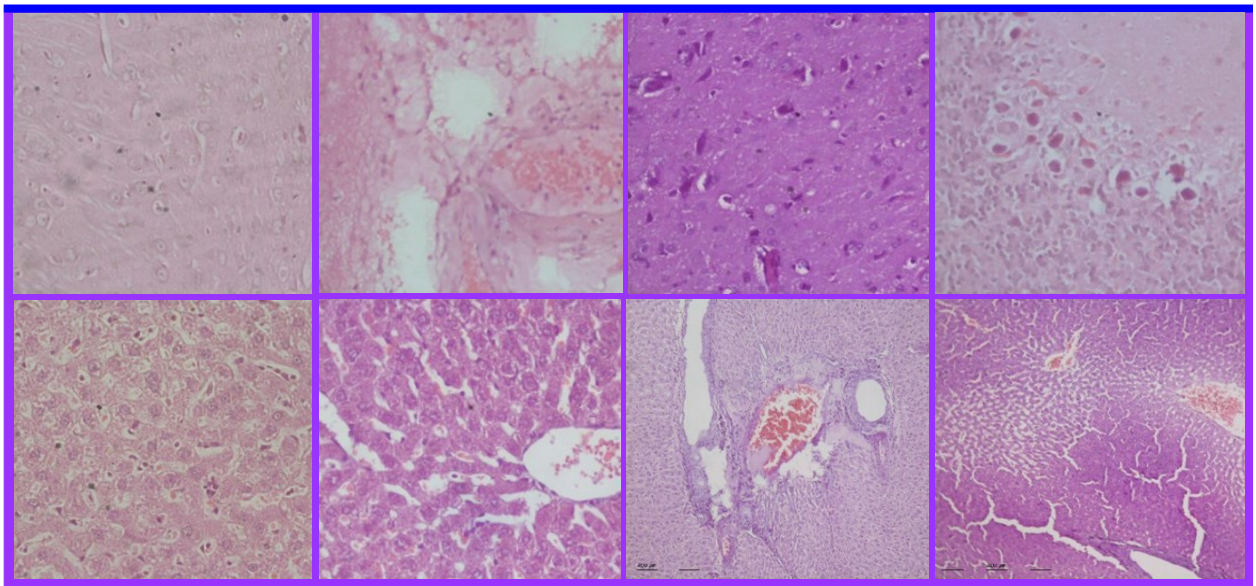

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Effect of *Phyllanthus amarus* in sodium arsenite-induced tissue damage

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Abstract

This study aims to evaluate the effect of alcoholic extract of *Phyllanthus amarus* leaves on arsenic-induced histological changes in Wistar rats. Rats were divided into 4 groups: Group I: normal control; Group II: rats received sodium arsenite (40 mg/kg); Group III: rats received sodium arsenite (40 mg/kg) + *P. amarus* extract (100 mg/kg); Group IV: rats received sodium arsenite (40 mg/kg) + extract (200 mg/kg). All groups were treated by oral gavage with sodium arsenite for 28 days and the animals were subsequently administered (oral) with 100 and 200 mg/kg extract, once daily for two weeks. Animals were sacrificed 24 hours after the last treatment and different organs were collected for histopathological analysis. Results revealed mild to severe type of necrosis and degenerative changes in brain, kidney, and liver of arsenic fed animals. In rats administered with extract showed significant improvements, and the normal histological feature of the cells was almost restored in rats. These suggest that *P. amarus* can be used to treat arsenic-induced multi-organ damages.

Introduction

Arsenic, the king of poisons, has influenced the human population more than any other element or toxic compound for thousands of years (Jolliffe, 2001). Arsenic exposure may cause severe health manifestations including cancers (Mabuchi et al. 1980), melanosis (hyperpigmentation or hypopigmentation), hyperkeratosis (hardened skin), black foot disease (peripheral vascular disorder), gangrene, diabetes mellitus, hypertension, ischemic heart disease, infant mortality, and reduction in birth weight, etc.

A lack of effective treatment options makes treating arsenic-mediated disorders a challenge.

The use of medicinal plants by man for the treatment of diseases has been in practice for a very long time. The presence of antioxidant molecules in plants is well documented and there is an ever-increasing demand for natural antioxidants over synthetic additives (Hashim et al., 2019). Several plants such as *Curcuma longa*, *Saffron crocus*, *Emblica officinalis* and *Silybum marianum* are suggested to reduce body arsenic load.

