Current Research Journal of Biological Sciences 4(2): 153-158, 2012
ISSN: 2041-0778

Submitted: October 28, 2011 Accepted: December 09, 2011 Published: March 10, 2012

Phytochemical Screening of the Dried Leaf Extract of *Cnidoscolus aconitifolius* and Associated Changes in Liver Enzymes Induced by its Administration in Wistar Rats

[1]J.C. Mordi and [2]M.A. Akanji
[1]Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Delta StateUniversity, Abraka, Nigeria
[2]Department of Biochemistry, Faculty of Science University of Ilorin, Ilorin, Nigeria

Abstract: *Cnidoscolus aconitifolius* (Euphorbiaceae) is used traditionally for the treatment of many disease conditions in Nigeria. So far, no safety studies have been carried out with this plant. This study attempts to determine the phytochemical constituents of the plants leaf extract as well as examination of its effect on some liver enzymes. Results show no significant difference ($p>0.05$) between the control and the *Cnidoscolus aconitifolius* administered rats (at doses of 100, 300, 500 and 800mg/kg body weight for 6 weeks) with respect to the changes in body weight as well as in the liver enzymes analyzed in serum. The non-toxic effect of the aqueous and ethanolic plant extracts were also confirmed by histological studies. Phytochemical investigation of both the (dry) aqueous and ethanolic leaf extracts of *Cnidoscolus aconitifolius* shows the presence alkaloids, tannins, phlobatannin, saponin and phenols. Phlobatannin and saponin were found in appreciable amounts in the aqueous extract than the ethanolic extract. While cardiac glycosides were only positive and present in the aqueous extract only. The ethanolic extract was found to contain flavonoids, anthraquinones, steroids, terpenes. These were not found in the aqueous extract. From this study, it may be concluded that *Cnidoscolus aconitifolius* showed absence of cumulative toxicity as reflected by the non-significant changes in the parameters studied as well as from the results of the histological investigation.

Key words: *Cnidoscolus aconitifolius*, enzymes, flavonoid, liver, phenol, saponins

INTRODUCTION

There are many under exploited native leafy plants with potential as a traditional source of food (NAS, 1975). With current renewal of interest in household gardens, attention is being focused on promoting some of these plants as leafy green vegetables among population in the developing countries (FAO, 1987).

Nigeria, an important nation of biodiversity, is enriched with herbal resources. One of the plant genera widely used traditionally for the treatment of many diseases is *Cnidoscolus aconitifolius* (Family: Euphorbiaceae). Colloquially, the plant is referred to as Chaya (Donkoh *et al*., 1990). In the western part of Nigeria it is called different names such as Efo Iyana Ipaja and Efo Jerusalem, while in the Niger Delta of Nigeria; it has been nick-named "Hospital Too Far" because of its numerous traditional claims.

Cnidoscolus aconitifolius belongs to a group of arbre scent shrubs. It is an evergreen drought deciduous shrub up to 6 m in height with alternate pinnate lobed leaves, milky sap and small flowers on dichotomously branched cymes. The leaves are large, 32 cm long and 30 cm wide on chartacious and succulent petioles. The crop originated as a domesticated leafy green vegetable in the Maya region of Guatemala, Belize, and Southeast Mexico during pre-Cambrian period (Ross-Ibarra and Molina-cruz, 2002). It has continued to be used as food, medicine and ornamental plant till date. Due to its ease of cultivation, potential productivity and substantial nutritional value, the plant has spread all over the world including the tropics. Colloquially the plant is referred to as Chaya (Donkoh *et al*., 1990).

Although the plant is mainly cultivated as food, it has continued to be an important medicinal plant. Much of its recent spread into new areas may likely be attributed to its medicinal value. A wide variety of claims have been made for its medicinal efficacy as a treatment for numerous ailments ranging from its ability to strengthen fingernails and darken gray hair to cure for alcoholism, insomnia, gout, scorpion stings, brain and vision improvement (Jensen, 1997; Atuahene *et al*., 1999).

Correspondindg Author: J.C. Mordi, Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Delta StateUniversity, Abraka, Nigeria, Tell.: +2348038612368.

