair growth [42]. Fruit part is carminative, purgatives, anti pyretics, anthelminitic and also used in tumors, ascities, asthma, bronchitis, elephantiasis tuberculous glans of neck and spleenomegaly. Its roots have been used in mastitis, rheumatlgia, viseromegaly in children and jaundice [43].

Fig 1: *Citrulus colocynthis* (Teixeira De-Silva and Hussain, 2017)

Its active principle is said to be glucoside colocinthin [42]. It also contains citrulluin, citrulluene and citrulluic acid. The peel free flesh of the ripe fruits contains a bitter oil, citbittol and a di-hydric alcohol, citrullol in the dried pulp [43].

It is normally considered that phytochemcials are safe which, however, is not the case. Herbs are known to cause drug induced liver injury (DILI). The damage is related to self medication and poor vigilance related to use of herbal medicine [44, 45]. It is known that the pulp of Citrullus is poisonous in excessive doses [42, 46]. Citrullus is known to have toxic effects on female reproductive system [47], heart [48], kidneys and liver [49]. Cases of rectorrahgia [50], colitis [51] and bloody diarrhea [52] have also been reported after Citrullus ingestion. It is also known to cause carcinogenicity [53], while on the other hand, its anticancer effect has also been documented [24]. However, a gross conflict exists regarding hepato protective and hepatotoxic effects of Citrullus.

Methodology

Objective

The objectives of this review were to see the hepatic effects of toxicity of *Citrullus colocynthis* and to identify gaps for further research in this area

Data Collection

Meticulous strategy regarding data sources, study section and data extraction was used for the collection of evidence. Literature search was done in two phases. In phase 1,original research papers were searched using primary sources available on selected data bases and using specific key terms followed by a scoping search into the secondary sources like review articles, conference proceedings and books. In phase 2, the bibliographies of all the selected articles or review papers were searched manually to find the relevant literature.

Data Sources

Research articles were retrieved using specific data basis which included: Google Scholar, Google, pubmed, Medline, Proquest. Data was also collected from secondary sources like review papers, chapters of books on Ayurvedi or herbal medicine available in local library. All the sources were searched using key words of *Citrullus colocynthis* Or Colocynth OR Tumbah OR Bitter Apple AND Toxicity OR phyto-toxicity AND Hepatic.

Study Selection

Specified inclusion and exclusion criteria was used to maximize the value of review through the collection of articles which were found to be relevant. Search was performed by the authors individually. All data collected was merged and any duplication was removed from the pool. The studies identified were thence organized for abstraction and critical appraisal. The inclusion criteria were pre decided. Original research papers based on animal or human trials for the evaluation of either the histological changes of liver or liver function markers or both due to treatment with any part of the plant of *Citrullus colocynthis*, published in journals in English were included in the review.

Data Extraction

All the selected articles were read critically and detailed description of the results along with the experimental animals, part of plant, type of extract, dosage, route and duration of the study was included.

Results

All researches have been produced from some areas of Asia including Iran (4studies), India (3), SaudiArabia (3), Iraq (2), Egypt (1), Jordan (1), UAE (2), Algeria (1) whereas only one study is produced from Europe, Germany. (Table 1, Fig. 2). Search found 18 studies (Table 2) in which different experimental animals like mice (2 studies), rabbits (2 studies) and rats (14 studies) were used. Twelve studies documented the hepatotoxicity of CCT while six studies have shown either non toxic nature or hepato protective nature of CCT. Different doses of various parts of *Citrullus colocynthis* like pulp (4 studies), full fruit (9), Pulp and seed (2) seeds (1) leave (1 study) were used in the experiments. Most of the studies evaluated markers of liver function like AST, ALT, ALP along with the morphological changes. In addition to it, two only abstracts were found which are not included in the data extraction. Hepato toxicity of Citrullus.

Table 1: The year wise detail of number of publications, country and journal of publication

Year	No	Authors	Country	Journal
2017	1	Shafaei *et al*	Algeria	International Journal of Pharmacognosy and Phytochemical Research
2014	1	Shafaei *et al*	Iran	Advanced Biomedical research
2013	2	Al-Gerwi *et al*	Egypt	Journal of American Science
2012	3	Shafaei *et al*	Iran	Journal of medicinal plant research
		Dar *et al*	India	International research of biological and pharmaceutical research
		Dar *et al*	India	American journal of Plant Sciences
2011	1	Khatibi	Iran	Journal of Agricultural Sciences
2010	2	Khalil *et al*	Jordan	American Journal of Biochemistry and Biotechnology
		Alabadi and Al-Ali	Iraq	-
2009	1	Atole *et al*	India	Veterinary World
2006	1	Deghani and PanjehShahin	Iran	Iranian journal of pharmacology and therapeutics
2004	1	Al-Ghaithi *et al*	UAE	Molecular and cellular biochemistry
2002	1	Barth *et al*	Germany	Experimental and Toxicological Pathology
2001	1	Adam *et al*	SA	Small Ruminant research
2000	2	Diwan *et al*	Iraq	Eastern Mediterranean Health Journal
		Al-Yahya *et al*	SA	Filoterapia
1994	1	Wasfi	UAE	Journal of Herbs, Spices and Medicinal Plants
1989	1	Shah *et al*	SA	Phytotherapy

