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EFFECT OF HYDROALCOHOLIC EXTRACT OF *SAMANEA SAMAN* BARKS IN VINCRISTINE-INDUCED PERIPHERAL NEUROPATHY IN RATS

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ABSTRACT

The aim of the present study is to evaluate the effect of hydro alcoholic extract of *Samaneasaman*in vincristine-induced peripheral neuropathy in Wister albino rats (200-220g). About 30 rats were procured and sorted into five groups (n=6). Group Iwas administered with routine food and normal saline, Group V served with pregabalin, Group II was treated with vincristine(50µg/kg,i.p).Two different dose (100/200mg/kg,orally) of HAESS were administered to group III and IV rats for 14 consecutive days. Vincristine was administered (50µg/kg, i.p) two hours after the administration of extracts to induce neuropathy. Paw cold allodynia, Haffner's tail clip and Von Frey hair test were assessed on alternative days. On 21st day the rats were sacrificed and sciatic nerve was isolated. Total proteins, calcium levels, reduced glutathione levels and lipid peroxidation were determined from the sciatic nerve homogenate. Kidney, liver and heart were isolated and were subjected to histopathological studies. Both dose of extracts showed significant decreased paw cold allodynia, hyperalgesia, and mechanical allodynia (Von Frey hair tests) in comparison with pregabalin. Both doses of extracts showed significant increased levels of proteins,reduced glutathione, lipid peroxidation levels and did not cause any significant change in calcium levelswhen compared with pregabalin. Hydro-alcoholic extract of *Samaneasaman*showed significant andmild protective effect against vincristine induced neuropathy in a dose–dependent manner when compared with pregabalin.

INTRODUCTION:

Peripheral neuropathic pain is frequently observed in patients with cancer, AIDS, long standing diabetes, lumbar disc syndrome, herpes infection, traumatic spinal cord injury, multiple sclerosis and stroke[1-3].

The physiological mechanisms underlying this kind of pain is poorly understood, although spontaneous activity in damaged sensory neurons due to ever expression or redistribution of voltage gated sodium channel is through to be a factor [4,5]. Its side-effects limit its use. It is essential and immediate to identify the alternative therapy to reduce its

usage. *Samaneasaman* (Jacq) Merrill is a large tropical tree growing as much as 60 m tall, with roughwrinkled bark and developing a symmetrical broad umbrella shaped crown about 80 m wide, making this species a beautiful choice for a shade tree. It is commonly known as rain tree belongs to family Mimosaceae. *Samaneasaman* (Jacq) Merrill is used for the treatment of rheumatism, constipation, leprosy, diabetes, microbial infection, inflammation and spasms[6].In folklore medicine of Venezuela, roots are used to treat skin diseases and bronchitis [7]. In India traditional practice of Chittakong hill region, barks are used to treat leucohorrea[8]. The phytochemical review of leaf, flower, stem bark, root and the whole plant revealed the presence of alkaloids, carbohydrates, sterols, terpenoids, tannins, proteins, flavonoids, gum and mucilage [9, 10].The pharmacological survey of bark revealed antioxidant effect, anti-fungal& anti-microbial[11-14], anti-ulcer, cytotoxic effect, analgesicand anti-diabetic activity[15-18.]

MATERIALS AND METHODS

Collection of Plant Material

The plant material was collected from medicinal garden, Madurai Medical College Campus, Madurai, during the month of August 2015. The plant was identified and authenticated by Dr. Stephen, M.Sc. Ph.D., Dept. of Botany, American College, Madurai.

Preparation of Plant Material

The barks were collected, dried in shade and coarsely powdered passed through sieve no 40 and stored in a closed container for further use. All reagents used of analytical grade were used.

Preparation of Hydro-alcoholic extracts (HAESS)

The coarsely powdered bark of *Samaneasaman*was defatted with petroleum ether (60-80˚c). further extracted with hydro alcohol (70% ethanol) by Soxhlet extraction until the complete extraction of the material. The extract was concentrated under reduced pressure to obtain solid residue.

Acute Toxicity

Acute toxicity studies were carried out using acute toxicity class method as per OECD guidelines 425[19]. The result showed no clinical sign and mortality of the animal and therefore an $LD_{50,} < 5000$mg/kg, body weight was assumed.

