# Antimicrobial and toxicological studies on fruit pulp of *Citrullus colocynthis* L.

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**Abstract**: The methanolic extract of dried fruit pulp of *Citrullus colocynthis* (Cucurbitaceae) has been studied with respect to antimicrobial and toxicological properties. The antimicrobial profile was investigated against thirty bacterial isolates (10 Gram +ve and 20Gram-ve) and five fungal species. None of the bacterial or fungal culture used in the study showed sensitivity against the extract. Acute toxicity studies carried out in Albino mice NMRI indicated the highly toxic nature of the colocynth. A very significant decrease in body weight of test animals was noted at $P<0.05$. The $LD_{50}$ was calculated as 1000mg/kg body weight. Within four days of experimentation mortality was 100%. Histopathological studies confirmed the toxic nature of extract. Gross changes in histology of Heart, Liver and Kidneys were noted. Section of spleen did not exhibit any abnormality.

**Keywords**: *Citrullus colocynthis*, antimicrobial profile, toxicology.

## INTRODUCTION

Though plants were used to treat human and animal ailments from time immemorial, scientific basis of the use of most of plants is still obscure. In current search for new drugs, researchers face problems that vast number of plant species are still unscreened with respect to their bioactivity.

*Citrullus colocynthis* (cucurbitaceae) is a native of arid soils. It is commonly found in Saudi Arabia, Syria, Jordan, Egypt, Iran, India and Pakistan. It is commonly known as Hanzal, Indrian, Tumma or Bitter apple. It is a large creeping herb, with deeply dissected lobulate leaves. Flowers, solitary, monoecious of yellow colour. Fruits rounded 7-9cm in diameter, green and white, striped, become yellow when ripe (Jafri, 1966). The dried pulp of mature fruits freed from rind and seeds constitute the drug "Colocynth" formerly official in Indian pharmacopeia and currently official in Homoeopathic pharmacopeia. In United States pharmacopeia it was included from 1829-1910 (Rizvi *et al* 2007). Colocynth is a powerful hepatic and intestinal stimulant (Ponder & Hooper 1910). It is also found useful in asthma, rheumatism, sciatica, gout, paralysis, leprosy, epilepsy and in expulsion of intestinal parasites. It is also used as purgative for chronic constipation and is a very well-known abortifacient (Usmanghani *et al* 1997).

In Mediterranean countries, infusion of *Citrullus colocynthis* is traditionally used as antidibetic medication. In a clinical study in Iran involving 50 patients, it was concluded that fruit pulp of the plant was effective in improving the glycemic effect of type II diabetic patients without severe adverse effects (Huseini *et al* 2009). Anticancer activity has also been reported in cucurbitacin glycosides isolated from leaves of *Citrullus colocynthis*. It was observed that combination of glycosides B and E (1: 1) inhibited growth of ER + MCF-7 and ER-MDA-MB-231 human breast cancer cell lines and thus can be used against human breast cancer cells (Tannin *et al.,* 2007). Pesticidal and insectidal activity has also been reported in extracts of fruit (Kantouch *et al* 1997, Patole & Mahajan 2007). The highest insecticidal effect was obtained from methanol extract of cucurbitacin E glycoside against *Aphis craccivora*** (Torkey *et al.,* 2009). The methanolic extract of fruits are reported to possess antioxidant (Kumar *et al.,* 2008) and antiallergic activity (Masayuki, 2007) where as aqueous extract possess anticholesterol activity (Khouri *et al.,* 2007).

Through the folklore uses of plants in treating different ailments are multifarious, the scientific literature on antimicrobial activity and toxicology of plant is very scarce. Present study is thus conducted to evaluate the fruit pulp of *Citrullus colocynthis* with respect to its antimicrobial and toxicological properties.

## MATERIALS AND METHODS

### *Plant material*

Ripe fruits of *Citrullus colocynthis* were collected from the herbal gardens of Hamdard University, Madina-tul-Hikma in the months of Sep-Nov 2011. The pericarp was removed and the fruits were sliced and dried in sun and afterwards stored in dry containers. The plant was identified by Dr. Anjum, Director Herbal Museum, University of Karachi. The voucher specimen No.86411 was deposited in the herbarium of the Karachi University.

