Drug Standardisation

Acorus calamus

Botanical name	:	*Acorus calamus* Linn.
Family	:	Araceae
Vernacular names		
Assamese	:	Bach
Bengali	:	Bach
English	:	Sweet Flag
Gujarati	:	Gandhilovaj, Ghoduvaj, Ghodvach, Godavaj, Vekhand
Hindi	:	Bach, Ghorbach, Gora-bach
Kannad	:	Baje, Narru Berua
Malayalam	:	Vashampa, Vayambu
Marathi	:	Vekhand, Vace
Punjabi	:	Bariboj, Wach, Ghodavaca, Varch
Sanskrit	:	Shadangrantha, Tikshnapatra, Ugra, Ugragandha, Vacha, Vijaya.
Tamil	:	Vashambu, Pillai maruntho
Telugu	:	Vasa
Urdu	:	Waja-e-Turki

Description

An erect, perennial, aromatic herb, with a creeping horizontal light brown or flesh coloured, branched rhizome. Leaves simple, alternate, distichous, very closely arranged, vertically oriented, linear to narrowly ensiform, 0.9-1.8 m long and 1.8-3.8 cm broad, glossy bright green with wavy margin in young, but entire when mature, apex acute, base broad ,amplexicaul. Inflorescence spadix, spathe 15-75 cm long, linear, foliaceous, narrowed into unequal shortly acuminate apex, pedicel (formed of connate pedicel and spathe) 3.2-3.8 cm broad, spadix 5-10 by 1.3-2 cm , cylindric, thick, greenish, slightly curved, obtuse and densely covered with mass of numerous pale green flowers. Flowers small, bisexual, fragrant; perianth 6; stamens 6, filaments flat, anthers yellow and reniform; carpels glabrous, stigma obtuse, ovary conical, 3-celled. Fruits fleshy berries, angular ,1-3 seeded . Seeds oblong with fleshy endosperm.

Part used : Rhizome

Distribution

A native of warm temperate regions of Europe, introduced into India, wild or cultivated, in marshy habitat of Naga hills, Manipur , Kashmir , Mysore, Sirmaur (Himachal Pradesh) and throughout India ascending upto 2200 m in Himalayas. It is regularly cultivated in Koratagere taluk in Karnataka.

Macroscopical

Rhizomes subcylindrical, vertically compressed, somewhat tortuous, rarely straight, sometimes branched, spongy within, 1-3 cm in thickness, light brown with pinkish tinge externally, buff coloured internally, upper surface marked with large triangular leaf scars that encircle the rhizome springing from each side alternately, longitudinally furrowed, lower surface with circular, pitted scars of rootlets arranged in irregular,

zigzag lines. Fracture short, granular and porous. Odour aromatic; taste pungent and bitter.

Microscopical

Transverse section exhibits a circular outline with broad cortex separated from large cylinder or stele by prominent endodermis. The diagnostic characteristics include: outermost single layered epidermis consisting of radially enlongated cells or cork of thin walled cells; hypodermis of 3-5 layers of compact parenchyma or somewhat collenchymatous cells; cortex composed of chains of thin walled parenchyma cells with large intercellular spaces and filled with starch grains, each chain contains one or more suberized sphaeroidal sacs with oil .Stele composed of parenchymatous ground tissue enclosing large air spaces similar to cortex. Vascular bundles of various sizes scattered in cortex, central ground tissue and form almost complete ring under the endodermis. Vascular bundles concentric, leptocentric, enclosed by complete or incomplete sclerenchymatous sheath, absent in bundles under the endodermis. Starch grains spherical, simple or compound with 2-6 components present in the cortex and ground tissue. Small prisms of calcium oxalate forming complete or imcomplete crystal sheath around the vascular bundle

Powdered drug

Buff coloured. Starch grains abundant small, spherical to ovoid, simple or compound with 2-6 components; fragments of parenchyma with cells composed of rounded, moderately thick- walled cells enclosing air spaces, oil cells round to oval slightly larger than the surrounding cells; small prisms of calcium-oxalate, vessels and fibres of fibrovascular bundles, fibres narrow lumened with tapering ends; vessels, reticulate or scalariform or rarely spiral and fragments of epidermis and hypodermis.