Experimental animals

Wister rats of either sex, weighing 200–220 g, were employed in the present study. The animals were given free access to food and water in laboratory conditions. The rats were exposed to 12-h light–dark cycles. The Institutional Animal Ethics Committee (5953/E1/5/2015) duly approved the experimental protocol.

Experimental protocol

Five groups, each comprising six Wister albino male rats were employed for the present study.

Group I (Normal group) Rats were subjected to normal diet were kept for 21 consecutive days.

Group II (Vincristine treated group) Rats were administered vincristine sulfate (50 µg/kg; i.p.) daily, for 10 consecutive days.

Group III (HAESS per se 200mg/kg) Rats were administered hydro alcoholic extract of SS (100 mg/kg; p.o.) daily, for 14 consecutive days.

Group IV (HAESS per se 400mg/kg) Rats were administered hydro alcoholic extract of SS (200 mg/kg; p.o.) daily, for 14 consecutive days.

Group V (Pregabalin per se 10mg/kg) Rats were administered with pregabalin (10 mg/kg; p.o.)daily, for 14 consecutive days. Vincristine was injected 2 hours after the administration of dose and pregabalin, for 14 consecutive days. The behavioral tests were performed at different time intervals, viz., 0, 1, 3, 6, 9, 12, 15, 18 and 21 days.

BEHAVIORAL EXAMINATION

Paw- Cold Allodynia Test (Allodynia)

Paw Cold-allodynia of the hind paw was assessed using acetone drop method as described method of Choi*et al.*, 1994.[20] with slight modification, for assessing the reactivity to non-noxious cold chemical stimuli. The rats were placed on the top of a wire mesh grid, allowing access to the hind paws. Acetone (0.1 ml) was sprayed on the plantar surface of left hind paw of rat. Cold chemical sensitive reaction with respect to either paw licking, shaking or rubbing the left hind paw was observed and recorded as paw lifting duration for 30 sec.

Haffner's Tail Clip Test (Hyperalgesia)

A metal artery clip was applied to the of the rat's tail to induce pain (Bianchi C, and Franceschimi J., 1954[21]. Sensitivity test was carried out and animals did not attempt to

dislodge the clip within 10s were discarded. The responsive mice were allotted to groups of six animals each. The tail clip was applied 60min after oral administration of extract (100mg/kg and 200mg/kg), Vincristine (50 µg/kg),wherecontrol remains unaltered.

Von FreyFrey Hair test (Mechanical allodynia)

Mechanic – tactile allodynia (non-noxious mechanical stimuli) was assessed as described by Chaplan et al., 1994[22]. Calibrated nylon filaments, in terms of different bending force, were applied to the mid plantar surface of left hind paw. The filaments were applied ten times, starting with the softest and containing in ascending order of stiffness. A brisk withdrawal of the hind limb was considered as positive response. The criterion for the threshold value, in gram, was equal to the filament evoking a withdrawal of the paw 5 times out of 10 trials i.e., 50% responses. All the animals were sacrificed according to CPCSEA guidelines at the end of the 21stdays.

Isolationof Sciatic Nerve: Sciatic nerve of the animals were isolated and a portion of the sciatic nerve was used to prepare a homogenate (10%) treated with 0.1M Tris HCl (pH 7.4) buffer to estimate biomarkers such as total protein and calcium per Lowry's et al., 1951[21]and Severinghaus and Ferrebee 1950[22].Oxidative stress markers such as reduced glutathione levels (GSH) and lipid peroxidationwere estimated as per Beutler et al., 1963 [23] and Okhawa et al., 1979[24] respectively.

Estimation of Total Protein: The standard curve was obtainedusing bovine serum albumin as a standard. The absorbance was determined spectrophotometrically at 750 nm.

Estimation of Total Calcium: The sciatic nerve homogenate was mixed with 1 mL of trichloroaceticacid (4 %) as in ice cold condition and centrifuged at 1500 g for 10 mins. The clear supernatant was used for the estimationof calcium by atomic emission spectroscopy at 556 nm.