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**Member of genus 'Aphis' insects that suck the juice of plant.

***Plant extract***

In 1500ml of methanol, 50gm of dried fruit pulp was soaked for 48 hrs at room temperature. Whattman filter paper (no. 1) was used for primary filtration to remove coarse particles and then suction filtration was done through bacterial filtration unit (Kontes-Vineland New Jersey: 9553827). Under reduced pressure, filtrate was evaporated to dryness. 6.1gm of crude extract was yielded (12% yield).

***Experimental animals***

Male and female NMRI mice (20-30 gm weight) were used in the study. The test and control group comprised of 16 mice each (8 male + 8 female). They were housed in "Dr. HMI Institute of Pharmacology Herbal Sciences" with standard diet and housing conditions. The temperature of animal house was maintained at 22±2$^{o}$C. International and institutional ethical guidelines were followed for all protocols of animal maintenance and handling.

***Antimicrobial studies***

In present study thirty identified bacterial cultures including ten Gram positive and twenty Gram negative and five cultures of fungi were used. They were collected from Liaqat National Hospital. The purity of cultures were checked. Culture of bacteria were maintained on Muller Hinton agar at 4$^{o}$C, whereas those of fungi on Sabouraud's dextrose agar at same temperature.

***Preparation of disc***

The test compound was dissolved in methanol to make a stock solution of 1000µg/ml for further dilution. Sterile blank filter paper disc of 6mm diameter were impregnated with 20µg of solution per disc. Commercially available discs of Augmentin 30µg/disc were used as positive control whereas methanol impregnated sterile disc were used as negative control.

***Determination of antibacterial activity***

For antibacterial assay, disc diffusion technique of Bauer *et al* 1966 was used. For inoculation 5-6 hours old culture of the organisms in Muller Hinton broth was used. The Muller Hinton agar plates were seeded with bacterial culture, the turbidity of which matched 0.5 MacFarland turbidity standards. The extract impregnated disc and discs of negative and positive control were placed on the surface of agar and plates were incubated at 37$^{o}$C for 16-18 hours. After incubation, diameters of growth inhibition zones around discs were measured. Triplicate means were used for result interpretation. The zone of inhibition of 8mm or above was considered as significant (Bauer *et al.*, 1966).

***Determination of antifungal activity***

For antifungal assay, fungal cultures were prepared by growing the organisms in Sabouraud's glucose broth tubes far three days at 25-30$^{o}$C. Sabouraud's glucose agar plates were seeded with the cultures and then discs of test extract, negative control and fungizone (20µg/disc standard drug) as positive control were placed on the plates with the help of sterile forceps. They were incubated at 25$^{o}$C and results were noted every 24hours for 5-10 days. Zone of inhibition of 8mm or above was considered as significant.

***Toxicological studies***

*a) Acute toxicity*

Animals were divided into two groups; test group "T" and control group "C". Animals of "T" group were given test dose of 1000µg/kg body weight per oral route via oro-gastric Gavage tube. The control group was given same amount of saline. Animals were kept under observation for three hours post administration and then for further 10 days. After 10$^{th}$ day all the surviving animals were dissected and organs removed.

*b) Histopathology*

For fixation of organs i.e. Heart, Liver, Spleen and Kidneys, 10% formalin was used. After usual process of dehydration, clearing and infiltration, tissues were embedded in paraffin wax and sectioned into 5-µm slices, with Rotation Microtome Leica RM2145 (Rubeena *et al.*, 2003).

The tissues were stained with haematoxylin and eosin dyes and photographed by Nikon Trinoculer research microscope equipped with photographic system and phase contrast N-plan.

## STATISTICAL ANALYSIS

All the results were analyzed by using student's T-test and were considered significant when $P<0.05$.

## RESULTS

***Antimicrobial activity***

The results of the sensitivity test indicated that cultures of all Gram positive and Gram negative bacteria and the fungi included in the study did not exhibit any sensitivity to methanolic extract of fruit pulp of *Citrullus colocynthis* (tables 1-2).