Organoleptic characters of powder

Colour	:	Light brown, buff
Touch	:	Smooth to slightly coarse.
Taste	:	Bitter.
Odour	:	Aromatic, pungent.

Substitutes and Adulterants

Alpinia officinarum Hance and *Alpnia galanga* Willd. are adulterants of Vacha and being sold in the name of *Bach* and *Ghorbach* in the local market apart from the genuine Calamus rhizomes.

Chemical constituents

Asarone, β-asarone, calamenol, calamene, calamenone, eugenol, methyl eugenol, α-pinene and camphene, various fatty acids, calamol, calamone acoradin, azulene, two selinane type sesquiterpenes acolamone and isoacolamone, sugars, glucosides-acorin, calameon, calamusenone, a flavone-luteolin-6, 8-C-diglucoside, new natural products acoramone, asaryladehyde, carcinogen, β-asarone and epoxyisoacoragermacrone are the main chemical constituents reported from this plant.

Physico-chemical standardization

i) Raw Drug Standardization

1. Moisture content (L.O.D. at 105 °C)	=	Not more than 13.0% w/w
2. Ash value (total)	=	Not more than 9.0 % w/w
3. Acid insoluble ash	=	Not more than 4.0 % w/w
4. Water soluble ash	=	Not more than 6 % w/w

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Acorus calamus Linn.

5. Extractive value in

a. Petroleum ether	=	1.7 – 5.0 % w/w
b. Chloroform	=	2 – 4.0 % w/w
c. Acetone	=	3.65 – 3.75 % w/w
d. Ethanol	=	10.8 – 11.0 % w/w
e. Methanol	=	9.7 – 10.0 % w/w
f. Distilled water	=	9.8 – 10.5 % w/w

ii) Qualitative test

Qualitative test indicates the presence of tannins, steroids, phenols and glycosides.

iii) Preparation of mother tincture

Mother tincture was prepared on the basis of Maximum Extractive value which was observed in 70% alcohol. Percolation method was used.

iv) Preparation

Drug strength 1/10

Acorus calamus in coarse powder Containing solids 100 gm and Moisture 13 %	=	113 g
Purified water	=	325 ml
Strong alcohol	=	700 ml

To make one thousand millilitres of the tincture.

v) Standardisation of mother tincture

1. Organoleptic properties

a. Appearance	=	Clear, non-viscous
b. Colour	=	Yellowish brown
c. Odour	=	Characteristics
2. Sediments	=	Absent
3. Wt. per ml/sp. gravity	=	0.94 – 0.96 g
4. Total solids	=	2.2 – 2.99 % w/v
5. Alcohol content	=	68-70% v/v
6. pH at R.T	=	5.5 – 6.5
7. λmax	=	252.2, 258.4, 286,300 nm.

vi) Identification (TLC Studies)

1. Carry out TLC of chloroform layer on silica gel 'G' plate using Toluene: Ethyl acetate (93:7 v/v) as mobile phase and vanillin sulphuric acid as spraying reagent. Five spots appear at Rf 0.96, 0.85, 0.55, 0.25 and 0.1.
2. Carry out TLC of chloroform layer on silica gel 'G' plate using chloroform: methanol (9:1 v/v) on mobile phase. Under U.V light, seven spots appear at Rf. 0.25, 0.36, 0.6, 0.67, 0.74, 0.8 and 0.85.
3. On spraying with antimony trichloride five spots appear at Rf. 0.35, 0.4, 0.67, 0.85, 0.88.
4. On spraying with vanillin sulphuric acid reagent three spots appear at Rf. 0.52, 0.75 and 0.92.
5. Carry out TLC of mother tincture using chloroform as solvent system. When sprayed with 10% Sulphuric acid reagent, eight spots appear at Rf 0.5, 0.14, 0.21, 0.28, 0.41, 0.47, 0.58 and 0.88.

Reported pharmacological and toxicological activities

Alcoholic extract of the plant causes sedative and analgesic effects, moderate depression in blood pressure and respiration; rhizome extract and essential oil effective against houseflies. Other pharmacological activities are hypothermic, hypotensive, spasmolytic, CNS depressant, anticonvulsant, carcinogenic, antimicrobial, anthelmintic, insecticidal, antibacterial and sedative tranquillizing.