Usually herbal medicines are widely perceived by the public as being natural, healthful and free from side effects, but that is speculations. Plants contain hundreds of constituents and some of them may elicit toxic side effects. A number of studies exist reporting the toxic effect of herbal medicines (Shaw *et al*., 1997; Kaplowitz, 1997; Calixto, 2000).

This present study however attempts to determine the phytochemical constitutes of the aqueous extract to pinpoint its active ingredients, and also assess its effects by considering changes in some serum liver enzymes in Wistar rats.

MATERIALS AND METHODS

Study center and period: This research was conducted at the Department of Chemical Pathology, University of Benin Teaching Hospital (UBTH), Benin City, Nigeria, between October and December, 2010.

Plant material: Fresh leaf samples of *Cnidoscolus aconitifolius* were collected form an uncultivated farmland at the University of Benin, Edo State, Nigeria. Botanical identification was carried out at the herbarium (FHI) Forestry Research Institute of Nigeria, Ibadan Oyo State. The voucher number obtained was FHI.108788.

Preparation of the aqueous plant extract: The preparation of the aqueous plant extract was carried out as described by Yakubu *et al*. (2008). The plants leaf materials were sundried and macerated into uniform powder using Thomas Contact Mill (Pyeunicam, Cambridge, England). Approximately 218 g of the powder was extracted with 500 mL distilled water using soxhlet apparatus and concentrated by rotator evaporator 50ºC. This was transferred into a suitable container and lyophilized (freeze dried). The yield of the crude aqueous plant extract was 8.75 g. The dried extract was stored in desiccators until required for use. The extract was dissolved in appropriate volume of distilled water to the desired concentration.

Preparation of the ethanolic plant extract: The method used was as described by Oyagbemi and Odetola (2010). Air-dried powder (1 kg) of fresh matured *Cnidoscolus aconitifolius* were extracted by percolation at room temperature with 70% ethanol (EtoH). Leaf extract of *Cnidoscolus aconitifolius* was concentrated under reduced pressure (bath temperature 50ºC) and finally defatted with n-hexane. The extract was evaporated to dryness. The dried mass yielded 69.9 g.

Phytochemical screening: Phytochemical screening for major constituents was undertaken using standard qualitative procedures as previously described (Sofowora, 1993; Trease and Evans, 1989; Harborne, 1973). The test for tannins was carried out by dissolving 0.5 g of the dried powdered plant extract in 20 mL distilled water, then filtered and 0.1% ferric chloride reagents was added to the filtrate. For cardiac glycosides, killer kiliani test (Trease and Evans, 1989) was adopted (0.5 g of extract was added to 2 mL acetic anhydrate plus H_2SO_4). The test for alkaloids was carried out by adding 0.5 g aqueous extract in 5 mL 1% HCl, boiled and filtered. Then Mayer's reagent was added (Harborne, 1973; Trease and Evans, 1989). The extract was subjected to frothing test for the identification of saponin. Haemolysis test was further performed on the frothed extracts in water to remove false positive results (Sofowora, 1993). The extract was also tested for free glycoside bound anthraquinones (Wall *et al*., 1952; Sofowora, 1993). Five gramme of the extract was added to 10 mL benzene, filtered and ammonia solution added. The presence of flavonoids was determined using 1% aluminum chloride solution in methanol concentrated HCL, magnesium turnings and potassium hydroxide solution (Kapoor *et al*., 1969; Earnsworth *et al*., 1974).

Animals and experimental designs: Sixty male Wistar rats (180-250 g) used for this study were purchased from the Animal Unit, College of Medicine, Ambrose Ali University, Ekpoma, Edo State Nigeria. The sixty rats were divided into two sets of thirty rats each for the assessment of the effect of the aqueous and ethanolic extract. Each set was divided into five experimental groups of six rats per group. Members of each group were housed in a standard rat cage and allowed to acclimatize to laboratory condition for one week. All rats were then allowed free access to drinking water and rat feed (chow) - product of Edo Feeds and Flour Mill (BFFM), Ewu Edo State, Nigeria.

Treatment of animal for chronic study: Rats in group I (control) received distilled water for a period of six weeks. Group II, III, IV and V were administered with *Cnidoscolus aconitifolius* extract at the doses of 100, 300, 500 and 800 mg/kg body weight per day for six weeks by gavages, respectively. The animals were observed daily for any signs of morbidity and mortality and their body weights were measured every two weeks during the experimental period.