Fig 2: Location of the countries in Asia (encircled) as source of publications

Hepato Toxicity

In 1989, Shah *et al*.z evaluated the toxicity profile of 6 plants used as traditional medicine in Arab, which included *Citrullus colocynthis*. They found CCT to be maximally lethal with percentage lethality of 45% and highest mortality. The mortality rate was 40, 60 and 100% in mice fed 500 mg/kg, 1 g/kg and 3 g/kg. After acute toxicity, animals were found to have dirraea, increased respiratory rate, increased motor activity, tremors and even convulsions and death. Chronic intake of 100mg/kg caused significant increase in liver weight but weight of heart lung, spleen, kidney and testis was not changed significantly. Wasfi [55] observed hepatorenal toxicity of 800mg/kg CCT pulp and leaves.

Two studies examined the effect of CCT rich diets. Al-Yahya *et al*. [56] examined the toxicity of diet having 10% CCT and 10% Nerium Oleander for 3 and 6 weeks. They found raised levels of AST and ALT at both time bounds, suggesting liver damage. Adam *et al*. [57] used crude powder of CCT as 10% of diet in rats for 3 and 6 weeks. After 3 weeks, mild hepatic congestion and degenerative changes in livers were observed. After 6 weeks of treatment, necrosis and cytoplasmic fatty vacoulation of centrilobular hepatocytes were observed in liver along with significant rise in AST and ALT.

Diwan *et al*. [5] used saponin extract as 200 mg/kg and examined livers after 48 hours. There was necrosis, central venous congestion and hemorrhages in liver samples. They also observed acute toxicity of CCT with 50, 100, 150, 250, 350 and 600 mg/kg of saponin extract showing high rate of mortality in groups fed doses higher than 150mg/kg. They observed dose dependent effect on appetite, gait, activity and diarrhea.

Deghani and Panjeh Shahin [59] used alcoholic extract of CCT as 50, 100, 200, 400 mg/k for 2 weeks intraperitoneally and observed liver damage showing sinusoidal and central venous congestion, Inflammation, hemorrhages, lymphocytic and leukocytic infiltration and fibrosis at 200-400mg/kg. However, fibrosis was evident particularly at 400mg/kg dose. CCT induced hepatotoxicity in also observed in rabbits. Shafaei *et al*. [60] studied the effect of CCT on rabbit liver tissues. They used 100 and 200 mg/kg of CCT pulp and seed extracts for one month in rabbits weighing 3kg. Animals taking higher dose of pulp extract didn't survive. Severe damage to the histological architecture of liver was observed along with kidneys. However, seed extract induced minor intestinal effects. Likewise, Soufane *et al*. [46] observed raised liver enzymes at 5th, 10th and 14th days of treatment with CCT 131mg/kg but no change in liver weight was observed.

Long term use of CCT extract is more deleterious even at lower doses. In evaluation of subchronic haematotoxicity and histotoxicity of CCT, Elgerwi and colleagues used CCT (1/4th of LD50) collected from three areas of Lybia. After oral admisnteration of 100 or 162mg/dl pulp extract every week for 10 weeks, they observed necrosis, fibrosis, chromatolysis, vacuolar degeneration of hepatocytes, central venous congestion, sinusoidal congestion, Inflammation with marked infiltration of lymphocytes and macrophages, focal hemorrhages, hypertrophy of kupffer cells in rat livers. They observed significantly raised liver function markers GPT, GOT and ALP. Total protein was also decreased. [61] More recently, Soufane *et al*. [62] used higher doses of CCT 265 mg/kg for 6 weeks and observed hepatic congestion, elevated liver enzymes, and significant reduction in liver weight

Hepato protective activity of Citrullus

However, contradictory evidence also exists. Experimental studies have shown various degrees of hepato protective action of *Citrullus colocynthis*. In four studies showing hepato protective effects of CCT, whole fruit extract has been used whereas two studies have used seed only or pulp only extracts. Dar *et al*. evaluated the effect of CCT on polluted water [27] and paracetamol induced liver damage(26). Dar *et al*. [27] gave ethanolic extract orally to rats as 100 and 200mg/kg which effectively decreased Polluted water induced raised liver enzymes and improved inflammation, necrosis and congestion in liver. In the second study they observed protective effect of CCT against paracetamol induced hepatotoxicity [26]. Paracetamol was given as 500mg/kg per oral every 72 hours for 7 days and rats were treated with 50, 100, 200 mg/kg CCT ethanolic extract. The higher dose (200mg/kg) was found to be effective in decreasing hepatocyte vacuolization and fatty change and decreasing the raised liver enzymes.