Calamus oil and extract are prohibited from use in human food due to its toxicity. Acute toxicity studies in rats revealed that near toxic doses of

asarone caused ataxia, hypnosis and loss of righting reflex whereas b-asarone failed to induce any of the three responses. LD 50 of volatile oil of root rhizome for guinea pigs found to be 0.6275 ml/100 gm body weight.

Pharmacological screening

The Therapeutic efficacy of this drug, Acorus calamus Q for various disorders/ailments which has been recorded in Materia Medica after its human-trials, but in most of the cases the mechanism of action of this drug in terms of Pharmacological including Endocrinological aspects remain unexplained. Hence, different experimental models have been designed/framed on laboratory animals in a scientific way to obtain full pharmacological spectrum viz. cardiovascular, anti-pyretic, anti-inflammatory, analgesic and anti-convulsant activities of the drug and accordingly an attempt has been made to explore the therapeutic efficacy including toxicity effects of A. calamus Q. The results obtained on experimental animals are important pre-requisites for the Clinical trials on human-beings.

Toxicity study

The mother tincture of A. calamus upto dose level of 0.2ml./100 g. b.w. in mice and up to 0.5ml./ 100 g.b.w. in rats did not exert any overt symptom of toxicity during observation period. None of the animals died nor was there any change in their dietary intake and general behavioural pattern and found safe, whereas, on increasing the doses to 0.6 ml./ 100g.b.w. in mice and 0.8 ml./100 g.b.w. in rats in respective groups of animals, the drug becomes toxic and animal were fatal. Histopathological studies also confirm the degenerative/atrophic changes at cellular, nuclear and neuronal levels of different organs including endocrine glands viz. testis showing desquamated and vacuolated seminiferous tubules, thyroid glands exhibited degenerated/ atrophic follicles predominantly vacuolated and ovary indicated atretic and scanty vesicular follicles in test drug and vehicle fed control groups. (PL.2 a-f & Table-I&II, Histogram-1,2).

Cardiovascular activity

The test drug, A. calamus Q showed fall in blood pressure to 23.8±0.04 mm. Hg at a dose level of 50µl/100 g.b.w. intravenously in comparison to vehicle administered control having 40% alcohol which exhibited fall in blood pressure to 14.8 ± 0.03 mm. Hg. Hence, preliminary studies indicate that the drug, A. calamus Q possessed significant response in blood pressure at a dose level of 50 µl/100 g.b.w., in albino rat.

Anti-pyretic activity

The test drug, A. calamus Q at a dose level of 50 µl/100 g.b.w., in albino rats produced fall in rectal temperature to 0.6°F at the end of 2 hrs. whereas, aspirin treated group exhibited the maximum fall in rectal temperature to 4.5°F at the end of 2 hrs. adopted Loux et al (1972) methodology. Furthermore, no observable fall in rectal temperature was noticed in normal saline treated group under identical condition. The vehicle treated group produced no fall in rectal temperature at the end of 2 hrs.

Statistical analysis of existing data discerned that the drug A. calamus Q does not possess anti-pyretic potentiality at a dose level of 50µl/100 g.b.w. in albino rats. (Table-III , Histogram-3).

Anti-inflammatory activity

The initial diameter of the arthritis area was found to be 4.4 mm.± 0.2 in GP.I, 4.5 mm.±0.03 in GP.II and 4.3 mm.±0.25 in GP.III. using Shirota et al (1984) method. Intramuscular administration of test drug, A calamus Q daily for 7 days exhibited no marked anti-inflammatory activity at a dose level of 50µl/100 g.b.w. corresponding to control. Whereas, hydrocortisone treated group at a dose level of 1.0 mg./100 g.b.w. exhibited perceptible anti-inflammatory activity. Henceforth, it is inferred that the drug, A. calamus Q at a dose level of 50

Table-1. Toxicity studies (on mice)