Collection of serum and liver samples for analysis: At the end of the experimental period (6 week) after an overnight fasting, all rats were sacrificed by decapitation. Blood was collected in tubes without anticoagulant to separate serum for various biochemical estimations. The samples were stored frozen until required for use. The liver were dissected out and cleared of blood. A portion of the live tissues were fixed in 10% formal saline for the histological studies.

Biochemical analysis: In the collected serum, the total protein and the activities of some liver enzymes such as Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP) and Acid phosphatase (ACP) were assayed using commercial kit (ALT, AST,ALP Randox Kit- Randox Laboratories Ltd, UK; ACP Roche Kits-Roche diagnostics, GmbH, Germany) in a Hitachi-912 auto-analyzer available in the Department of Chemical Pathology , University of Benin Teaching Hospital (UBTH), Benin City, Nigeria. Total protein was determined by following the method of Lowry *et al*. (1951) using Bovine Serum Albumin (BSA), at 660 nm.

Histological studies: The histopathological procedure adopted was as described by Ragavan and Krishna kumara (2006) and Dapar *et al*. (2007). A portion of all the liver specimens fixed in 10% formal saline were processed routinely overnight using histokinette. Then, they were embedded in paraffin wax. Three sections, each 4 μ in thickness were cut from each paraffin block. One section from each sample was stained with Haematoxylin and Eosin (H$E) stain by the standard method for light microscopic (histological) examination.

Statistical evaluation: The results of the biochemical analysis were expressed as Mean±SD for six animals in each group. The difference between the Control and *Cnidoscolus aconitifolius* extract administered groups were analysed by Student's t-test. p-value<0.05 was considered as significant.

RESULTS AND DISCUSSION

Herbal medicines are very popular in developing and underdeveloped countries. Therefore, a clear understanding of potential adverse effects of herbs used is necessary for implementing safety measures. In the case of *Cnidoscolus aconitifolius*, no systematic safety study had been done so far, hence a study on their toxicity is required. This present study tends to investigate the phytochemical content of the aqueous extract as well as the chronic toxicity of *Cnidoscolus aconitifolius.*

The phytochemical analysis carried out on the dry leaf aqueous extract and ethanolic extract showed the presence of some bioactive compounds in the plant. In the two forms of the extract, twelve bioactive constituents were tested for, out of which five were present in the two extracts (Table 1). An appreciable amount of alkaloids and tannins were obtained from the ethanolic extract than the aqueous extract. The presence of tannins suggests the ability of this plant to play a major role as antidiarrhoec and antihaemorrhagic agent (Asquith and Butler, 1986),

Table 1: Phytochemical constituents of *Cnidoscolus aconitifolius*

Phytochemicals	Water extract	Ethanolic extract
Alkaloids	+	+ + +
Tannin	+	+ + +
Phlobatannin	+ + +	+
Saponin	+ + +	+ +
Flavonoids	-	+
Anthraquinones	-	+ +
Steroids	-	+
Terpenes	-	+
Cardenolides	-	-
Phenol	+ +	+ + +
Chalcones	-	-
Cardiac glycosides	+	-

+ + +: appreciable amount; + +: moderate amount; +: minute amounts; -: not detected

while alkaloids has been implicated for its detoxifying and antihypertensive properties (Trease and Evans, 1989; Zee-cheng, 1997). A higher intensity of saponin was obtained from both aqueous and ethanolic extracst and this compound has since shown to have immense significance as antihypercholesterol, hypotensive and cardiac depressant properties (Trease and Evans, 1989; Price *et al*., 1987). Further more, a moderate amount as well as an appreciable quantity of phenol was observed in the aqueous and ethanolic extract respectively. This is an indication that the plant might play an important role as dietary antioxidants. Phenolic compounds prevent oxidative damage in living systems (Block, 1992; Hertog and Feskens, 1993). Flavonoids, anthraquinones, steroids and terpenes were negative for the aqueous extract but positive for the ethanolic extract. The possible reasons that can be adduced for this, is the mode of extraction. Chalcones and Cardenolides were absent in both extracts. Thus the absence may not be a minus for the medical efficacies of *Cnidoscolus aconitifolius.*

Histological features of the liver: The mean body weight gain of the aqueous and ethanolic extract of *Cnidoscolus aconitifolius* administered groups has shown no appreciable difference when compared with the control after 6weeks duration of the study (Table 2 and 3).