***Acute toxicity studies***

No significant change in general behavior of test and control animals was noted except decrease in motor activity (table 3).

All animals were weighted daily. As indicated in Table 04, test animals exhibited prominent decrease in body weight at $P<0.05$ (table 4).

In addition, fifty percent of the test animals (4 male + 4 female) expired within twenty-four hours of treatment. Hence $LD_{50}$ was calculated as 1000mg/kg body weight. Within four days of experimentation, mortality rate was 100% in treated mice. In control group only one female

**Table 1**: Antibacterial activity of fruit pulp of *Citrullus colocynthis* against Gram negative (n=20) and Gram positive (n=10) bacteria

| S. No. | Code | Species name | Diameter of zone of inhibition (mm) | | |
|---|---|---|---|---|---|
| | | | Test extract | Augmentin 30µg | Negative control |
| 1 | $E_1$ | *Escherichia coli* | _ | _ | _ |
| 2 | $E_2$ | *Escherichia coli* | _ | _ | _ |
| 3 | $E_3$ | *Escherichia coli* | _ | _ | _ |
| 4 | $E_4$ | *Escherichia coli* | _ | _ | _ |
| 5 | $E_5$ | *Escherichia coli* | _ | _ | _ |
| 6 | $E_6$ | *Escherichia coli* | _ | _ | _ |
| 7 | $E_7$ | *Escherichia coli* | _ | _ | _ |
| 8 | $K_1$ | *Klebsiella pneumoniae* | _ | _ | _ |
| 9 | $K_2$ | *Klebsiella pneumoniae* | _ | _ | _ |
| 10 | $K_3$ | *Klebsiella pneumoniae* | _ | _ | _ |
| 11 | $K_4$ | *Klebsiella pneumoniae* | _ | _ | _ |
| 12 | $K_5$ | *Klebsiella pneumoniae* | _ | _ | _ |
| 13 | $P_1$ | *Proteus vulgaris* | _ | _ | _ |
| 14 | $P_2$ | *Proteus vulgaris* | _ | _ | _ |
| 15 | $Ps_1$ | *Pseudomonas aeroginosa* | _ | _ | _ |
| 16 | $Ps_2$ | *Pseudomonas aeroginosa* | _ | _ | _ |
| 17 | $Sl_1$ | *Salmonella typhi* | _ | _ | _ |
| 18 | $Sl_2$ | *Salmonella typhi* | _ | _ | _ |
| 19 | $Sl_3$ | *Salmonella typhi* | _ | _ | _ |
| 20 | $SlA_1$ | *Salmonella para typhi A* | _ | _ | _ |
| 21 | $Sp_1$ | *Staphylococcus aureus* | _ | 15 | _ |
| 22 | $Sp_2$ | *Staphylococcus aureus* | _ | 12 | _ |
| 23 | $Sp_3$ | *Staphylococcus aureus* | _ | 15 | _ |
| 24 | $Sp_4$ | *Staphylococcus aureus* | _ | 10 | _ |
| 25 | $Sp_5$ | *Staphylococcus aureus* | _ | 12 | _ |
| 26 | $Sp_6$ | *Staphylococcus aureus* | _ | 12 | _ |
| 27 | $SpE_1$ | *Staphylococcus epidermidis* | _ | 14 | _ |
| 28 | $SpE_2$ | *Staphylococcus epidermidis* | _ | 12 | _ |
| 29 | $B_1$ | *Bacillus cereus* | _ | 8 | _ |
| 30 | $B_2$ | *Bacillus cereus* | _ | 8 | _ |

**Table 2**: Antifungal activity of fruit pulp extract of *Citrullus colocynthis* (n=05)

| S. No. | Code | Species Name | Diameter of growth inhibition zone (mm) | | |
|---|---|---|---|---|---|
| | | | Test extract | Fungizone 25µg | Negative control |
| 1 | $C_1$ | *Candida albicans* | _ | 14 | _ |
| 2 | $C_2$ | *Candida albicans* | _ | 16 | _ |
| 3 | $C_3$ | *Candida albicans* | _ | 14 | _ |
| 4 | $A_1$ | Aspergillus Species | _ | _ | _ |
| 5 | $Pe_n$ | Penicillium Species | _ | _ | _ |

mouse expired on forth day. No significant change in weight of animal of control group was noted.