The leaf extract of *Phyllanthus amarus* has potent free radical scavenging activity in various *in vitro* and *in vivo* studies (Devi et al., 2017; Patel et al., 2011).

The present study aimed to see the effects of arsenic on the brain, liver, and kidney and to evaluate the effect of feeding ethanolic extract of *P. amarus* leaves on histopathological changes induced by sodium arsenite in the tissues of Wistar rats.

Materials and Methods

Plant materials

The *P. amarus* was collected from locality, Kannur, Kerala, India. The plant was identified and authenticated by the plant taxonomist at the Department of Botany, Payyanur College, Kannur, Kerala.

Chemicals

Sodium arsenite was purchased from the Durga laboratory (prepared in distilled water), and all the reagents were of analytical grade.

Preparation of P. amarus ethnolic leaf extract

P. amarus leaves were cleaned and washed repeatedly, air-dried at room temperature (~32°C) in a cool dry place, in the shade for 30 days, and finally ground to a coarse powder. Dried leaf powder (350 g) was taken and extracted with ethanol (90%) in a Soxhlet apparatus. The filtrates were then concentrated using vacuum rotary evaporator at a temperature of 60°C (yield 20% w/w), and a semi-solid gummy mass was obtained. The total weight of crude extract obtained was 23.6 g. It was stored in a sterile amber-colored storage bottle in the refrigerator until used for the experiment. The ethanol extract was be dissolved in distilled water before use (2 g in 50 mL distilled water).

Animal maintenance

Adult Wistar rats of either sex (n = 24), weighing 150-170 g were used. The experimental animals were supplied by the University and used for experiments after 1 week of acclimatization. The animals were maintained in an air-conditioned animal house with constant 12 hours light and 12 hours dark cycles. Animals were fed on standardized pellets for rodents and water *ad libitum*. The rats were separated randomly into 4 groups of 6 rats each. Group I: Control rats were administered with sterile distilled water as a vehicle for 42 days. The animals of Group II acted as a positive control, received

Box 1: Histopathology of tissue

Principle

Hematoxylin and eosin has special affinity for tissues. Hematoxylin gives bluish purple color to acidic parts of the tissues like nucleic acids, chromatin, ribosomes and RNA. Whereas eosin, the acidic part imparts pinkish red and orange to cytoplasm, muscle, collagen and red blood cells.

Requirements

Automatic tissue processor (Leica, Germany); Eosin; Ethanol; Hot air woven (Rotek, India); Isopropanol; Harris hematoxylin; Tissue floatation bath (Lab plus, India); 10% Neutral buffered formalin; Rotator microtome (Spencers, India); Xylol

Procedure

Step 1: Samples of brain, liver and kidney were collected and fixed in neutral buffered formalin.

Step 2: The amount of fixative was approximately 10 times to bulk of tissue fixed. Size of the samples was 1 cm^3.

Step 3: The fixed samples were taken out with a forceps from the bottle and placed separately in a perforated plastic holder which was covered by perforated still plates.

Step 4: Marking was done with dark pencil in perforated plastic holder.

Step 5: The samples were then arranged in a still rack and placed in an automatic tissue processor machine for dehydration, clearing and infiltration.

Step 6: Alcoholic series of higher concentrations, xylene and paraffin wax were used in the processor according to the time schedule as mentioned next:

Step 7: After tissue processing, the samples were embedded with melted wax, perforated plastic holder and still mold.

Step 8: Proper care was taken for the orientation of the samples in the still mold during the embedding period.

Table I: Time schedule in the automatic tissue processor

Process	Container	Chemicals used	Time (min)
Dehydration	1	50% methylated spirit	60
	2	60% methylated spirit	60
	3	70% methyalted spirit	60
	4	80% methyalted spirit	60
	5	90% methyalted spirit	60
	6	100% Alcohol	60
	7	100% Alcohol	60
Clearing	8	Xylene	20
	9	Xylene	20
Infiltration	10	Molten wax	45
	11	Molten wax	45

Step 9: The solid blocks were placed in a refrigerator (deep freeze) for half an hour and paraffin blocks were separated from the still molds.