Estimation of Reduced Glutathione levels

To 0.01 mL of this supernatant, 2mL of phosphate buffer (pH-8.4). 0.5 mL of 5'5 dithiobis (2- nitrobenzoic acid) and 0.4mL of distilled water were added. Mixture was vortexed and the absorbance was taken at 415 nm with 15 min. The concentration of reduced glutathione was expressed as µg/mg of protein.

Estimation of Lipid Peroxidation levels

To each test tube, 0.5 mL of supernatant,0.5 mL normal saline, 1 mL of 20 % tricholoroacetic acid (TCA) and 0.25 mL of TBA reagent (200 mg of thiobarbituric acid in 30 mL distilled water and 30 mL of acetic acid) were added. The test tubes were kept for boiling at 95˚ c for one hour. To each test tube, 3 mL of n-butanol was added and mixed well. These test tubes were centrifuged at 3000 rpm for 10 minutes. The separated butanol layer was collected and read in a spectrophotometer against blank at 535 nm. Concentration of thiobarbituric reactive substance was expressed in terms of malondialdehyde per mg of protein.

HISTOPATHOLOGICAL STUDIES

Distal portion of the sciatic nerve,heart,liver and kidney were isolated and were subjected to histopathological studies as per Yukari et al., 2004[25].All the results were expressed as mean ± standard error of means (SEM). The data from the behavioral results were statistically analyzed by two-way analysis of variance (ANOVA) test.

RESULTS

Effect of HAESS on Paw Cold Allodynia test

Vincristine treatment leads to the development of paw cold-allodynia indicated by decrease in the nociceptive threshold, when compared to normal control group of animals. Treatment of HAESS at 100 and 200 mg/kg, *p.o.*improved the nociceptive threshold in a dose dependent manner. Similar result was obtained with pregabalin treated animals. Normal control and per se animals did not show any effect on paw cold allodynia test (Figure 1).

Effect of HAEBS on Haffner's Tail Clip test: Administration of vincristine caused significant development noxious thermal hyperalgesia noted by decrease in hind paw withdrawal threshold after 3rd day of vincristine administration when compared to normal. Vincristine treated showed decrease in nociceptive threshold for hyperalgesia andwas improved by the administration of HAESS (100 and 200 mg/kg, *p.o.*) in a dose dependent

manner. Pregabalin treated animals also produced similar effects. Normal control and per se group of animals did not show any significant effect on paw hyperalgesic test (Figure 2).

Effect HAESS on Von Frey Hair test: Vincristine treatment leads to the development of mechanical-allodynia indicated by decrease in the nociceptive threshold, when compared to normal control group of animals. Treatment of HAESS at 100 and 200 mg/kg, *p.o.*improved the nociceptive threshold in a dose dependent manner. Similar result was obtained with pregabalin treated animals. Normal control and per se animals did not show any effect on mechanical allodynia test (Figure 3).

Effect of HAESS on vincristine-induced tissue on Total Proteins&, Calcium levels: Sciatic nerve showed decrease calcium levels were in the vincristinetreated group Administration of HAESS 100 and 200 mg/kg, b.w, *p.o*did not cause significant change in calcium levels and doubled the quantity of proteins in comparision with control amd standard.

Oxidative stress markers: Administration of lower dose (100mg/kg) of HAESS showed increased levels of reduced glutathione levels and lipid peroxidation levels when compared with control andpregabalin treated used as standard. Higher dose (200mg/kg) of HAESSshowed elevatedlipid peroxidase levels and decreased in reduced glutathione levels when compared with control and pregabalin treated used as standard**.**

HISTOPATHOLOGICAL STUDIES:

Effect of HAESS in vincristine induced histopathological change (sciatic nerve): Histopathological observation of the sciatic nerve showed nerve derangement, axonal degeneration and axonal swellingin vincristine treated group while the extract treated remains normal when compared standard.It is found that extract treated group showed protected the effects of vincristine induced histopathological alterations. pregabalin treated group and control group remains normal and the observations are shown in figure **4.**

Effect of HAESS in vincristine induced histopathological change in liver: Histopathological observation of the liver showed degeneration of central vein and vascular zadion, inflammation (over activation of kubffer cells), sinusoidal dilation, heptocytesin vincristine treated group while the extract treated remains normal when compared standard.. It is found that extract treated group showed unaltered the effects of vincristine induced histopathological alterations when compared with pregabalin group while control group remains normal and is shown in figure **5.**