Liver is an organ involved in many metabolic functions and is prone to xenobiotic induced injuries because of their central role in xenobiotics metabolism (Sturgill and Lambert, 1997). Liver contains a host of enzymes such as AST, ALT, ACP and ALP. The activities of these enzymes are used to assess the functional status of the liver and as the biochemical markers of liver damage (Moss and Ralph Handerson, 1999). The results from this study (Table 4 and 5) showed that there were no increased activities of ALT, AST, ALP and ACP upon administration of both ethanolic and aqueous leaf extract of *Cnidoscolus aconitifolius.* The results of the biochemical estimation were also confirmed

Table 2: Mean body weight changes before and after the 6 weeks treatment with *Cnidoscolus aconitifolius* aqueous extract

	Body weight changes (g)		
Treatment	Initial	Final	Change (%)
Group I (control)	194.93±6.2	216.25±4.7	9.86*
Group II (100 mg/kg aqueous extract)	202.44±4.9	221.19±2.4	8.48*
Group III (300 mg/kg aqueous extract)	199.56±6.7	220.86±3.3	9.87*
Group IV (500 mg/kg aqueous extract)	218.17±8.0	237.42±6.5	8.11*
Group V (800 mg/kg aqueous extract)	196.60±7.4	219.74±4.3	10.01*

n: 6; values were expressed as Mean±SD; *: p>0.05, not significantly different from Control

Table 3: Mean body weight changes before and after the 6 weeks treatment with *Cnidoscolus aconitifolius* ethanolic extract

	Body weight changes (g)		
Treatment	Initial	Final	Change (%)
Group I (control)	244.53±7.0	276.60±3.3	11.50
Group II (100 mg/kg ethanolic extract)	252.13±4.3	281.99±2.9	10.59*
Group III (300 mg/kg ethanolic extract)	220.22±5.5	249.86±4.6	11.86*
Group IV (500 mg/kg ethanolic extract)	198.55±6.6	222.52±2.5	10.54*
Group V (800 mg/kg ethanolic extract)	222.71±4.9	250.74±4.3	11.18*

n: 6; values were expressed as Mean±SD; *: p>0.05, not significantly different from Control

Table 4: Levels of total protein and activities of serum liver enzymes for control and *C. aconitifolius* aqueous extract of wistar rats

Treatment	Total protein (mg/100 g)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	ACP (IU/L)
Group I	38.2±19.3	67.7±6.4	38.2±3.5	90.0±7.6	146.0±3.6
Group II	37.5±16.4*	69.8±5.7*	37.9±3.0*	88.3±11.5*	147.2±5.9*
Group III	35.4±10.1*	69.1±6.4*	37.3±2.6*	87.5±8.8*	146.6±7.1*
Group IV	35.0±13.3*	68.5±9.2*	36.7±3.5*	88.1±8.9*	144.8±4.7*
Group V	37.2±10.9*	66.3±6.5*	36.1±2.7*	85.9±7.2*	145.2±5.4*

n: 6; values were expressed as Mean ± SD; *: p>0.05, not significantly different from control

Table 5: Levels of total protein and activities of serum liver enzymes for control and *C. aconitifolius* ethanolic extract of wistar rats

Treatment	Total protein (mg/100 g)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	ACP (IU/L)
Group I	19.6±10.4	35.6±3.0	18.2±5.5	111.3±14.1	161.3±5.0
Group II	19.8±13.5*	36.8±5.1*	17.1±6.0*	113.7±13.5*	160.5±7.3*
Group III	19.4±12.1*	36.1±4.4*	17.7±6.6*	111.5±15.1*	162.3±9.0*
Group IV	20.0±11.3*	37.5±5.9*	16.3±3.9*	110.3±10.8*	160.7±8.7*
Group V	18.7±15.8*	36.3±5.5*	19.3±7.7*	110.9±19.2*	161.4±9.5*

n: 6; values were expressed as Mean±SD; *: p>0.05, not significantly different from control