Step 10: Sections were taken from the blocks at a thickness of 5 micrometers by using the microtome machine.

Step 11: The ribbon with section was placed on a water bath at a temperature of 40°C.

Step 12: Fine sections were selected and separated from the ribbon with the help of needle and forceps which were finally picked up over glass slides.

Step 13: The glass slides were then marked by a diamond marker and kept over hot plate (at 42°C for 8 hours to fix the sections properly with the slides.

Step 14: The sections were then stained with hematoxylin and eosin (H & E).

Step 15: The H and E-stained slides were examined using light microscope (Olympus, Japan) and the lesions were documented by using Zeiss micrphotocamera

References

Bancroft et al., 2019

sodium arsenite @ 40 mg/kg in drinking water once daily for 42 days. While animals of Groups III and IV received sodium arsenite @ 40 mg/kg in drinking water once daily for 28 days, which was replaced with *P. amarus* ethnolic leaf extract @ 100 and 200 mg/kg, respectively, once daily in drinking water for a period of next 14 days. The doses used in this study were selected based on preliminary experiments in this laboratory using acute toxicity study.

All the animals were sacrificed 24 hours after the last treatment following protocols and ethical procedures. Tissues were surgically excised and rinsed thoroughly with ice-cold saline. For microscopic evaluation tissues were fixed in 10% formalin solution for 48 hours. Then, two blocks from each tissue were processed by an automatic tissue processor, followed by embedding in paraffin. In this experiment, 5.0 µm tissue sections were stained with hematoxylin and eosin. These sections were examined under light microscopy (Olympus, Japan) and documented by Zeiss microphotocamera.

Results

Histopathological results revealed mild to severe type of necrosis and degenerative changes in the brain, kidney, and liver of arsenic-fed animals. Oral treatments with *P. amarus* showed significant improvements, and the normal histological feature of the cells was almost restored in rats.

Role of extract on different tissue architecture

Histopathological analyses of the brain, liver, and kidney were done to assess the role of *P. amarus*.

Effect of extract on the brain of the arsenic-induced neurotoxicity

Histopathology of the brain tissue of the study groups showed that Group I had a normal number and distribution of neurons and glial cells in the white matter and grey matter (Figure 1A). Figure 1B shows that Group II had varying types of lesion on different parts of the brain, such as: i) In the cerebrum grey