Al-Gaithi *et al*. [63] evaluated the effect of CCT aqueous seed extract on biochemical parameters in diabetic rats. They used doses of 0.25ml, 0.5ml and 1ml/kg of extract. No significant effect in normal rats was observed, however, significant decrease in plasma AST level was observed in diabetic rats. Likewise, Alabadi and AL-Ali [64] used 300mg/kg aqueous fruit extract in alloxan treated diabetic rats for 3 weeks and evaluated its effect on biochemical markers. They documented decrease in serum ALT and ALP levels in diabetic rats. Atole *et al*. [65] conducted a safety evaluation study of CCT and used 50mg/kg and 100mg/kg CCT fruit extract for 28 days in rats. They did not observe any noticeable change in liver enzymes and histological structure and have documented these doses of CCT as safe in diabetes.

Barth *et al*. [66] evaluated the liver toxicity of CCT extract by estimating tissue GSH, LPO levels and ROS production. However, they did not observe histological changes or liver enzymes. CCT extract in doses of 100 µg/ml reduced lipid peroxidation but this concentration could not recover CCl4 induced damage in rat liver slices.

In another study, Khalil *et al*. [67] have observed hepatoprotection in CCT treated diabetic rats. They used pulp only extract as 300mg/kg and observed alleviation of diabetes induced hepatic inflammation, hepatocyte vacoulation, lipid accumulation and sinusoidal damage in liver tissue of rats.

Table 2: Selected articles showing Hepatic effect of *Citrullus colocynthis*

No	Study Population	Weight of Animals	Part of Plant	Preparation	Route	Dose	Duration of treatment	Results	References
Hepato Toxicity									
1	Rats	180-210 g	Pulp	Methanolic extract	Oral	265mg/kg	For 6 weeks	Reduction in liver weight Elevated liver enzymes Hepatic congestion	Soufane *et al*., 2017
2	Rabbits	3 kg	Pulp seeds	Pulp and seed extract		100, 200mg/kg	1 month	Increased glycogen content of hepatocytes in alloxanized rabbits	Shafaei *et al*., 2014
3	Rats	180-210 g	Pulp	Methanolic extract	Oral	131mg/kg	24hr, 5, 10 and 14 days	No change in weight of liver Elevated liver enzymes	Soufane *et al*., 2013
4	Rats	180-250g	Pulp	Alcoholic extract	Oral	100mg/kg, 162mg/kg 1/4th of LD50	Every week for 10 weeks	Necrosis, fibrosis, chromatolysis, central venous congestion, sinusoidal congestion, Inflammation, Hemorrhages, hypertrophy of kupffer cells, vacuolar degeneration of hepatocytes Significantly raised GPT, GOT	Al-Gerwi *et al*., 2013
5	Rabbits	3 kg	Pulp seeds	Methanolic Pulp extract and seed extract		100, 200mg/kg	1 month	Dilatation of sinusoids and bile ducts in pulp extract treated rats whereas no hepatorenal toxicity was observed with both doses of seed extract	Shafaei *et al*., 2012
6	Rats	200-250g	Fruit	Alcoholic Extract	IP	50,100,200, 400mg/kg	Two weeks	Sinusoidal and Central venous congestion, Inflammation, Hemorrhages, lymphocytic and leukocytic infilteration	Deghani and PanjehShahin, 2006
7	Rats	60 days old	Seeds	Ethanolic	Incubation of liver tissue slices	CCT extract for incubation in doses of 10 µg/ml, 100 µg/ml	22 hours exposure	Concentrations upto 100 micrograms/ml inhibits LPO and ROS production but did not alleviate CCl4 induced liver damage	Barth *et al*., 2002
8	Rats	150±10	Fruit	Crude powder	Oral	10% diet	3-6 weeks	After 3 weeks: Mild hepatic congestion, degenerative changes, After 6 weeks: Necrosis, fatty vacoulation of centrilobular hepatocytes Significant rise in AST and ALT	Adam *et al*., 2001
9	Mice	20-30g	Fruit	Methanolic Saponin extract	Bucco gastric cannula	200mg/kg	48 hours	Necrosis, Central venous congestion, Hemorrhages	Diwan *et al*, 2000
10	Rats	165±10g	Fruit	10% diet	Oral	10% diet	3,6 weeks	Significant rise in AST, ALT, ASP	Al-Yahya *et al*., 2000
11	Rats	200-250g	Pulp Leaves	Chloroform and methanolic extract	Oral	800mg/kg	24 hours	Central vein dilatation and congestion Sinusoidal dilatation and congestion	Wasfi *et al*., 1994
12	Mice	25-30g	Fruit		Oral	100mg/kg	3 months	Reduction in weight of liver	Shah *et al* 1989
Non Toxic or Hepato Protective action									
13	Rats	150-200g	Fruit	Ethanolic	Oral	100, 200mg/kg	10 days	200mg/kg effectively decreases raised liver enzymes and Improved infalamation necrosis and congestion	Dar *et al*., 2012a
14	Swiss Rats	150-200g	Fruit	Ethanolic	Oral	50, 100, 200mg/kg	7 days	Decrease vacuolization and fatty change and effectively decreases raised liver enzyme	Dar *et al*., 2012b
15	Rats		Fruit	Aquous extract	Oral	300mg/kg	3 weeks	Lowers ALP in alloxanized rats	Alabadi and Al-Ali, 2010
16	Rats	200-250g	Pulp	Ethanolic extract	Oral	1ml/kg (equivalent to 300mg/kg)	30 days	Alleviation of hepatocyte cytoplasmic vacuolar degeneration, pyknosis, steatosis severity grade and sinusoidal damage	Khalil *et al*., 2010
17	Rats	170-250g	Seeds	Aqueous	Oral	0.25, 0.5, 1 ml/kg	14 days	No significant effect in normal	Al-Gaithi *et al*.,

				extract	intubation			rats Reduce AST and LDH in diabetic rats	2004
18	Rats	100-200g	Fruit	Aqueous extract		50,100mg/kg	28 days	No significant effect on liver morphology and enzymes	Atole *et al*., 2009