Autopsy of all animals was carried out. Organs including Heart, Kidney, Liver and Spleen were examined together with abdominal, pericardial and pleural cavities. No gross change or abnormality was observed.

***Histopathology***

*Effect of Citrullus colocynthis fruit pulp extract on Liver.*

The sections of the liver tissue revealed preserved architecture with hexagonal lobules centered on central vein and surrounded by portal tracts. The hepatocytes were arranged in single cell thick hepatic cords along with scattered kupffer cells. The portal tracts contained bile

**Table 3**: Hourly observation for sign of toxicity after oral administration of extract of dried fruit pulp of *Citrullus colocynthis*

| Observation Hour | Observation |
|---|---|
| 1st hour | Animals were active, exploring the cage without any obvious sign of toxicity. |
| 2nd hour | Decreased activity – with grooming |
| 3rd hour | Corner sitting and sleep. |

**Table 4**: Weight change of animals treated with extract of dried fruit pulp of *Citrullus colocynthis*

| Test animal | Control (gm) | Treated (gm) |
|---|---|---|
| Male | 30.180±2.026 | 25.80±4.696 |
| Female | 30.510±3.377 | 24.40±3.651 |

Results are shown as average ± SD. n=16 for control group and n=16 for treated group

duct, hepatic artery and portal vein. Markedly dilated and congested central vein with hepatocytes showing pale wispy cytoplasm with reactive nuclear changes (figs. 1-2).

***Effect of Citrullus colocynthis fruit pulp extract on heart***

The sections from the heart revealed myocardium composed of cardiac muscle fibers, which were long cylindrical cells with 1-2 elongated and centrally located nuclei. Between the muscle fibers, delicate collagenous tissue was seen containing capillary size blood vessels. Surrounding fibroadipose tissue revealed dilated and congested blood vessels. Large areas of parenchymal haemorrhage seen along with few markedly dilated and congested blood vessels (figs. 3-4).

***Effect of Citrullus colocynthis fruit pulp extract on kidneys***

Sections of the kidneys revealed glomeruli, tubules and interstitium with few blood vessels. The glomerulus revealed a globular network of densely packed anastomosing capillaries invaginating into Bowman's capsule. Renal tubules were variably lined by tall cuboidal epithelium in the region of the proximal convoluted tubules to simple cuboidal epithelium of collecting tubules. The delicate interstitial supporting tissue contained little collagen with scattered fibroblast. Focal areas revealed mild mononuclear inflammatory infiltrate (figs. 5 & 6).

***Effect of Citrullus colocynthis fruit pulp extract on spleen***

The sections revealed splenic tissue composed of white pulp and red pulp. White pulp was made up of lymphoid cells forming lymphoid follicles. The red pulp was made up of venous sinuses along with splenic cords containing macrophages. Foci of extra medullary hematopoiesis were present along with scattered hemosiderophages (figs. 7-8).

Kidneys: Congestion / Inflammation
Heart: Foci of parenchymal haemorrhage with congestion.
Liver: Dilated and markedly congested central veins with reactive hepatocytes.
Spleen: Unremarkable.

**DISCUSSION**

The drug "Colocynth" which is the dried pulp of fruit is very extensively used in Unani system of Medicine as a diuretic, purgative, in ascites and jaundice. It is also prescribed in asthma, rheumatism, paralysis, gout epilepsy and sciatica (Usman Ghani *et al.*, 1997). The above-mentioned condition for which the drug is commonly used is not caused by any organism.