Effect of HAESS in vincristine induced histopathological change in kidney

Histopathological observation of the kidney showed renal parenchyma, perivascular cells mononuclear, and infiltration only in vincristine treated group while the extract treated remains normal when compared standard. It is found that extract treated group showed reversed the effects of vincristine induced histopathological *alterations* when compared with pregabalin group while control group remains normal and is shown in figure 6**.**

Effect of HAESS on vincristine induced histopathological change in heart

Histopathological observation of the heart showed acute myocardial necrosis, increased mitotic rate and neutrophiliain vincristine treated group while the extract treated remains normal when compared standard. It is found that extract treated group showed protected the effects of vincristine induced histopathological *a*lteration swhen compared with pregabalin group while control group remains normal and is shown in figure **7**.

DISCUSSION

In the present study both dose *Samaneasaman* (HAESS) showed unaltered levels of total calcium supports the physiological fact of calcium that plays key role for neurotransmitter release, cell membrane excitability, activation of intracellular proteins and pain thresholds[28.] In this study lower dose of HAESS and vincristine treated group showed increased levels of reduced glutathione levels implicated the anti-oxidant potential effect of Samaneasaman. Both dose of HAESS showed increased levels of proteins and lipid peroxidation when compared with control and standard which indirectly the phyto constituents alters the basic metabolism to correct its illness at the tissue levels.