GP. No.	No. of Animals of either sex B.W. range 30-40gms.	Control, Normal Control and Test Drug Administration	Doses Attempted / 100 g.b.w. I.P.	Percent (%) Safe/Mortality
I	10	**Control** 40% alcohol (v/v)	25µl, 50µl, 1.0ml,1.5ml, 0.2ml. 0.2ml, 0.25ml, 0.3ml, 0.4 ml. 0.45ml, 0.5ml, 0.6 ml.	100% Safe 20%, 30%, 40%, 50% Mortality 70%, 100% Mortality
II	10	**Normal Control** 0.9% saline (w/v)	25µl to 0.6 ml.	100% Safe
III	10	**Test Drug** Acorus calamus Q (w/v)	25µl, 50µl, 1.0ml,1.5ml, 0.2ml. 0.2ml, 0.25ml, 0.3ml, 0.4 ml. 0.45ml, 0.5ml, 0.6 ml.	100% Safe 20%, 30%, 40%, 50% Mortality 60%, 100% Mortality

Table-2. Toxicity studies (on Albino rats)

GP. No.	No. of Animals of either sex B.W. range 30-40gms.	Control, Normal Control and Test Drug Administration	Doses Attempted / 100 g.b.w. I.P.	Percent (%) Safe/Mortality
I	10	**Control** 40% alcohol (v/v)	25µl, 1.0ml, 2.0ml, 0.3ml, 0.4ml, 0.5 ml.0.55ml, 0.6ml, 0.7 ml.0.75ml, to 0.8ml	100% Safe 30%, 40%, 50% Mortality 100% Mortality
II	10	**Normal Control** 0.9% saline (w/v)	25µl to 0.8 ml.	100% Safe
III	10	**Test Drug** Acorus calamus Q (w/v)	25µl, 1.0ml, 2.0ml, 0.3ml, 0.4ml, 0.5 ml. 0.55ml, 0.6ml, 0.7 ml. 0.75 ml. to 0.8 ml.	100% Safe 20%, 30%, 40% 50% Mortality 70%, 100% Mortality

Table-3. Influence of Acorus calamus Q on the rectal temperature in the pyrexia induced male albino rats (Mean ± S.E. Value)

Sl. No.	Rectal temp. after Pyrexia & vehicle/ saline drug admn.	No.of Animals	Dose/ 100g.b.w. I.P.	GP-I Control 40% Alcohol (V/V)	GP-II Normal Control 0.9% Saline (W/V)	GP-III Drug Acorus Calamus Q (w/v) Admn.	GP-IV Aspirin (60mg./ 100mg. b.w.) (w/v)	Test of Signifi-cance P value
1.	Rectal Temp. After Pyrexia Mean ± S.E. value	10	50µl	102.7± 0.2	103.4± 0.3	103.4± 0.2	103.5± 0.4	
2.	Rectal Temp. After vehicle/saline/ Drug admn. °F	10	50µl	102.7± 0.4	103.5± 0.3	102.8± 0.3	99.0± 0.2	A2:B2:NS A2:C2:NS A2:D2:<0.05

NS – Not significant P<0.05 In-significant Response Versus Control.

µl/100 g.b.w. in albino rats does not possess anti-inflammatory effects.

Analgesic activity

Analgesia (Mean tail withdrawal time 6.7±0.05) seconds was detected at a dose level of 0.1 ml./ 100 g.b.w. followed Woolfe & MacDonald (1944) technique. Therefore, it is inferred that the test drug, A. calamus Q possesses perceptible Analgesic potentiality at a dose level of 0.1 ml./100 g.b.w. intraperitoneally in albino rats.

Anti-convulsant activity

The mother tincture of the test drug, A. calamus Q does not possess anti-convulsant activity at a dose level of 0.1/100 g.b.w. in albino rats against the electric shock (150 M.A. for 0.2 seconds) and metrazol induced seizures adopted Lippa et al (1979) methodology in albino rats when compared to control and normal control groups.

Conclusion

Pharmacological study of drug, A. calamus Q was undertaken on the experimental animals for acute and sub-acute toxicity activities and doses determined were selected for systemic effects and it was discernible that the drug A. calamus Q is safe, non-toxic and having no any overt symptoms and changes in behavioural pattern and dietary intake at a dose level of 0.2 ml./100 g.b.w. in mice and 0.5 ml./100 g.b.w. in rats. Histopathological studies of various organs including endocrine glands exhibited no degenerative changes/atrophy at cellular, nuclear and neuronal levels. The drug also possessed significant analgesic and hypotensive response upto dose of 50 µl/100 g.b.w. in rats. P value <0.001 was discernible, whereas, no observable anti-inflammatory, anti-convulsant and anti-pyretic activities, P value <0.05 were recorded.

