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EFFECT OF HYDROALCOHOLIC EXTRACT OF *BOUGAINVILLEA SPECTABILIS* LEAVES IN VINCRISTINE-INDUCED PERIPHERAL NEUROPATHY IN RATS

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Key Words

Bougainvillea spectabilis, Pregabalin, Neuropathy

ABSTRACT

The aim of the present study is to evaluate the effect of hydro alcoholic extract of *Samanea saman* in vincristine-induced peripheral neuropathy in Wister albino rats (200-220g). About 30 rats were procured and sorted into five groups (n=6). Group I was administered with routine food and normal saline, Group V served with pregabalin, Group II was treated with vincristine (50µg/kg,i.p). 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 levels when compared with pregabalin. Hydro-alcoholic extract of *Bougainvillea spectabilis* showed significant and mild protective effect against vincristine induced neuropathy in a dose–dependent manner when compared with pregabalin.

INTRODUCTION:

As per The International Association for the Study of Pain (IASP) define neuropathic pain (NP) as initiated or caused by a most important lesion or dysfunction of the nervous system"[1]. Neuropathic pain is not based on a single pathophysiological procedure resulting from peripheral or central nerve injury either due to acute events (like spinal cord injury) or systemic disease (like diabetes, alcoholism, kidney or thyroid diseases, viral infection and cancer, Poor nutrition or vitamin deficiency, AIDS etc.) and characterized by pathological symptoms, such as hyperalgesia and allodynia to mechanical and thermal (heat or cold)

stimuli, as well as spontaneous pain[2]. Its side-effects limit its use. It is essential and immediate to identify the alternative therapy to reduce its usage.

Bougainvillea spectabilis Willd is aclimbing shrub with spikes grow as an evergreen ornamental woody plant in gardens and inhabiting in warmer climate. It is commonly known as *Bougainvillea* belongs to family Nyctaginaceae [3]. The ethno medicinal information reveals that leaves are used to treat diabetes by tribals of Chittoor and Nagaland, India. Leaf, flower, and stem preparation are used by the tribals of Mandasaur, India to treat stomach acidity and leuchorrea. Fresh leaves are used by tribals of Sriharikata, Andra Pradesh to treat wound. In folklore practice of Assam and Maharashtra, leaves are used to treat fungal infection, jaundice and dysentery[3-8] . The phytochemical review of leaf, flower and root revealed the presence of alakoids, terpenoids, tannins, saponins, flavonoids and glycosides. Pinitol, a mehyl ester of chiroinsitol was isolated [9-l1].The plant exhibited anti-hyperglycemic activity, anti- ulcer, anti-bacterial, thromobolytic and antioxidant effect[12-18].

MATERIALS AND METHODS

Collection of Plant Material

The plant material was collected from Poovanthi village, 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 leaves 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 extract

The shade dried and coarsely powdered leaves of *Bougainvillea spectabilis* was defatted with petroleum ether (60-80˚c). The defatted marc was 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. The coarsely powdered bark of *Bougainvillea spectabilis* 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 $LD_{50,} <$ 5000mg/kg, body weight was assumed.

Experimental animals

Wister rats of either sex (200–220 g), were employed in the present study. The animals were given free access to food and water in laboratory cnditions.. 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 (HAEBS per se 200mg/kg) Rats were administered hydro alcoholic extract of SS (100 mg/kg; p.o.) daily, for 14 consecutive days.

Group IV (HAEBS 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), whereas control remains unaltered.

Von Frey Frey 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 and sciatic nerve was isolated and subjected to histopathological changes.

Isolation of 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 [23] and Severinghaus and Ferrebee 1950[24]. Oxidative stress markers such as reduced glutathione levels (GSH) and lipid peroxidation were estimated as per Beutler et al., 1963[25] and Okhawa et al., 1979[26]. Respectively.

Estimation of Total protein

The standard curve was obtained using 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 trichloro acetic acid (4 %) as in ice cold condition and centrifuged at 1500 g for 10 mins. The clear supernatant was used for the estimation of 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 [27] . 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 HAEBS 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 and was shown in 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 control group. Vincristine induced, decrease in nociceptive threshold for hyperalgesia was improved by the administration of HAEBS (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 and was shown in figure 2.