Discussion

History of using Folk medicine to treat various illnesses dates back to earlier times. Its only recently that herbal remedies are being analyzed for scientific validation of the toxicity profile [68]. Herbal remedies used for various purposes like psoriasis, slimming, liver tonics, rheumatism etc are well recognized for their hepatotoxicity [69]. However the scientific data about the hepatic profile of CCT in therapeutics is controversial and supports both the hepato protective and hepatotoxic effects.

Earlier researchers used higher doses of CCT as compared to the more recent studies. This change in trend looks like due to the evolving concept of toxicity potential of herbal medicines. This review shows that CCT induced hepato toxicity is dose and time dependent. Higher doses of CCT, as 800mg/kg [55], 400mg/kg and 200mg/kg [59] are more toxic. Most of the researchers documenting the antidiabetic, anti-oxidant, hypolipedemic, anti-inflammatory effect of CCT have used 50-300 mg/kg/day; wherein 300mg/kg is used for duration of 3-4 weeks and 50 mg/kg, considering a safer dose, is used for more than a month.

However, discrepancies exist regarding dose dependent effect. Shafaei *et al*. [70] and Soufane *et al*. [62] observed hepato toxicity of 200mg/kg CCT; Whereas, Dar *et al*. [26, 27] observed hepatoprotective effects of CCT and found 200mg/kg effective in reducing inflammation and congestion of liver in polluted water induced hepatic damage and decreased hepatic vacoulation in paracetamol induced hepatic injury. One difference between hepato protective and hepato toxic studies is the type of extract. In studies showing hepatoprotective effect, ethanolic extract was used; whereas, methanolic extract was used in most of the studies showing hepatotoxicity. Hence, it can be speculated that methanolic extracts might be hepatotoxic, however, it is a less likely justification and needs further exploration.

CCT fruit extract is considered safe if given as 50 mg or 100mg/kg when given for 28 days. [65] However, significant increase in rat liver weight was observed by Shah *et al*. [65] after chronic ingestion (3 months) of even 100mg/kg CCT which contradicts the finding of Soufane *et al*. [51] who did not observe significant change in weight of liver with 131mg/kg. This discrepancy may be due to the use of mice or longer duration of treatment. In studies showing hepto protective effect, CCT is used in different dosses from 50 to 300 mg/kg. However, Soufane *et al*. [71] observed raised liver enzymes even at 1/10th of LD50 131mg/kg. This controversy in dose dependent effect needs further exploration. However, it is said that LD50 can vary according to animal, species, age, bedding, time of the day, ambient temperature etc. [46]

Moreover, hepato protective effect was observed in 7-10 days treatment and hepatoxicity was observed when CCT was given for 1-6 months. However, Diwan *et al*. [72] have reported acute toxicity with 200 mg/kg but they used saponin extract. Saponin may be the responsible phytochemical for hepatotoxicity. Soufane *et al*. [71] have attributed the lethality of CCT extract to its saponin content or glycosides and alkaloids. Saponins are used in pharmaceuticals and are known for their cytotoxicity. On the other hand, colocynth is included in group of RIP (Ribosome Inhibiting Proteins) expressing plants [68]. RIPs are known for their cytotoxic effect along with anticancer, anti HSV and anti-HIV effect [73, 74].

Toxicity of CCT is not only dose and time dependent rather, it also depends upon the specific part (Fruit, pulp, seeds, leaves, roots) of the plant used. Various parts exert different effects. Experimental results of Shafaei *et al* [70] showed that animals treated with seed extract of Citrullus survived as compared to pulp extract. Pulp extract is not considered safe in higher doses and for longer duration [75].

This difference in the toxicity potential of seeds and pulp may be due to the difference in phytochemicals or bioactive constituents, as according to Uma and Sekar [76], pulp of CCT lack flavonoids which are otherwise present in seeds, leaves, roots. Other element responsible for the hepato toxicity may be the presence of sterol and terpinoids. Terpinoids inhibit the release of autacoids and prostaglandins [76]. Diterpines also inhibit cellular proliferation by inhibiting topoisomerase II [77] or inducing hepatocyte apoptosis [78] and monoterpines may cause hepatotoxicity by disrupting anti oxidant defense mechanism [79].

The cytotoxic effects of CCT can be attributed to the cucurbitcin content. Curcurbitacins are oxidized triterpinoids, known for cytotoxic and anticancer activity [80]. It may be speculated that cytotoxic effect may harm the normal non cancerous cells halting the normal functioning. It may be helpful if furure research is focused on isolation and evaluation of the individual constitutents of CCT like saponins, cucurboitacins and terpinoids. It may also allow to attain targeted actions and to avoid unwanted effect [76].