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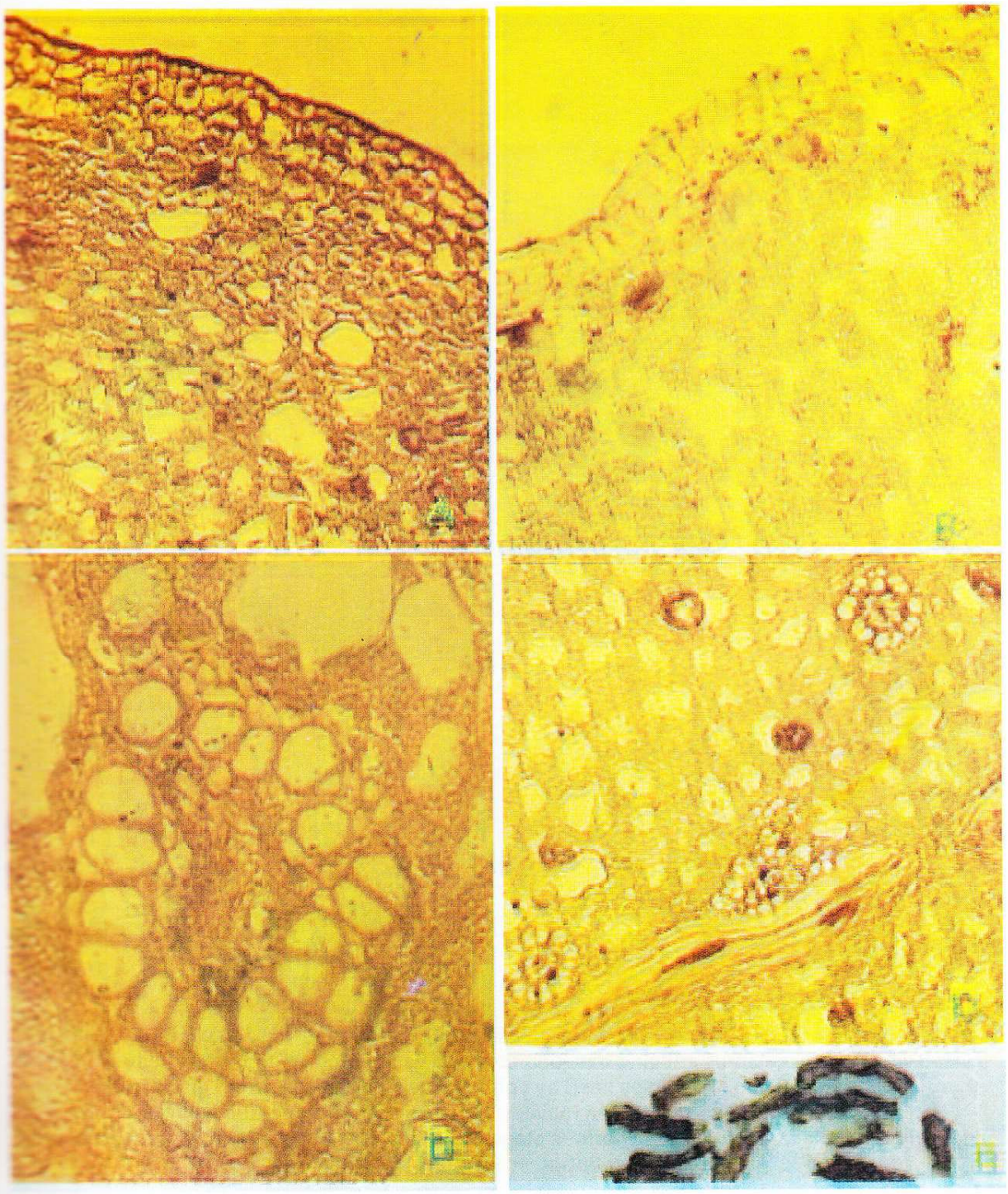


Plate 1. A). T.S. of root at periphery. B). T.S. of root cork tissue. C). T.S. of root at endodermis with vacular bundles. D). Vascular bundle (enlarged). E). Rhizomes.