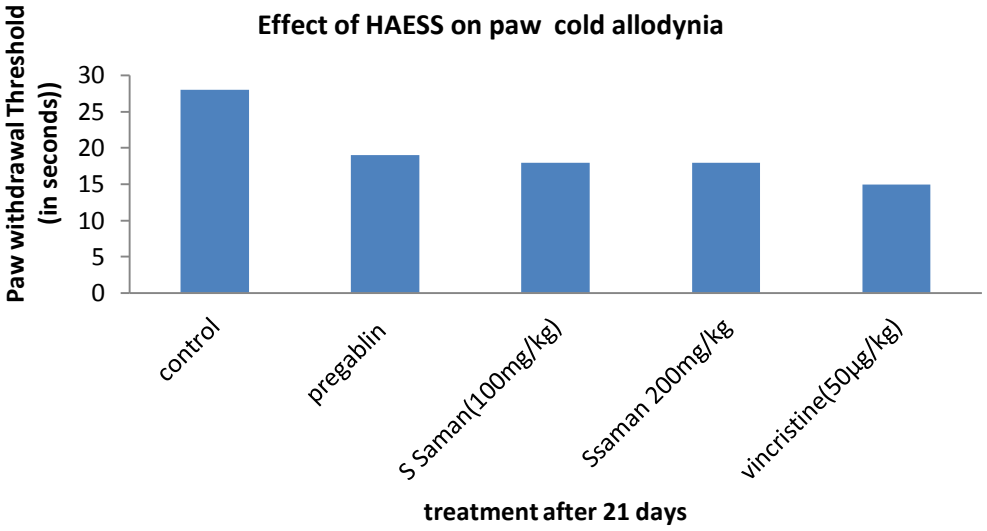


Fig-1: Effect of HAESS on Paw Cold Allodynia test

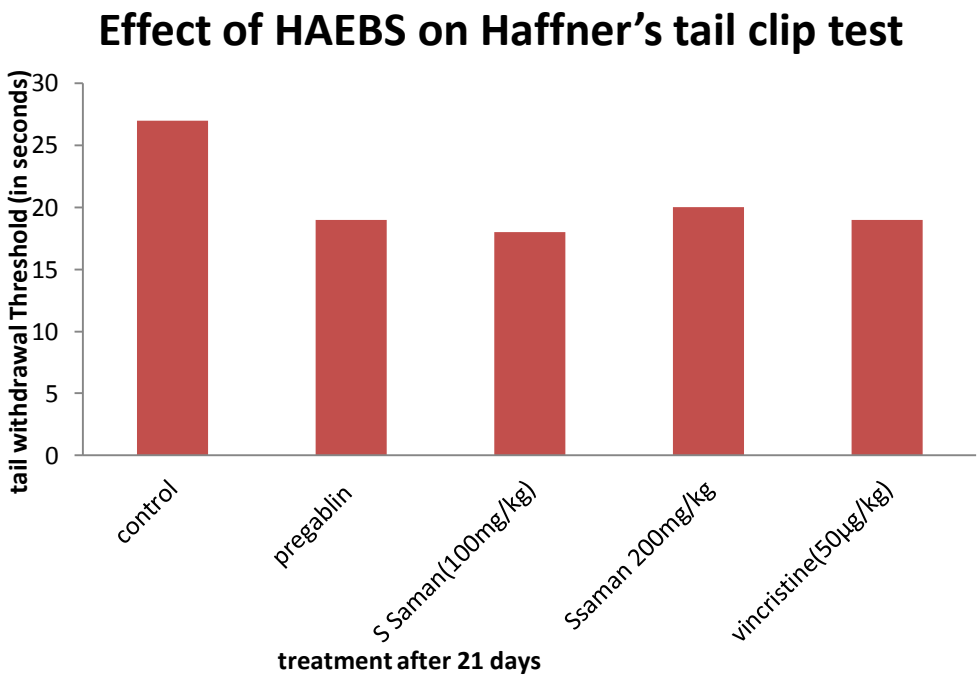


Fig-2: Effect of HAESS on Haffner's Tail Clip test

Table1 Effect of HAESS on Total Proteins and calcium levels

S.no	Groups	Total Protein levels (mg/g of tissue)	Total Calcium levels (mg/dl)
1	Control	5.86 ± 0.07	4.56 ± 0.08
2	Pregabalin-(10mg/kg)	5.40 ± 0.108	4.43 ± 0.12
3	Vincristine-(50µg/kg)	17.77 ± 0.08 [a]	4.6 ± 0.05 [a]
4	HAESS-(100mg/kg)	11.9 ± 0.03 [b]	4.66 ± 0.08 [b]
5	HAESS-(200mg/kg)	10.28 ± 0.03 [b]	4.633 ± 0.12 [b]

[a] Vincristine received group statistical significance ($p<0.05$) difference when compared to normal control group.

[b]hydroalcohol extract received group statistical significance ($p<0.05$) difference when compared to vincristine control group.