Moreover, in order to combat this expanding problem of herbal medicine induced tissue injury, early diagnosis, physician awareness, public awareness and astringents criteria for licensure of herbal drugs need to be ensured [45]. Moreover, at the time of registration of herbal medicine, toxicity profile of herbs based on quality evidence must be required4 [44].

Conclusion

Citrullus colocynthis is hepato toxic unless given in small (50-100mg/kg) doses and smaller duration. Its seed extracts are safer as compared to pulp. However, controversies exist in literature and further research is needed in this area. Isolation and evaluation of hepato toxicity of the individual constituents of *Citrullus colocynthis* needs to be done further at molecular level.

Declaration

There is no conflict of interest between the authors

References

1. Burrows Burrows GE, Shaik RS. Comparative developmental anatomy of the taproot of the cucurbitaceous vines *Citrullus colocynthis* (perennial), *Citrullus lanatus* (annual) and Cucumis myriocarpus (annual). Australian journal of botany. 2015; 62(7):537-45.
2. Da Silva JAT, Hussain AI. *Citrullus colocynthis* (L.) Schrad. (colocynth): Biotechnological perspectives. Emirates Journal of Food and Agriculture. 2017; 29(2):83.

3. Hussain AI, Rathore HA, Sattar MZ, Chatha SA, Sarker SD, Gilani AH. *Citrullus colocynthis* (L.) Schrad (bitter apple fruit): A review of its phytochemistry, pharmacology, traditional uses and nutritional potential. Journal of ethnopharmacology. 2014; 155(1):54-66.
4. Dallak M, Bin-Jaliah I. Antioxidant activity of *Citrullus colocynthis* pulp extract in the RBC's of alloxan-induced diabetic rats. Pak J Physiol. 2010; 6(1):1-5.
5. Lloyd JU, Cincinnati O. *Citrullus colocynthis*: Engelhard, 1898.
6. Sebbagh N, Cruciani-Guglielmacci C, Ouali F, Berthault M-F, Rouch C, Sari DC, *et al*. Comparative effects of *Citrullus colocynthis*, sunflower and olive oil-enriched diet in streptozotocin-induced diabetes in rats. Diabetes & metabolism. 2009; 35(3):178-84.
7. Schafferman D, Beharav A, Shabelsky E, Yaniv Z. Evaluation of *Citrullus colocynthis*, a desert plant native in Israel, as a potential source of edible oil. Journal of Arid Environments. 1998; 40(4):431-9.
8. Jeyanthi K. Antioxidant effect of *Citrullus colocynthis* on alloxan induced diabetic rats. International Journal of Pharmaceutical & Biological Archive. 2011; 2(2).
9. Amin A, Tahir M, Lone KP. Effect of *Citrullus colocynthis* aqueous seed extract on beta cell regeneration and intra-islet vasculature in alloxan induced diabetic male albino rats. Journal of Pakistan Medical Association. 2017; 67(5):715-21.
10. Houcine BRA, Rabah D, Farid L, Nabila B, Boufeldja T. Effect of saponosides crude extract isolated from *Citrullus colocynthis* (L.) seeds on blood glucose level in normal and streptozotocin induced diabetic rats Journal of Medicinal Plants Research. 2011; 5(31):6864-8.
11. Jayaraman R, Shivakumar A, Anitha T, Joshi V, Palei N. Antidiabetic effect of petroleum ether extract of *Citrullus colocynthis* fruits against streptozotocin-induced hyperglycemic rats. Rom J Biol Plant Biol. 2009; 4:127-34.
12. Huseini HF, Darvishzadeh F, Heshmat R, Jafariazar Z, Raza M, Larijani B. The clinical investigation of *Citrullus colocynthis* (L.) schrad fruit in treatment of Type II diabetic patients: a randomized, double blind, placebo-controlled clinical trial. Phytotherapy Research. 2009; 23(8):1186-9.
13. Benariba N, Djaziri R, Zerriouh BH, Boucherit K, Louchami K, Sener A, *et al*. Antihyperglycemic effect of *Citrullus colocynthis* seed aqueous extracts in streptozotocin-induced diabetic rats. Metab Funct Res Diab. 2009; 2:71-6.
14. Amin A, Tahir M. Alpha cells a 'therapeutic target': effect of *Citrullus colocynthis* on alpha cell count in healthy and alloxan induced diabetic male albino rats Worls Journal of Pharmaceutical Research. 2016; 5(11):329-39.