Effect HAEBS 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 HAEBS 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 and was shown in figure 3.

Histopathological Studies: Distal portion of the sciatic nerve were subject to histopathological studies (Yukari et al., 2004) [27] Sciatic nerve was subjected to bio-marker and oxidative stress marker estimation.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.

Effect of HAEBS on Total proteins and calcium levels & Oxidative stress markers: Administration of lower dose (100mg/kg) and higher dose (200mg/kg) of HAEBS showed increased levels of reduced glutathione levels and lipid peroxidation levels when compared with control and pregabalin treated used as standard. Administration of lower dose (100mg/kg)and higher dose (200mg/kg) of HAEBS showed significant increase in total protein levels but caused significant changes in total calcium levels and its results were displayed in table 1.

Effect of HAEBS in vincristine induced histopathological change in sciatic nerve

Histopathological observation of the sciatic nerve showed nerve derangement, axonal degeneration and axonal swelling in vincristine treated group while the extract treated remains normal when compared standard.

Figure1: Effect of HAEBS on Paw Cold Allodynia test

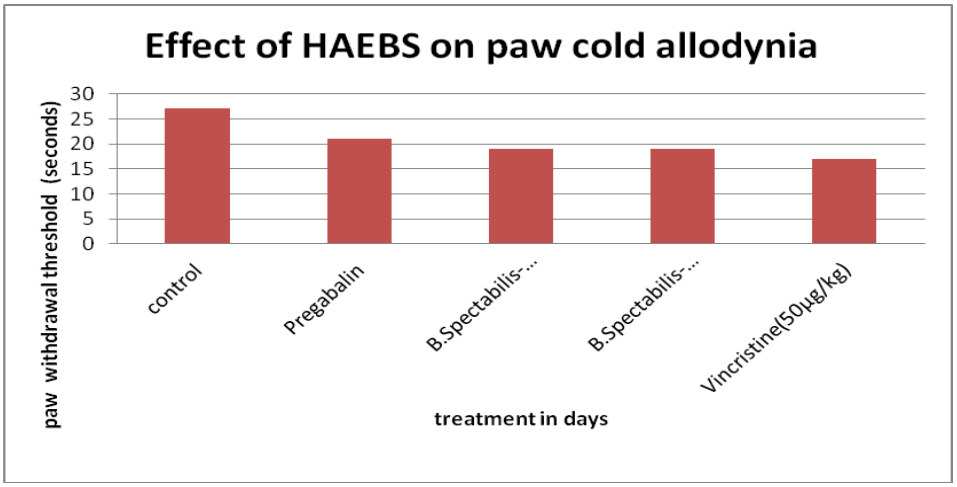
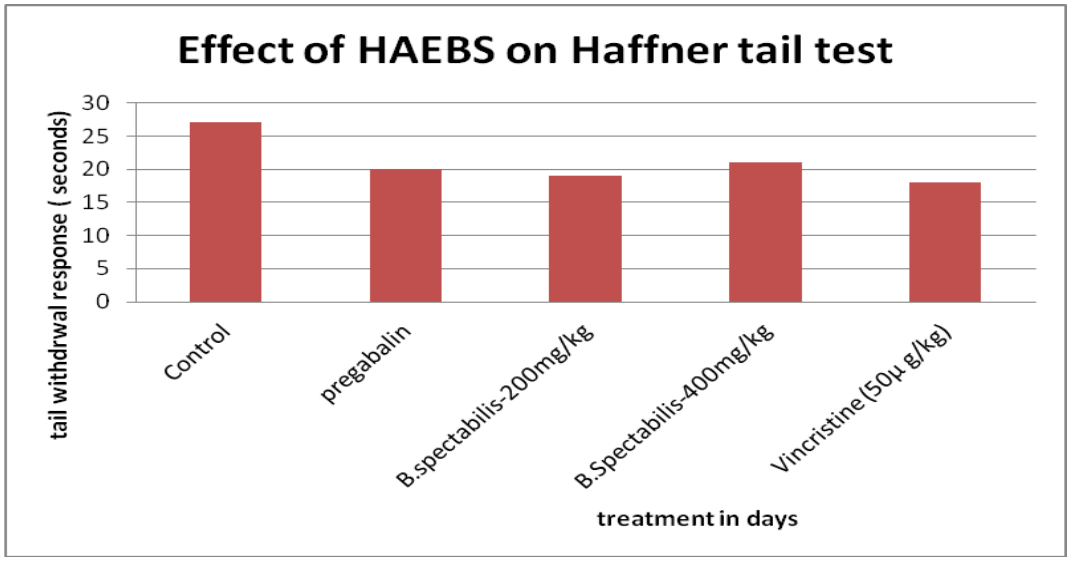