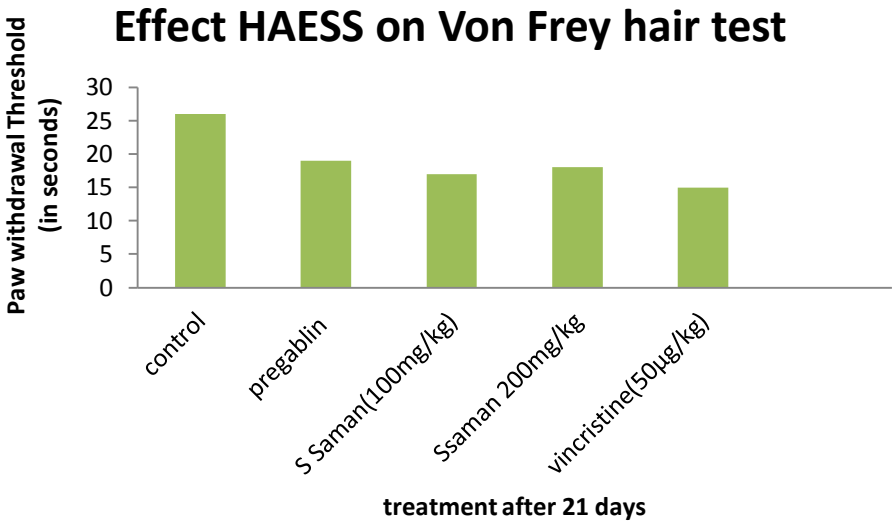


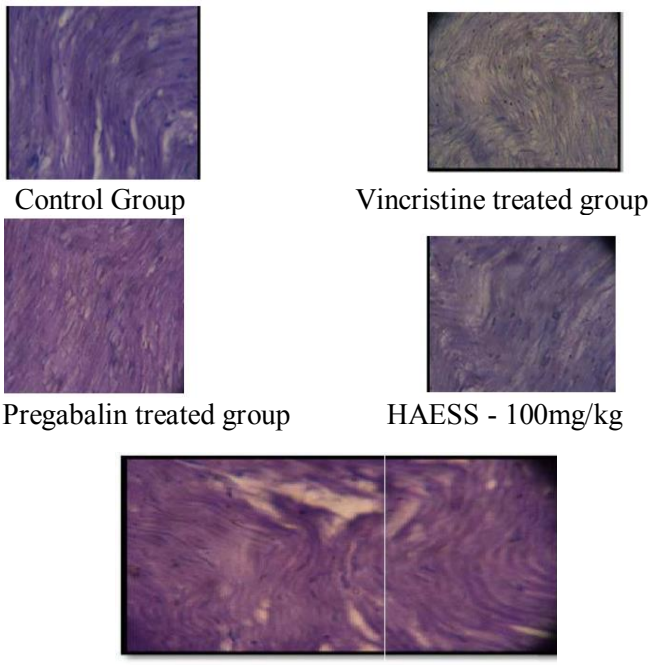
Fig-3Effect HAESS on Von Frey Hair test

Table 2: Effectof HAESS on Reduced glutathione and lipid peroxidation levels

S.no	Groups	Reduced glutathione (µg/mg of protein)	Lipid peroxidase (nmol/mg of protein)
1	Control	24.9 ± 0.23	27.04 ± 0.62
2	Pregabalin-(10mg/kg)	24.72 ± 0.24	33.64 ± 0.13
3	Vincristine-(50µg/kg)	36.44 ± 0.33[a]	43.08 ± 0.9[a]
4	HAESS-(100mg/kg)	40.47 ± 0.33[b]	40.88 ± 0.42[b]
5	HAESS-(200mg/kg)	23.08 ± 0.42[b]	44.65 ± 1.15 [b]

[a] Vincristine received group statistical significance (p<0.05) difference when compared to normal control group.

[b]hydroalcohol extract received group statistical significance (p<0.05) difference when compared to vincristine control group.



Control Group

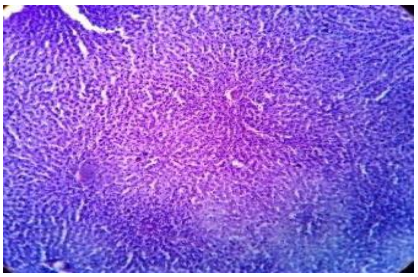
Vincristine treated group

Pregabalin treated group

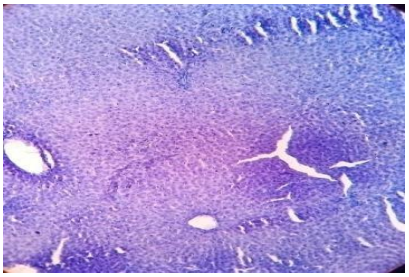
HAESS - 100mg/kg

HAESS - 200mg/kg

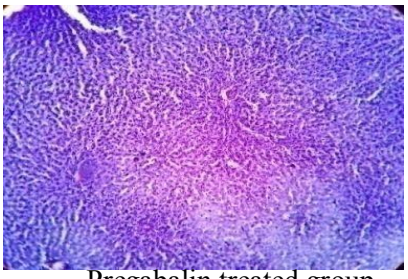
Fig: 4 Effect of HAESS in vincristine induced histopathological change (Sciatic nerve).