15. Nmila R, Gross R, Rchid H, Roye M, Manteghetti M, Petit P, *et al*. Insulinotropic effect of *Citrullus colocynthis* fruit extracts. Planta Medica. 2000; 66(05):418-23.
16. Ebrahimi E, Bahramzadeh S, Hashemitabar M, Mohammadzadeh G, Shirali S, Jodat J. Effect of hydroalcoholic leaves extract of *Citrullus colocynthis* on induction of insulin secretion from isolated rat islets of Langerhans. Asian Pacific Journal of Tropical Disease. 2016; 6(8):638-41.
17. Heydari M, Homayouni K, Hashempur MH, Shams M. Topical *Citrullus colocynthis* (bitter apple) extract oil in painful diabetic neuropathy: A double-blind randomized placebo-controlled clinical trial. Journal of diabetes. 2016; 8(2):246-52.
18. Kumar S, Kumar D, Jusha M, Saroha K, Singh N, Vashishta B. Antioxidant and free radical scavenging potential of *Citrullus colocynthis* (L.) Schrad. methanolic fruit extract. Acta Pharmaceutica. 2008; 58(2):215-20.
19. Akhzari M, Mirghiasi S, Vassaf M, Bidgoli M, Tari Z. The Effect of *Citrullus colocynthis* on the Reduction of Inflammatory Agents in Osteoarthritis. Mol Biol. 2015; 4(4).
20. Marzouk B, Marzouk Z, Haloui E, Fenina N, Bouraoui A, Aouni M. Screening of analgesic and anti-inflammatory activities of *Citrullus colocynthis* from southern Tunisia. Journal of ethnopharmacology. 2010; 128(1):15-9.
21. Sanadgol N, Najafi S, Ghasemi LV, Motalleb G, Estakhr J. A study of the inhibitory effects of *Citrullus colocynthis* (CCT) using hydro-alcoholic extract on the expression of cytokines: TNF-and IL-6 in high fat diet-fed mice towards a cure for diabetes mellitus. Journal of Pharmacognosy and Phytotherapy. 2011; 3(6):81-8.
22. Abd El-Baky AAA, Abd El-Mawgoud H, Abd El-Hay E. Hypoglycemic and Hypolipidaemic Action of Bitter Melon on Normoglycemic and Hyperglycemic Diabetic Rats. Research Journal of Medicine and Medical Sciences. 2009; 4(2):519-25.
23. Dhalak M. In vivo, hypolipidemic and antioxidant effects of *Citrullus colocynthis* pulp extract in alloxan-induced diabetic rats African Journal of Biotechnology. 2011; 10(48).
24. Seif-Eldin N. Ayyad AA-L, Walied M. Alarif, Francesca R. Patacchioli, Farid A. Badria, Saleh T. Ezmirly. Invitro and invivo study of Cucurbitanics type triterpene glucoside from *Citrullus colocynthis* growing in Saudi Arabia agaisnt hepatocellualr carcinoma. Environmental Toxicology and Pharmacology. 2012; 32:245-51.
25. Tannin-Spitz TGS, Dovrat S, Gottlie HE, Bergman M. Growth inhibitory activity of cucurbitacin glucosides isolated from *Citrullus colocynthis* on human breast cancer cells. biochemical pharmacology. 2007; 73:56-67.
26. Dar AI Saxena RC, Bansal SK. Hepatoprotection: a hallmark of *Citrullus colocynthis* L. against paracetamol induced hepatotoxicity in swiss albino rats. American Journal of Plant Sciences. 2012; 3(7):1022.
27. Dar AI, Saxena R, Bansal S, Matadeen B, Saxena R. Protective effect of *Citrullus colocynthis* L. against polluted water induced hepatotoxicity in albino rats. International Journal of Biological & Pharmaceutical Research. 2012; 3(2):240-4.
28. Marzouk B, Marzouk Z, Décor R, Mhadhebi L, Fenina N, Aouni M. Antibacterial and antifungal activities of several populations of Tunisian *Citrullus colocynthis* Schrad. immature fruits and seeds. Journal de Mycologie Médicale/Journal of Medical Mycology. 2010; 20(3):179-84.
29. Prasad MP. Phytochemical and Antifungal Activity of *Citrullus colocynthis* Seeds Solvent Extracts International Journal of Science and Research (IJSR). 2014; 3(10):1156-60.
30. Eidi S, Azadi HG, Rahbar N, Mehmannavaz HR. Evaluation of antifungal activity of hydroalcoholic extracts of *Citrullus colocynthis* fruit. Journal of Herbal Medicine. 2015; 5(1):36-40.