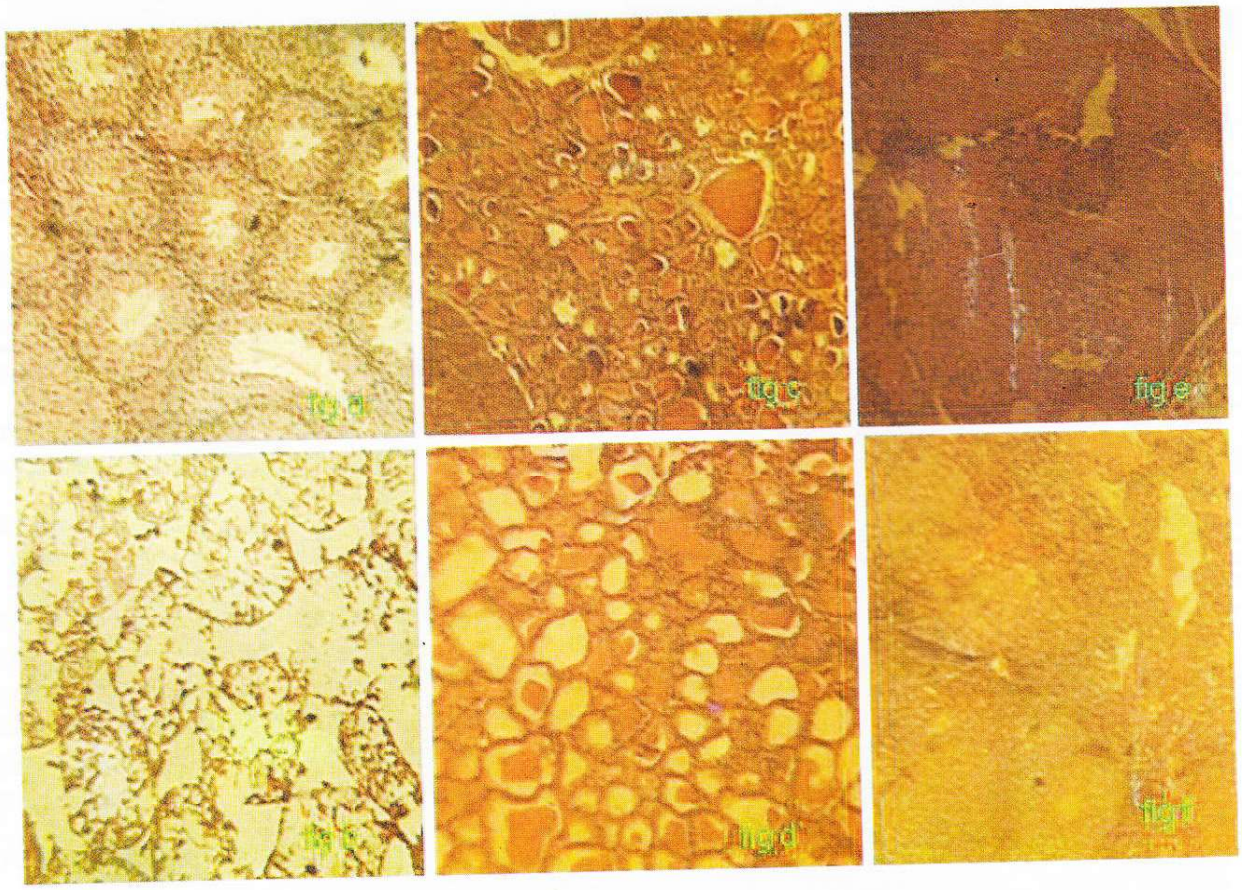