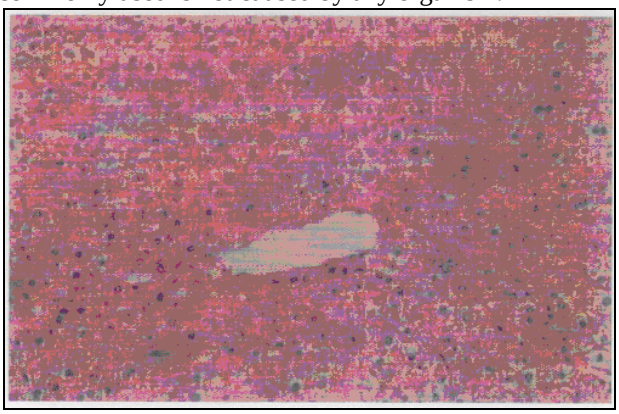

**Fig. 1**: T.S of liver of control mice (x40)

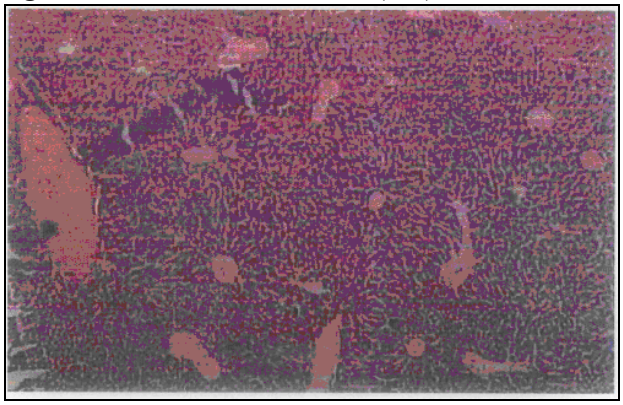

**Fig. 2**: T.S of liver of mice treated with fruit pulp of citrullus colocynthis (x40)

In the literature cited for antimicrobial activity of the plant, Marzouk *et al.* in 2009 indicated that aqueous and diluted acetone extracts of the root, stem, leaves and fruits showed activity against four bacterial species i.e.

*Staphylococcus aureus, Pseudomonas aeroginosa, Esherichia coli, Enterococcus faecalis* and various Candida species. In another report, Patel and Trivedi 1955 indicated (*Indian J. Pharm.,* 1995, 17, 228-reported in Wealth of India) that the extract of the dried pulp of fruit inhibited growth of *Salmonella paratyphi*. With the exception of these two reports, no other report on the antimicrobial activity of the plant is available. However present report in which the activity of Colocynth is determined against thirty bacterial and fungal cultures clearly indicated that methanolic extract of dried fruit pulp of plant has no activity against micro-organisms used in the study (tables 1-2).