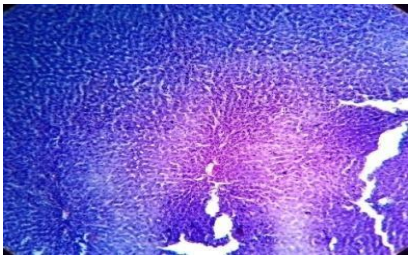
Control Group



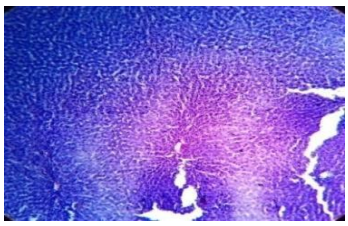
Vincristine treated group



Pregabalin treated group

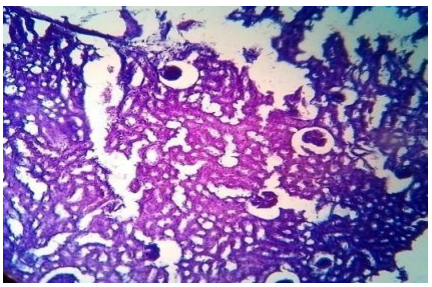


HAESS - 100mg/kg

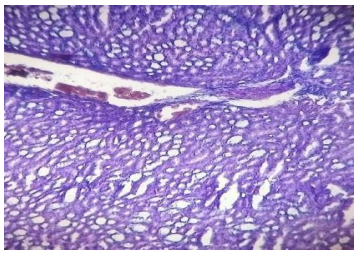


HAESS 200mg/kg

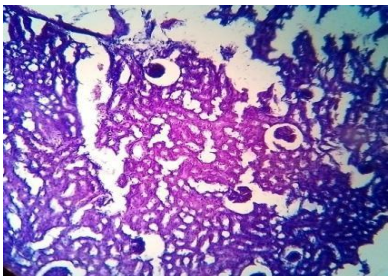
Fig: 5 Effect of HAESS in vincristine induced histopathological change (liver)



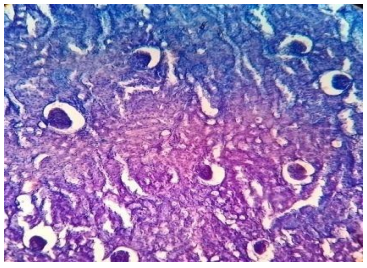
Control Group



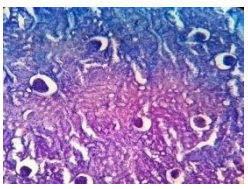
Vincristine treated group



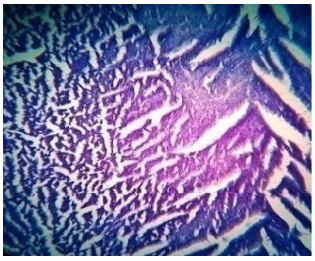
Pregabalin treated group



HAESS - 100mg/kg

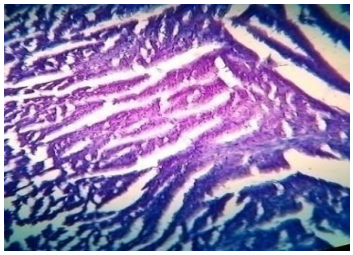


HAESS - 200mg/kg

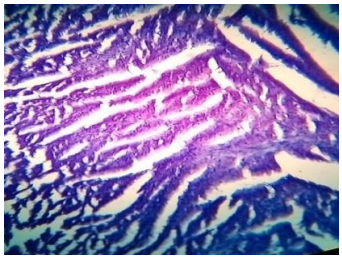


Control Group

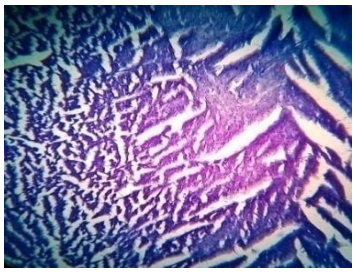
Vincristine treated group



Pregabalin treated group



HAESS - 100mg/kg



HAESS-200mg/kg

Fig: 7 Effect of HAESS in vincristine induced histopathological change (heart)

This study revealed that antioxidant is also one among the mechanism for the neuro protective effect of this extract. It indicates that investigations on herbal plants point out those natural products could be exploited to discover some novel neuropathic pain agent. Therefore, scope of the new herbal medicine to combat the management of neuropathic pain syndromes is expected. Traditional herbal plants have been used throughout the world for the treatment of neuropathic pain. This review will underline the importance of phyto chemicals on neuro protective function and, in particular their mechanism of action and therapeutic potential [29].

CONCLUSION

This study revealed that *in vivo* anti oxidant effect of hydro alocoholic extract and calcium channel modulating property of the plant. Hence extensive studies required to explore the exact mechanism responsible for the management of neuropathy.

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