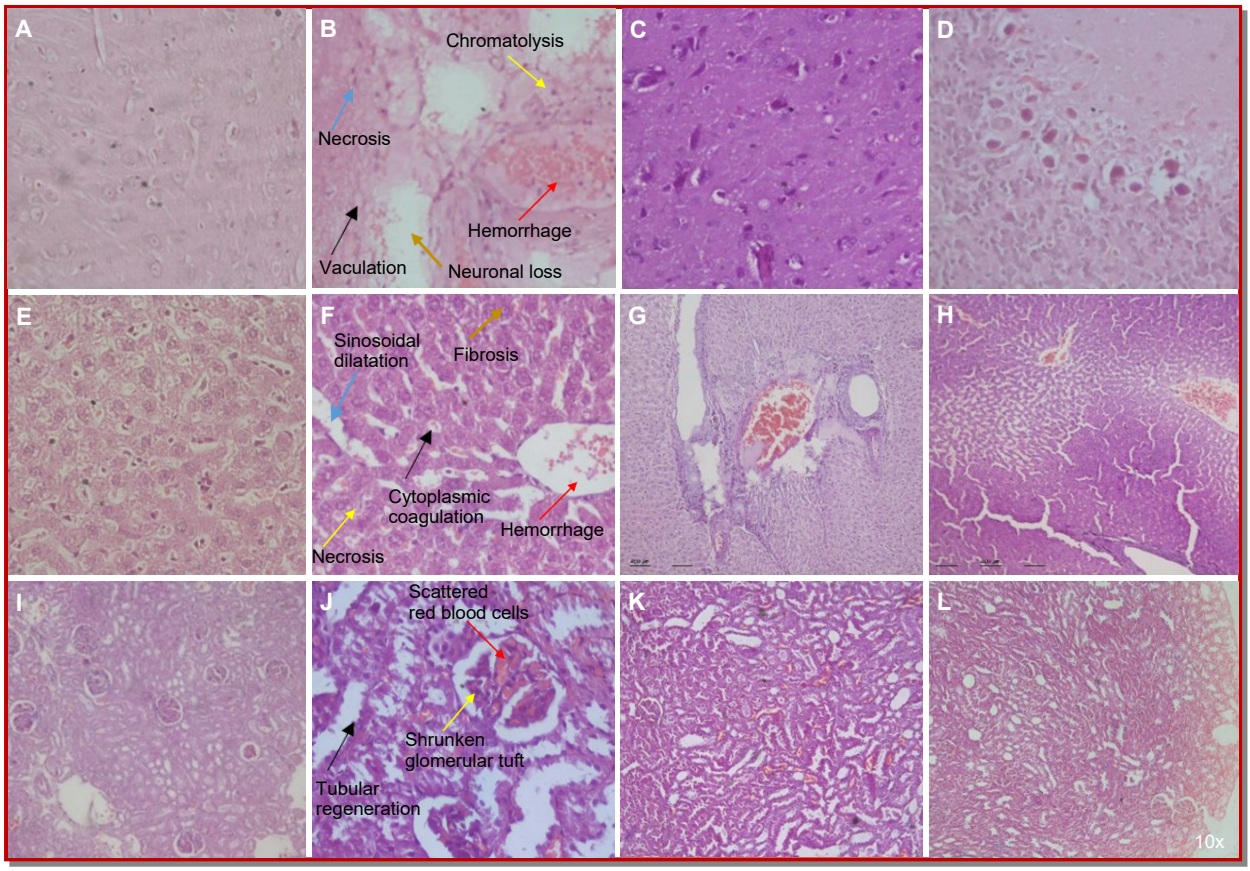

Figure 1: Histopathology of the brain (A-D), liver (E-H) and kidney (I-L) of sodium arsenite-induced hepatotoxicity

Longitudinal sections were taken and a Leica microtome was used to cut the paraffin block to a 4-5 µm thickness. After H&E staining the slides are viewed under the light microscope. Group description, A, E, I) Group I – normal control; B, F, J) Group II - Sodium arsenite control (40 mg/kg); C, G, K) Group III – Sodium arsenite (40 mg/kg) + *Phyllanthus amarus* (100 mg/kg; D, H, L) Group IV - Sodium arsenite (40 mg/kg) + *P. amarus* (200 mg/kg). Pathological findings and arrows indicating them in the picture have the same color and different colors indicate different pathological findings. Magnification of the lens is mentioned in white color. Camera used – Zeiss microphotocamera

matter had diffuse vacuolation (perineuronal, periglial, and perivascular vacuolation), focal aggregation of glial cells-gliosis, severe hemorrhage, extravasated erythrocytes in the neuropil surrounding the ventricles, engorged blood vessels of the choroid plexus in the ventricles, and infarction focal necrosis with perivascular vacuolation; ii) The neurons showed extensive chromatolysis, focal necrosis, and neuronal loss; iii) Cerebellum showed chromatolysis of Purkinje cells with eosinophilic cytoplasm, hemorrhage- extravasated erythrocytes in the molecular layer, and focal loss of granular neurons.

However, in *P. amarus*-treated groups, Group III and Group IV, the pathological findings were less severe than Group II (Figures 1C and D).

Curative effect of *P. amarus* on the liver of the arsenic-induced hepatotoxicity

Histopathology of the liver tissue of the study groups showed that Group I had radiating hepatic cords around the central vein with almost normal types of hepatic cells (Figure 1E). Figure 1F shows that Group II had variable degrees of degenerative to necrotic changes, such as increased cytoplasmic coagulation, and indistinct nuclear membrane. Extensive periportal necrosis is seen at frequent focal areas. The sinusoids were dilated mainly in centrilobular locations. Portal veins showed proteinaceous exudate in some of the sections. Mild sinusoidal dilatation and diffuse hemorrhage were seen occasionally. Centrilobular degeneration and initial bridging necrosis were evident. Mild fibrosis was also visible surrounding the portal triad. Focal areas of sinusoidal dilatation were also noticed adjacent to the hepatic blood vessels.

However, in *P. amarus*-treated groups, Group III and Group IV, the pathological findings were less severe than Group II (Figures 2C and D).