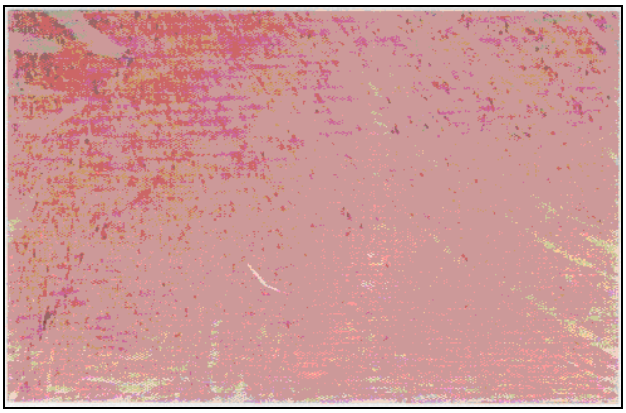

**Fig. 3**: T.S of heart of control mice (x40)

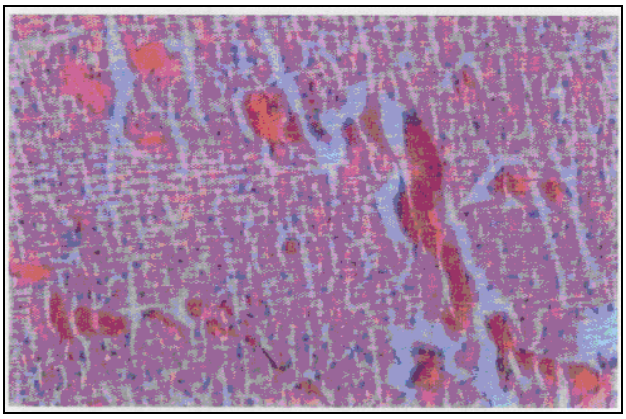

**Fig. 4**: T.S of heart of mice treated with fruit pulp of citrullus eolocynthis (x40)

***Acute toxicity studies***

Toxicity of *Citrullus colocynthis* fruit pulp is well documented in literature. Even the death of Roman Emperor Tiberius Claudius was attributed to poisoning due to eating of the fruits of *Citrullus colocynthis*. But the available reports in literature of the toxicity of plant are quite contradictory. A number of researcher conducting experiments on mice, rats and sheep confirmed the toxic effects of Citrullus (Stimpson 1926, Elawad *et al.*, 1984, Al-Qarawi and Adam 2003, Yahya *et al.*, 2010). But there are reports that indicated that Colocynth pulp is non-toxic and thus can be used without any significant risk (Lorenz *et al.*, 2005, Kalhoro *et al.*, 2002).

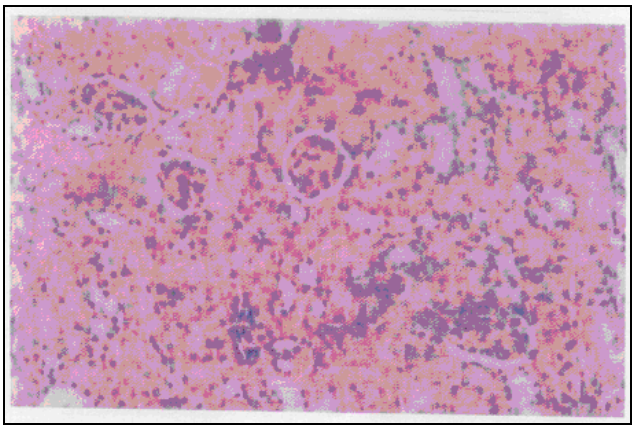

**Fig. 5**: T.S of kidneys of control mice (x40)

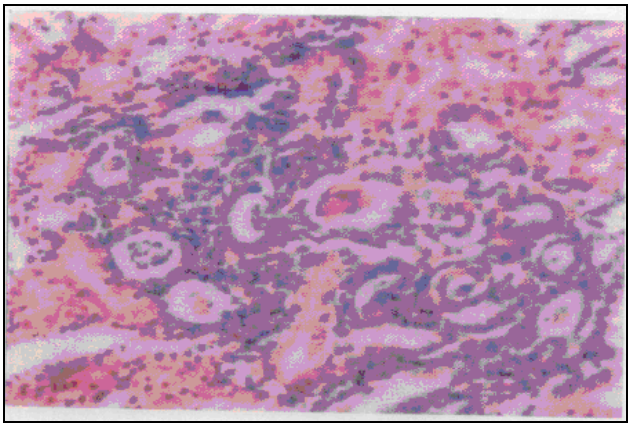

**Fig. 6**: T.S of kidneys of mice treted with fruit pulp of citrullus eolocynthis (x40)

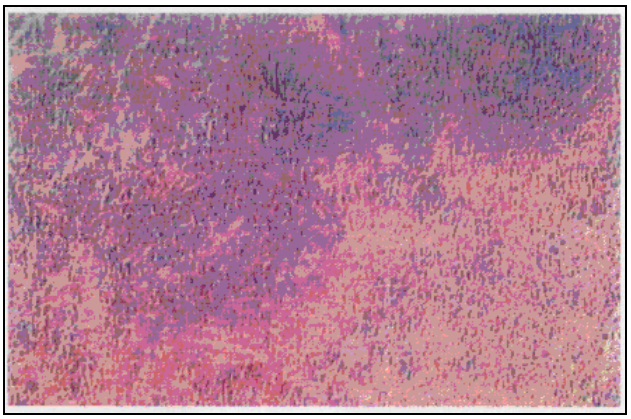

**Fig. 7**: T.S of spleen of control mice (x40)

Present study on acute toxicity in mice confirmed the highly toxic nature of the fruit pulp of *Citrullus colocynthis*. Fifty percent of mice that were administered a test dose of 1000mg/kg died within 24 hours, hence $LD_{50}$ was calculated as 1000mg/kg body weight. Within four days of experimentation, all treated mice died. In treated group the decrease in body weight was significant at $P<0.05$ (table 4). Histopathological studies indicated gross changes in the histology of Heart, Liver and kidneys. Sections of Liver of the treated animals showed that central veins were markedly dilated and congested