Figure 2: Effect of HAEBS on Haffner tail test



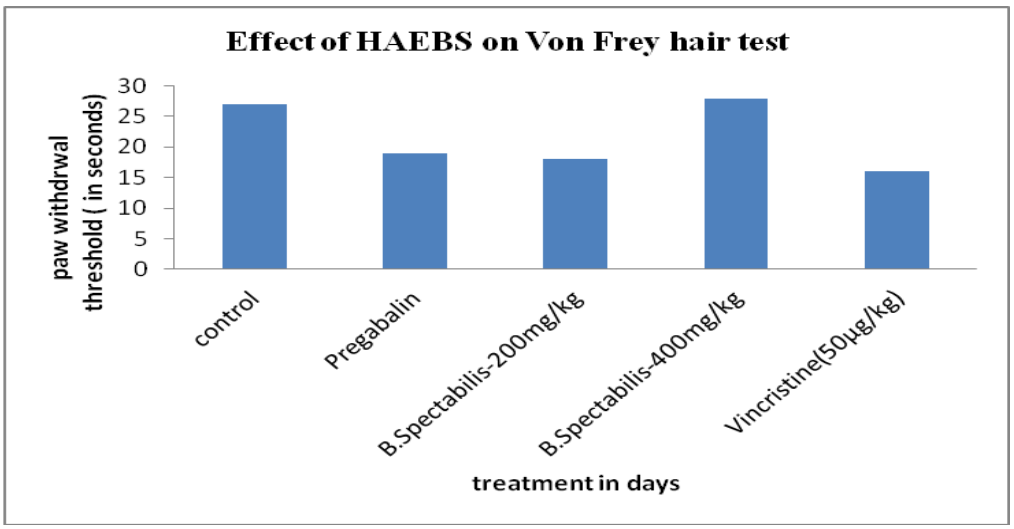


Figure 3: Effect of HAEBS on Haffner tail test

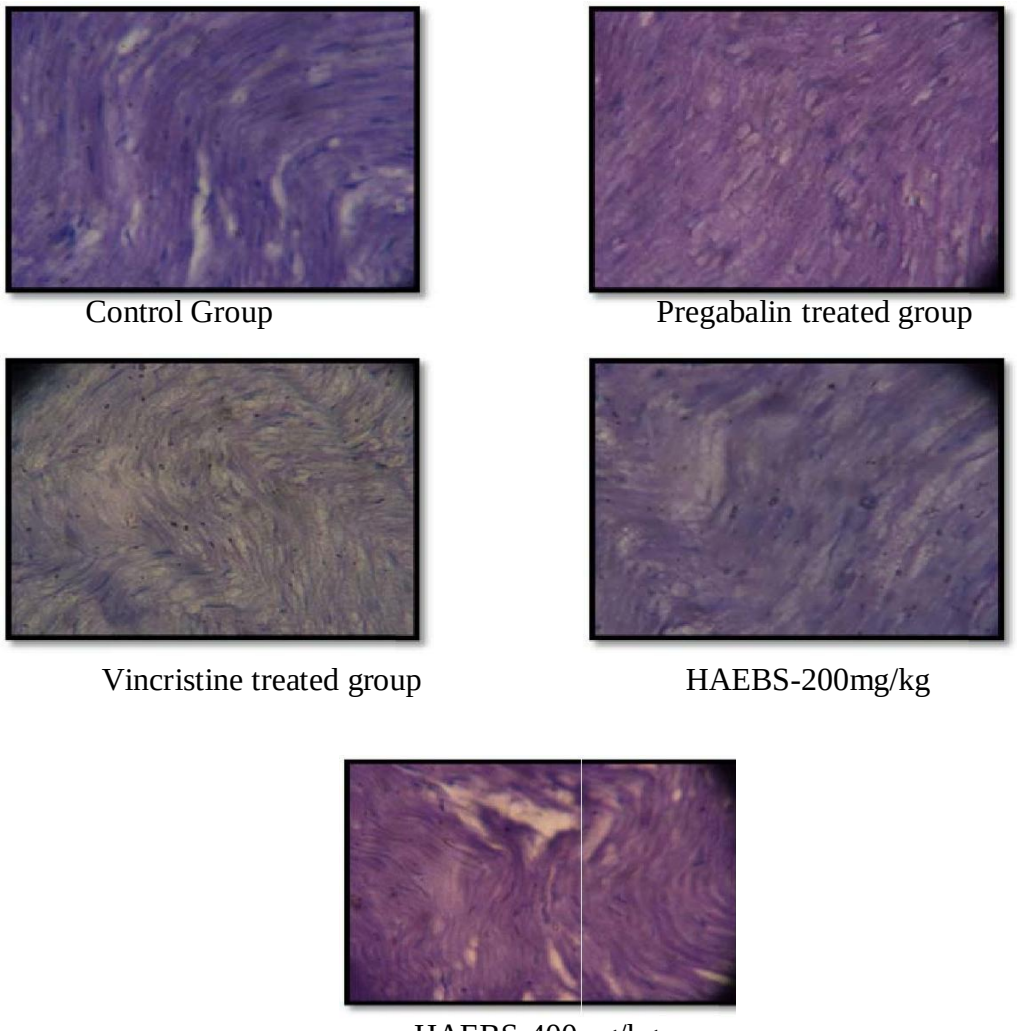
Table1. Effect of HAEBS on Total proteins and calcium levels &reduced glutathione and lipid peroxidation levels.

S. no	Groups	Total protein levels (mg/g of tissue)	Total calcium levels (mg/dl)	Reduced glutathione (µg/mg of protein)	Lipid peroxidase (nmol/mg of protein)
1	Control	5.86 ± 0.07	4.56 ± 0.08	24.9 ± 0.23	27.04 ± 0.62
2	Pregabalin (10mg/kg)	5.40 ± 0.11	4.43 ± 0.12	24.72 ± 0.24	33.64 ± 0.13
3	Vincristine-(50µg/kg)	17.77 ± 0.08 [a]	4.6 ± 0.05 [a]	36.44 ± 0.33[a]	43.08 ± 0.9[a]