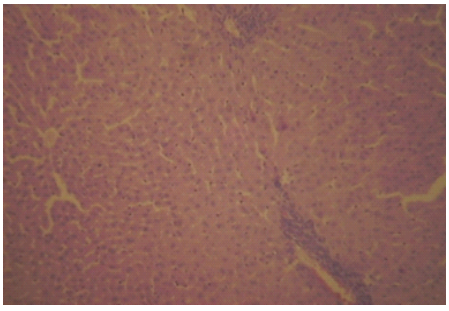

Plate 1: Control group showing normal cells. H & E x 100

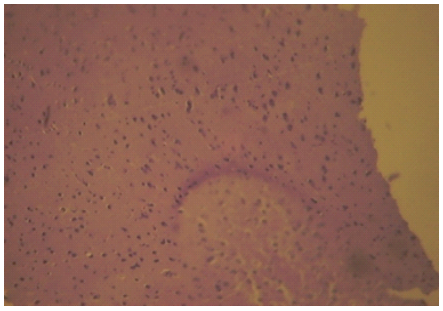

Plate 2: 800 mg aqueous extract per kg body weight for 6 weeks treated group: H & E x100 No degenerative change observed

by the histological studies (light microscopic study). For the histological examination of the liver specimen, there were no observable or degenerative changes observed between the control (Plate 1) and administration of the ethanolic (Plate 2) and the aqueous (Plate 3) extract of *Cnidoscolus aconitifolius* extract at a dose of 800 mg/kg. This indicates that the plant extract might be non-hepatotoxic in nature.

From this study, *Cnidoscolus aconitifolius* leaf extract administration at doses of 100, 300, 500 and 800 mg/kg body weight, may be safe. It is suggestive to say that *Cnidoscolus aconitifolius* showed absence of cumulative toxicity as reflected by the non-significant changes in the parameters studied as well as from the

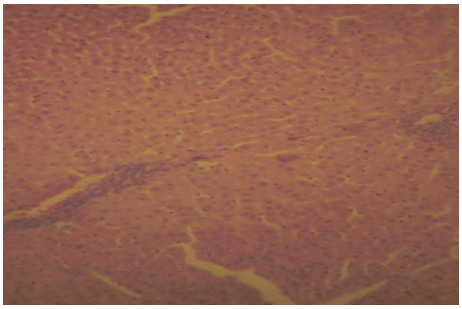

Plate 3: 800 mg ethanolic extract per kg body weight for 6 weeks treated group: H & E x100 No degenerative change observed

results of the histological investigation. It is thus recommended that further studies be carried out with this plant grown in Nigeria to assess it antidiabetic, anticancerous as well as antihepatotoxic properties.

CONCLUSION

The phytochemical analysis of the plant revealed the presence of alkaloids, tannins, phlobatannin, saponins and phenol among others both in the aqueous extract and to greater extent in the ethanol extract. These bioactive agents may contribute to the medicinal efficacy of the plant. Furthermore, the presence of phenols and flavonoids as detected from the ethanolic extract shows that the aqueous and ethanolic extract of *Cnidoscolus aconitifolius* might be able to manage oxidative stress. Finally, results obtained from the liver maker enzyme assay did not show any significant difference between the extract and the control and this was confirmed by the histopathological examination of the liver.

REFERENCES

Asquith, T.N. and L.G. Butler, 1986. Interaction of condensed Tannins with selected proteins. Phytochem., 25 (7): 1591-1593.

Atuahene, C.C., B. Poku-Prempeh and G. Twun, 1999. The nutritive values of chaya leaf meal (*Cnidoscolus aconitifolius*) Studies with broilers chickens. Anim. Feed Sci. Technol., 77: 163-172.

Block, G., 1992. The data support a role of antioxidants in reducing cancer risk. Nutr. Rev. 50: 207-213.

Calixto, J.B., 2000. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). Braz. J. Med. Biol. Res., 33:179-189.

Dapar, L.P.M., C.J. Aguiyi, N.N. Wannang, S.S. Gyang and M.N. Tanko, 2007. The histopathologic effects of *Securidaca longepedunculata* on heart, liver, kidney and lungs of rats. Afr. J. Biotechnol., 6(5): 591-595.

Donkoh, A., A.G. Kese and C.C. Atuahene,1990. Chemical composition of chaya leaf meal (*Cnidoscolus Aconitifolius*) and availability of its amino acids to chicks. Anim. Feed Sci. Technol., 30: 155-162.