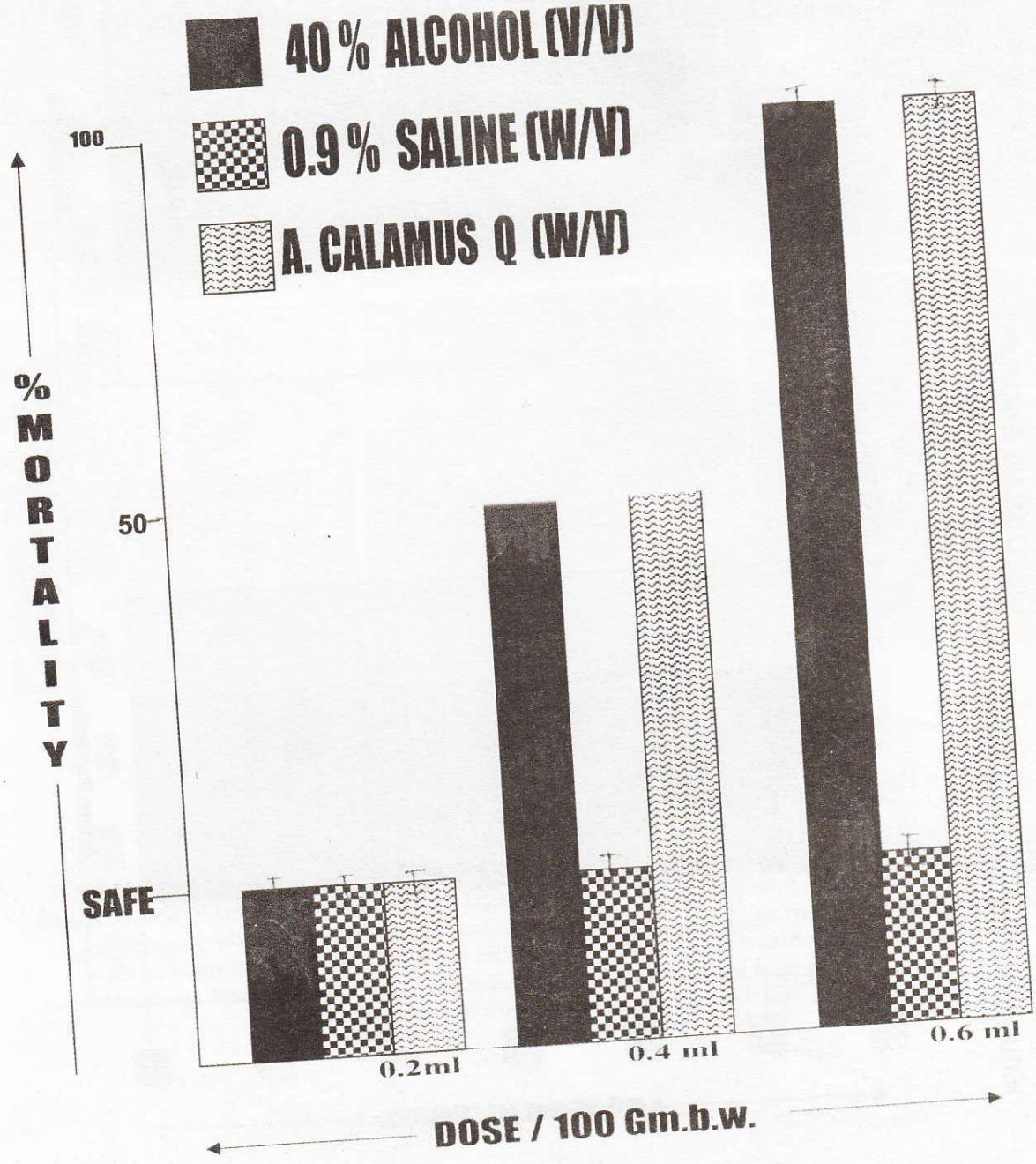