31. Khatibi R, Teymorri J. Anticandidal screening and antibacterial of *Citrullus colocynthis* in South East of Iran. Journal of Horticulture and Forestry. 2011; 3(13):392-8.
32. Marzouk B, Marzouk Z, Décor R, Edziri H, Haloui E, Fenina N, *et al*. Antibacterial and anticandidal screening of Tunisian *Citrullus colocynthis* Schrad. from Medenine. Journal of ethnopharmacology. 2009; 125(2):344-9.
33. Gurudeeban SRT, Satyavani K, Dhinesh T. Antimicrobial effect of coastal medicinal plant – *Citrullus colocynthis* against pathogenic microorganisms African Journal of Pure and Applied Chemistry. 2011; 5(5):119-22.
34. Fatima L-D, Mohamed K. Phytotherapy as new approach to treat scorpion envenomation: Experimental study. International Journal of Pharmaceutical Sciences and Research. 2014; 5(5):1682.
35. Dhanotia R, Chauhan NS, Saraf DK, Dixit VK. Effect of *Citrullus colocynthis* Schrad fruits on testosterone-induced alopecia. Natural product research. 2011; 25(15):1432-43.
36. Roy R, Thakur M, Dixit V. Effect of *Citrullus colocynthis*. On hair growth in albino rats. Pharmaceutical biology. 2007; 45(10):739-44.
37. Jeon JH, Lee HS. Biofunctional Constituent Isolated from *Citrullus colocynthis* Fruits and Structure−Activity Relationships of Its Analogues Show Acaricidal and Insecticidal Efficacy. J Agric Food Chem, 2014.
38. Reddy VP, Sudheshna G, Afsar S, Saran S, Kumar SN, Ram CR, *et al*. Evaluation of anti-ulcer activity of *Citrullus colocynthis* fruit against pylorus ligation induced ulcers in male wistar rats. Int J Pharm Pharm Sci. 2012; 4(2):446-51.
39. Chaturvedi M, Mali P, Ansari A. Induction of reversible antifertility with a crude ethanol extract of *Citrullus colocynthis* Schrad fruit in male rats. Pharmacology. 2003; 68(1):38-48.
40. Dehghani F, Talaei-Khozani T, Mesbah F, Azizi M, Panjehshahin M. Toxic effects of hydroalcoholic extract of *Citrullus colocynthis* on pregnant mice. Iranian Journal of Veterinary Research. 2008; 9(1):42-5.
41. Qazan W, Almasad MM, Daradka H. Short and long effects of *Citrullus colocynthis* L. on reproductive system and fertility in female Spague-Dawley rats. Pakistan journal of biological sciences: PJBS. 2007; 10(16):2699-703.
42. Nadkerni KM. Indian plants and drugs with their medical properties and uses. New Delhi: Srishti book Distributorss, 2004.
43. Parajapati ND PS, Sharma AK, Kumar T. A handbook of medical plants: A complete source book. Agrobios, Jodhpur, India, 2007.
44. Moreira DDL TS, Monteiro MHD D, De-Oliveira ACAX, Paumgartten FJR. Traditional use and safety of herbal medicines. Brazilian Journal of Pharmacognosy. 2014; 24:248-57.
45. Chitturi SFG. Herbal hepatotoxicity: An expanding but poorly defined problem. Journal of Gastroenterology and Hepatology. 2000; 15:1093-9.
46. Soufane SBA, Mahdeb N, Bouzidi A. Acute Toxicity study on *Citrullus colocynthis* fruit methanol extract in Albino rats. Journal of Applied Pharmaceutical Science. 2013; 3(6):88-93.
47. Dehghani FAM, Panjehshahin MR, Talaei-Khozani T, Mesbah F. Toxic effects of hydroalcoholic extract of *Citrullus colocynthis* on pregnant mice Iranian Journal of Veterinary Research. 2008, 9.
48. Banerjee SP DP. Smooth Muscle and Cardiovascular Pharmacology of ~~-Elaterin-2-D-glucopyranoside Glycoside of CitruZZus colocynthis Journal of Pharmaceutical Sciences, 1967.
49. Dehghani FPM. The Toxic Effect of Alcoholic Extract of *Citrullus colocynthis* on Rat Liver. Iranian Journal Of Pharmacology & Therapeutics. 2006; 5(2):117-9.
50. Javadzadeh HR DA, Davoudi F, Valizadegan G, Goodarzi H, Mahmoodi S, Ghane MR, Faraji M. (2013). *Citrullus colocynthis* as the Cause of Acute Rectorrhagia. Case reports in emergency medicine.
51. Goldfain DLA, Galian A, Chauveinc L, Prudhomme F. Peculiar acute toxic colitis after ingestion of colocynth: a clinicopathological study of three cases. Gut. 1989; 30:1412-8.
52. Khan SA SH, Bhat AR, Bhat KS. A possible cause of bloody diarrhea. Saudi medical Journal. 2003; 24(8):904-6.
53. Habs MJS, Schmahl D. Carcinogenic Activity of Condensate from Coloquint Seeds (*Citrullus colocynthis*) after Chronic Epicutaneous Administration to Mice. J Cancer Res Clin Oncol. 1984; 108:154-6
54. Shah A, Qureshi S, Tariq M, Ageel A. Toxicity studies on six plants used in the traditional Arab system of medicine. Phytotherapy Research. 1989; 3(1):25-9.
55. Wasfi I. Some pharmacological studies on *Citrullus colocynthis*. Journal of Herbs, Spices & Medicinal Plants. 1994; 2(2):65-79.
56. Al-Yahyaa MA, A-FA, Adam SEI. Preliminary toxicity study on the individual and combined effects of *Citrullus colocynthis* and Nerium oleander in rats Fitoterapia. 2000, 71.
57. Adam S, Al-Yahya M, Al-Farhan A. Effects of dietary mixtures of *Citrullus colocynthis* L. schrad. and Rhazya stricta Decne in rats. Journal of herbs, spices & medicinal plants. 2001; 8(4):9-18.
58. Diwan F, Abdel Hassan I, Mohammed S. Effect of saponin on mortality and histopathological changes in mice, 2000.
59. Dehghani F, Panjehshahin MR. The toxic effect of alcoholic extract of *Citrullus colocynthis* on rat liver. Iranian Journal of Pharmacology & Therapeutics. 2006; 5(2):117-9.
60. Shafaei H, Esmaeili A, Rad JS, Delazar A, Behjati M. *Citrullus colocynthis* as a medicinal or poisonous plant: A revised fact ournal of Medicinal Plants Research. 2014; 6(35):4922-492.
61. Elgerwi A, Benzekri Z, Awaidat S, El-Magdoub A, Abusnina A, El-Mahmoudy A. Subchronic haemotoxicity and histotoxicity of *Citrullus colocynthis*. Journal of American Science. 2013; 9(5).
62. Soufane S, Bouzidi A, Mahdeb N, Krache S. Evaluation of Acute and Subacute Toxicity of Fruit Methanolic Extract from *Citrullus colocynthis* in male Albino rats. International Journal of Pharmacognosy and Phytochemical Research. 2017; 9(4):567-80.
63. Al-Ghaithi F, El-Ridi MR, Adeghate E, Amiri MH. Biochemical effects of *Citrullus colocynthis* in normal and diabetic rats. Molecular and cellular biochemistry. 2004; 261(1):143-9.
64. Alabadi UAM, Al-Ali AJR. Effect of Hot Aqueous Extract of *Citrullus colocynthis* L. Fruit on some