Curative effect of *P. amarus* on the kidney of the arsenic-induced nephrotoxicity

Histopathology of the kidney tissue of the study groups showed that group I had showed normal architecture of the cortex and medulla (Figure 3A). Figure 3B shows that group II had severe tubular degeneration and glomerular changes. The brush border was completely lost in the tubules all over the sections. The tubular epithelial cells are lost or desquamated into the lumen of the tubules. Some of the tubules were dilated with eosinophilic proteinaceous fluid. Focal intertubular hemorrhage was also seen in some of the sections. The glomerular tuft was shrunken and scattered red blood cells were seen in some of the glomerular space

However, in *P. amarus*-treated groups, Group III and Group IV, the pathological findings were less severe than Group II (Figures 3C and D).

Discussion

P. amarus was among these plants that are scientifically proved to possess neuro-, hepato- and nephroprotective effects (Devi et al., 2017; Patel et al., 2011; Mao et al., 2016). In the present study, even after stopping the arsenic on 28th day, the histological alterations did not come back to normal on 42nd day; there was a massive necrosis and other degenerative changes in the brain, liver and kidney. These finding are comparable with other studies on arsenic-induced tissue damages (Roy et al., 2006; Gaim et al., 2015; Ferzand et al., 2008; Centeno et al., 2002). Subsequent treatment with *P. amarus* reduced arsenic-induced tissue damages. The histopathological findings of the brain showed mild hemorrhage, congestion in ventricles, mild neuronal degeneration in the cerebrum, and mild pyknosis in the neurons were present after the 28-day dosages of arsenic. The liver showed less pronounced lesions. Only very little centrilobular degeneration and sinusoidal dilatation were noticed in the same experimental animals. Treatment with *P. amarus* increases antioxidant activities (SOD, CAT, GPx, vitamin E, and vitamin C) in the liver, reduces LPO, and protects against hepatitis-C induced free radical damage (Nikam et al., 2011). The kidney showed less severe lesions when compared to the arsenic control group. Those fed with *P. amarus* 100 mg/kg, and *P. amarus* 200 mg/kg plant extract showed sporadically dilated tubules and milder tubular degeneration in the histopathological investigation. *P. amarus* significantly attenuated acetaminophen- and gentamicin-induced acute renal injury. This might be due to the inherent antioxidant and free-radical-scavenging principle(s) in the extract (Adeneye et al., 2008). Aqueous extract of *P. niruri* has significant diuretic activity, and it has significantly increased the excretion of sodium, potassium, and chloride as compared to that of the control (Udupa et al., 2010). In the present study, the histological examination revealed minimal damage to the brain, liver, and kidney in animals that received *P. amarus* after the termination of arsenic in the current study, it is evident that *P. amarus* can cause regeneration of tissues caused by arsenic. The regenerative effect of *P. amarus* extract is possibly due to the presence of various phytoconstituents present in the ethanolic extract. There is evidence that *P. niruri* extracts possess antioxidative and anti-inflammatory activities (Lee et al., 2016; Karuna et al., 2009). The regenerative activity of these plants can be due to the phytochemical-mediated modulation of the growth factors or due to their antioxidant property. Nirphyllin, a second active component, belongs to the group of lignans that proved to have a regenerative effect in liver damage (Arasan et al., 2010; Divya et al., 2011). Apart from the above factors, neurotransmitters also play an important role in tissue regeneration, which is not yet established.

Conclusion

Subsequent treatment with *P. amarus*, the histological findings showed less damage to the architecture of the brain, liver, and kidney in animals that received *P. amarus* after the termination of arsenic. This regenerative effect of plant extracts may be due to the presence of various phytoconstituents in the ethanolic extracts.

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Ethical Issue

Experimental procedure was followed according to the guidelines of American's national Institute for the care and use of rats. The present study was conducted in 2018 after obtaining ethical clearance from the Institutional Animal Ethics Committee (YU-IAEC 3a/ 19.2016).

Conflict of Interest

Authors declare no conflict of interest

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References

Adeneye AA, Benebo AS. Protective effect of the aqueous leaf and seed extract of *Phyllanthus amarus* on gentamicin and acetaminophen-induced nephrotoxic rats. J Ethnopharmacol. 2008; 118: 318-23.