with hepatocyte. The cytoplasm appeared pale wispy with reactive nuclear changes (figs. 1-2).

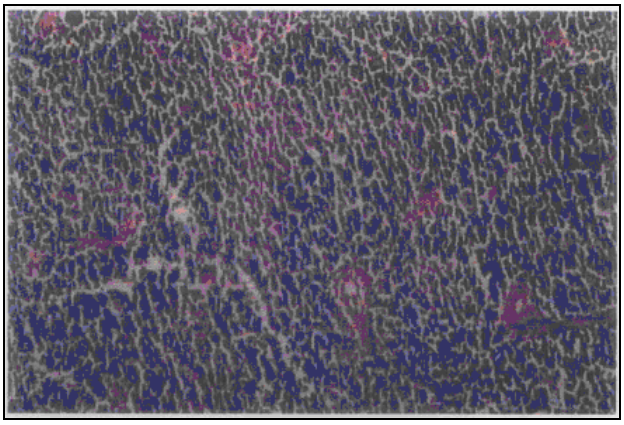

**Fig. 8**: T.S of spleen of mice treated with fruit pulp of citrullus eolocynthis (x40)

Section of Heart of treated mice exhibited major changes with large areas of parenchymal hemorrhage along with markedly dilated and congested blood vessels (figs. 3-4). Sections of treated kidneys revealed mild mononuclear inflammatory infiltrate in focal areas (figs. 5-6). Section of spleen did not exhibit any abnormality (fig 7-8).

## CONCLUSION

The antimicrobial profile of the methanolic extract of "Colocynth" (dried fruit pulp of fruits) was determined against thirty bacterial and five fungal species. The extract failed to show any activity against any organism used in the study. The drug is used in ayurvedic system of medicine for a number of ailments but none of these conditions is caused by any etiologic agent.

Reports on toxicity of the fruit pulp are overwhelming but quite contradictory (Al-Qarawi and Adam 2003, Yahya *et al.*, 2010, Lorenz *et al.*, 2005, Kalharo *et al.*, 2002). Present study confirms that "Colocynth" is highly toxic as indicated by acute toxicity studies in white mice. The $LD_{50}$ was calculated as 1000mg/kg body weight. All test animals expired within four days of experimentation. Histopathological studies on Heart, Kidneys and Liver also indicated Gross changes in histology of Heart, Liver and Kidneys. As the drug is used in a number of herbal drug formulations it is advisable that it should be used very cautiously or where ever possible its use should be avoided.

## REFERENCES


Al-Qrawi AA and Adam SEI (2003). Effect of combination of *Capsicum frutescence* and *Citrullus colocynthis* on growth, haematological and pathophysiological parameters of rats. *Phytother. Res.*, **17**(1): 92-95.

Bauer AW, Kirby WMM, Sherris JC and Turk M (1966). Antibiotic susceptibility testing by a standardized single disc method. *Am. J. Clin. Pathol.*, **45**: 493-496.

CLSI. Clinical Laboratory Standard Institute (2009). Performance standard for antimicrobial disc susceptibility test, Approved test 2009. 19th Edition, CLSI document 2009 M 100-519, Wanye PA.

Elawad AA, Abdel B, Ekhlas M, Mahmoud OM and Adam SEI (1984). The effects of *Citrullus colocynthis* on sheep. *Vet. Hum. Toxicol.*, **26**(6): 481-485.