Earnsworth, N.R., J.P. Berderka and M. Moses, 1974. Screening of medicinal plants. J. Pharm. Sci., 63: 457-459.

FAO, 1987. Promoting under-exploited food plants in Africa: A brief for policy markers. Food and Agriculture Organization, Food Policy Nutr. Div., Rome, pp: 23-25

Harborne, J.B., 1973. Photochemical Methods: A Guide to Modern Techniques of Plant Analysis.Chapman A. & Hall. London, pp: 279.

Hertog, M.G.L., and E.J.M. Feskens, 1993. Dietary antioxidant flavonoids and risk of coronary heart disease the Zutphen Elderly Study. Lancet, 342: 1007-1011.

Jensen, S.A., 1997. Chaya, the Mayan miracle plant. J.Food Sci., 51: 234-244.

Kaplowitz, N., 1997. Hepatotoxicity of herbal remedies. Insight into the intricacies of plant - animal warfare and death. Gastroenterology, 113: 1408-1412.

Kapoor, L.D., A. Singh, S.L. Kapoort and S.N. Strivastava, 1969. Survey of Indian Medicinal Plants for Saponins, Alkaloids and Flavonoids. Lloydia, 32: 297-302.

Lowry, O.H., N.J. Rosenbrough, A. Farr and R.J. Randall,1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193: 265-275.

Moss, D.W. and A. Ralph Handerson, 1999. Clinical Enzymology. In: Burtis, C.A. and Ashword, E.R., (Eds.), Tietz Text book of clinical chemistry, WB Saunders Company, 3rd edition, Philadelphia, pp: 651-683.

NAS. 1975. Chaya. In: Underexploited tropical plants with promising economic value. National Academy of Science, Washington, DC, pp: 45-48.

Oyagbemi, A.A. and A.A. Odetola, 2010. Hepatoprotective effects of ethanolic extract of *Cnidoscolus aconitifolius* on paracetamol-induced hepatic damage in rats. Pakistan J. Biol. Sci., 13(4): 164-169.

Price, K.R., I.T. Johnson and G.R. Fenwick, 1987. The Chemistry and biological significance of saponins in food and feeding stuffs. Crit. Rev. Food Sci. Nutr., 26: 22-48.

Ragavan, B. and S. Krishna kumara, 2006. Effect of *T. Arjuna* stem bark extract on histopathology of liver, kidney and pancrease of Alloxan-induced diabetec rats. Afri. J. Biomedical. Res, 9: 189-197.

Ross-Ibarra, J., and A. Molina-cruz, 2002. The ehnobotany of chaya (*Cnidoscolus aconitifolius*): A nutritious maya vegetable. J. Ethnobothany, 56: 350-364.

Shaw, D., C. Leon, S. Koleu and V. Murray, 1997. Traditional remedies and food supplements. A five-year toxicological study (1991-1995). Drug Safety,17: 342-356.

Sofowora, A. 1993. Medicinal Plants and Traditional Medicines in Africa. Chichester John Wiley & Sons New York, pp: 97-145.

Sturgill, M.G. and G.H. Lambert, 1997. Xenobiotics-induced hepatotoxicity; Mechanism of liver injury and method of monitoring hepatic function. Clin. Chem., 43: 1512-1526.

Trease, G.E. and W.C. Evans, 1989. Pharmacology 11th Edn., Bailliere Tindall Ltd., London, pp: 60-75.

Wall, M.E., C.R. Eddy, M.L. Mc Clenna and M.E. Klump. 1952. Detection and estimation of steroid and sapogenins in plant tissue. Anal. Chem. 24:1337-1342.

Yakubu, M.T., M.A. Akanji, A.T. Oladiji, A.O. Olatinwo, A.A. Adesokan, M. Oyenike, R.N. Yakubu, B.V. Owoyele, T.O. Sunmonu and S.M. Ajao, 2008. Effect of *Cnidoscolus aconitifolius* leaf extract on reproductive hormones of female rats. Iranian J. Rep. Med., 6: 149-155.

Zee-cheng, R.K., 1997. Anticancer research on Loranthaceae plants. Drugs Future, 22(5): 515-530.