Arasan E, Vijayalakshmi M, Devalarao G. Anthelmintic screening of various crude extracts of *Phyllanthus simplex* Retz. Res **J** Pharm Biol Chem Sci. 2010; 1: 425-28.

Bancroft JD, Gamble M. Theory and practice of histopathological techniques. 8th ed. Philadelphia, Churchill Livingstone Elsevier, 2019, p 55.

Centeno JA, Mullick FG, Martinez L, Norbert P, Gibb H, Longfellow D. Pathology related to chronic arsenic exposure. Environ Health Perspect. 2002; 110 (Suppl 5): 883-86.

Duzguner V, Kaya S. Effect of zinc on the lipid peroxidation and the antioxidant defense systems of the alloxan-induced diabetic rabbits. Free Radical Biol Med. 2007; 42: 1481-86.

Devi S, Rashid R, Kumar M. Phytochemistry and pharmacological properties of *Phyllanthus amarus* Schum: A review. Pharma Innov J. 2017; 6: 169-72.

Divya K, Ankur R, Seema Ro, Khan MU. Pleiotropic multifaceted therapeutic potential of *Phyllanthus amarus*. Int J Pharm Biol Arch. 2011; 2: 610-14.

Flora SJS, Mehta A, Mital M. Heavy metal-induced oxidative stress and its possible reversal by chelation therapy. Ind J Med Res. 2008; 128: 221-24.

Flora SJS. Arsenic-induced oxidative stress and its reversibility. Free Rad Biol Med. 2011; 51: 257-81.

Ferzand R, Gadahi JA, Saleha S, Ali Q. Histological and haematological disturbance caused by arsenic toxicity in mice model. Pakistan J Biol Sci. 2008; 11: 1405-13.

Gaim K, Gebru G, Abba S. The effect of arsenic on liver tissue of experimental animals (fishes and mice): A review article. Int J Sci Res Publ. 2015; 5: 1-9.

Hashim A, Manikoth S, Pai VR. Role of medicinal herbs in the treatment of arsenic-induced toxicity. Int J Med Toxicol Legal Med. 2019; 22: 29-33.

Jolliffe D. A history of the use of arsenicals in man. J Royal Soc Med. 1993; 86: 287.

Kasote DM, Katyare SS, Hegde MV, Bae H. Significance of antioxidant potential of plants and its relevance to therapeutic applications. Int J Biol Sci. 2015; 11: 982.

Karuna R, Reddy SS, Baskar R, Saralakumari D. Antioxidant potential of aqueous extract of *Phyllanthus amarus* in rats. Indian J Pharmacol. 2009; 41: 64-67.

Lee NY, Khoo WK, Adnan MA, Mahalingam TP, Fernandez AR, Jeevaratnam K. The pharmacological potential of *Phyllanthus niruri*. J Pharm Pharmacol. 2016; 68: 953-69.

Mabuchi K, Lilienfeld AM, Snell LM. Cancer and occupational exposure to arsenic: A study of pesticide workers. Prev Med. 1980; 9: 51-77.

Mao X, Wu LF, Guo HL, Chen WJ, Cui YP, Qi Q, Li S, Liang WY, Yang GH, Shao YY, Zhu D. The genus Phyllanthus: An ethnopharmacological, phytochemical, and pharmacological review. Evid Based Complement Alternat Med. 2016; 2016.

Nikam PS, Nikam SV, Sontakke AV, Khanwelkar CC. Role of *Phyllanthus amarus* treatment in hepatitis-C. Biomed Res. 2011; 22: 319-22.

Patel JR, Tripathi P, Sharma V, Chauhan NS, Dixit VK. *Phyllanthus amarus*: Ethnomedicinal uses, phytochemistry, and pharmacology. J Ethnopharmacol. 2011; 138: 286-313.

Roy S, Bhattacharya S. Arsenic-induced histopathology and synthesis of stress proteins in liver and kidney of *Channa punctatus*. Ecotoxicol Environ Saf. 2006; 65: 218-29.

Udupa AL, Sanjeeva, Benegal A, Prusty V, Prabhath G, Kodancha P, et al. Diuretic activity of *Phyllanthus niruri* (Linn) in rats. J Health. 2010; 2: 511-12.

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