Biochemical and Haematological Parameters in Alloxan –Induced Diabetic Rats, 2010.
65. Atole S, Jangde C, Philip P, Rekhe D, Aghav D, Waghode H, *et al*. Safety evaluation studies of *Citrullus colocynthis* for diabetes in rats. Veterinary World. 2009; 2(11):423-5.
66. Barth A, Müller D, Dürrling K. In vitro investigation of a standardized dried extract of *Citrullus colocynthis* on liver toxicity in adult rats. Experimental and Toxicologic Pathology. 2002; 54(3):223-30.
67. Khalil M, Mohamed G, Dallak M, Al-Hashem F, Sakr H, Eid R, *et al*. The effect of *Citrullus colocynthis* pulp extract on the liver of diabetic rats a light and scanning electron microscopic study. American Journal of Biochemistry and Biotechnology. 2010; 6(3):155-63.
68. Polito L BM, Maiello S, Battelli MG, Bolognesi A. Plants Producing Ribosome Inactivating Proteins in Traditional Medicine. Molecules. 2016, 21.
69. Chitturi S, Farrell GC. Herbal hepatotoxicity: an expanding but poorly defined problem. Journal of gastroenterology and hepatology. 2000; 15(10):1093-9.
70. Shafaei HEA, ad JS, Delazar A, Behjati M. *Citrullus colocynthis* as a medicinal or poisonous plant: A revised fact Journal of Medicinal Plants Research. 2012; 6(35):4922-7.
71. Soufane S, Bedda A, Mahdeb N, Bouzidi A. Acute Toxicity study on *Citrullus colocynthis* fruit methanol extract in Albino rats. Journal of Applied Pharmaceutical Science. 2013; 3(6):88.
72. Diwan FH A-HI, Mohammad ST. Effect of Saponin on mortality and histopathological changes in mice. Estern Mediterranian Jealth Hournal. 2000; 6(2):345-51.
73. Ng TB CW, Yeung HW. Proteins with abortifacient, ribosome inactivating, immunomodulatory, antitumor and anti-AIDS activities from Cucurbitaceae plants. Gen Pharmacol. 1992; 23:579-90.
74. Zhang YH WY, Yusufali AH, Ashby F, Zhang D, Yin ZF, Aslanidi GV, Srivastava A, Ling CQ, Ling C. Cytotoxic genes from traditional Chinese medicine inhibit tumor growth both in vitro and in vivo. J Integr Med. 2014; 12:483–94.
75. Soufane S BA, Mahdeb N, Krache S. Evaluation of Acute and Subacute Toxicity of Fruit Methanolic Extract from *Citrullus colocynthis* in male Albino rats. International Journal of Pharmacognosy and Phytochemical Research. 2017; 9(4):557-86.
76. Uma C, Sekar K. (year). Phytochemical analysis of a folklore medicinal plant *Citrullus colocynthis* L (bitter apple) Journal of Pharmacognosy and Phytochemistry. 2(6):195-202.
77. Miyata S WL, Yoshida C, Kitanakac S. Inhibition of cellular proliferation by Di terpenes, Topoisomerase II inhibitors. Bioorganic & Medicinal Chemistry. 2006; 14:2048-51.
78. Fau DLM, Farrell G. Diterpenoids from germander, an herbal medicine, induce apoptosis in isolated rat hepatocytes. Gastroenterology. 1997; 113:1334-46.
79. Zárybnický OBI, Ambrož M, Skálová L. Hepatotoxicity of monoterpenes and sesquiterpenes Arch Toxicol, 2017.
80. Chung SO KY, Park SU. An updated review of cucurbitacins and their biological and pharmacological activities EXCLI Journal. 2015; 14:562-6.