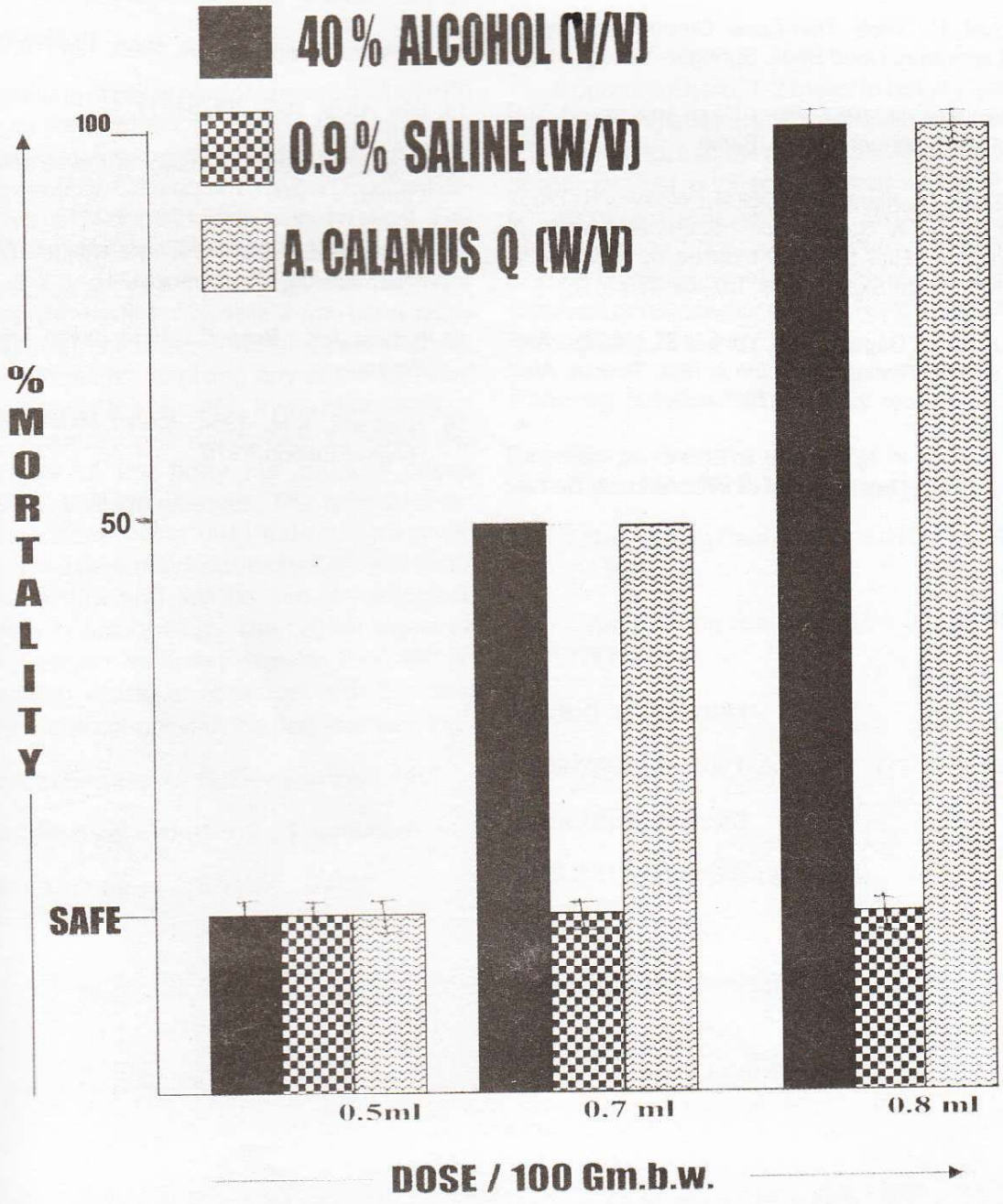
Plate 2. A). Mice testis treated with saline showing normal seminiferous tissue; B). Mice testis treated with Acorus calamus shoeing desquamated and vacuolated seminiferous tissue; C). Mice thyroid gland treated saline follicles lined by epithelial cells; D). Mice thyroid gland treated with Acorus calamus showing atrophied follicles; E). Mice ovary treated with saline showing development of ovarian follicles at various stages; F). Mice ovary treated with Acorus calamus showing atretic and scanty vesicular follicles.

HISTOGRAM SHOWING
Influence of Drug Acorus calamus Q on the Rectal Temp. in the Pyrexia Induced Male Albino Rats
(Mean + S/E. Value)

INFUENCE OF A. CALAMUS Q DOSE RESPONSE IN TERMS OF % MORTALITY ON MICE



INFUENCE OF A. CALAMUS Q DOSE RESPONSE IN TERMS OF % MORTALITY ON RATS



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