Huseini HF, Darvishzadeh F, Heshmat R, Jafariazar Z, Raza M and Larijani B (2009). The clinical investigation of *Citrullus colocynthis* (L) fruit in treatment of type II diabetic patients: A randomized, double blind, placebo-controlled clinical trial. *Phytother. Res.*, **23**(8): 1186-1189.

Jafri SMH (1966). The flora of Karachi (Coastal West Pakistan). *The book Corp. Kar. Pak.*, p.23.

Kalhoro MA, Afza N, Saleem M and Malik A (2002). Pharmacochemical studies of the oil aerial parts pulp and peel of *Citrullus colocynthis*. *J. Chem. Soc. Pak.*, **24**(4): 274-276.

Kantouch A, Abdel Fattah S, Allam O and EL-Sayed HZ (1997). Some studies on mothproofing of wool Fabrics. *Egypt. J. Text. Polym. Sci. Tech.*, **1**(1): 85-98.

Khouri NA, EL-Akawi Z and Daradka H (2007). Effect of short-term treatment with *Citrullus colocynthis* L on Lipid profile and other biochemical parameters in albino rats. *Asian J. Chem.*, **19**(2): 1468-1472.

Kumar S, Kumar D, Manjusha S, Saroha K, Singh N and Vashishta B (2008). Antioxidant and free radical scarvenging potential of *Citrullus colocynthis* (L) schard methanolic extract. *Acta. Pharmaceut. (Zagreb-croatia).*, **58**(2): 215-220.

Lorenz PR, Lippmann F, Duerrling K, Solf M and Gelssler J (2005). Pharmaco-toxicological and clinical studies with colocynth pulp extracts. *Arzneimittel-Forsch.*, **55**(11): 621-633.

Marzouk B, Marzouk Z, Haloui E, Fenina N, Bouraoui A and Aouni M (2010). Analgesic and Anti-inflammatory activities of *Citrullus colocynthis* from southern Tunisia. *J. Ethano. Pharmacol.*, **128**(1): 15-19.

Masayuki Y, Morikawa T, Kabayashi H, Nakamura A, Matsuhira K, Nakamura S and Matsuda H (2007). Bioactive saponins and glycosides. Structure of new cucurbitane type triterpene glycosides and antiallergic constituents from *Citrullus colocynthis*. *Chem. Pharm. Bull.*, **55**(3): 428-434.

Patole SS and Mahajan RT (2007). Evaluation of some indigenous plants of khandesh region as mosquito larvicides. *Himalayan J. Environ. Zool.*, **21**(2): 257-264.

Ponder CF and Hooper H (1910). An introduction to Materia Media for India. Thacker Spink and Co. Calcutta, India, p.88.

Rizvi MA, Saeed A and Zubairy HN (2007). Medicinal plants. History, cultivation and uses 1st edition.

Hamdard Institute of Advanced Studies and Research, Hamdard University.

Rubeena S, Syed IA, Mohammad A, Zareen F, Sadia ZR, Muhammad A and Shaheen F (2003). Hypotensive Activity and Toxicology of constituents from *Bombax ceiba* Stem Bark. *Bio. Pharm. Bull*., **26**(1): 41-46.

Stimpson HS (1926). "Colocynthis". *J. Am. Inst. Homeopath.,* **19**: 501-510.

Tannin T, Grossman S, Bergman M and Dovart S (2007). Cucurbitacin glucosides and use thereof in treating cancer. *PCT Int. Appl.,* p.35.

Torkey HM, Abou-Yousuf HM, Adbel Azeiz Az and Hoda EAF (2009). Insecticidal effect of cucurbitacin E glycoside isolated from *Citrullus colocynthis*. *Aust. J. Basic & Appl. Sci.,* **3**(4): 4060-4066.

Usmanghani K, Saeed A and Alam MT (1997). "Indusyunic Medicine" Department of Pharmacognosy, Faculty of pharmacy, University of Karachi, Karachi Pakistan.

Yahya MA, Al-Farhan AH and Adam SE (2010). Preliminary toxicity study on the individual and combined effects of *Citrullus colocynthis* and *Nerium oleander* in rats. *Fitoterapia*., **71**(4): 385-391.