4	HAEBS-(200mg/kg)	11.64± 0.07 [b]	3.46 ± 0.08 [b]	31.13 ± 0.366[b]	40.56± 0.4219[b]
5	HAEBS-(400mg/kg)	10.28 ± 0.06 [b]	4.5± 0.06[b]	27.83 ± 0.372[b]	34.90±0.4219 [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.

Figure 4: Effect of HAEBS in vincristine induced histopathological change in sciatic nerve



Control Group

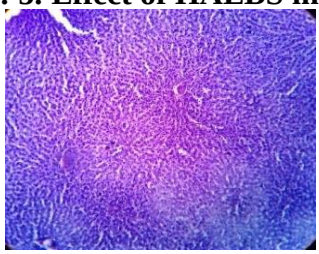
Pregabalin treated group

Vincristine treated group

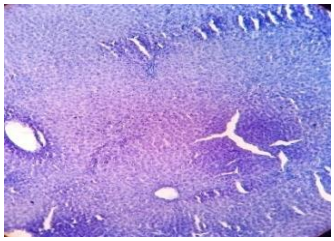
HAEBS-200mg/kg

HAEBS-400mg/kg

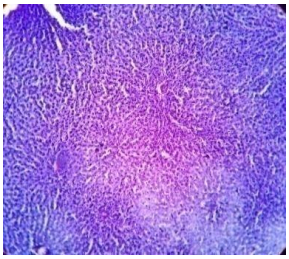
Figure: 5. Effect of HAEBS in vincristine induced histopathological change in liver



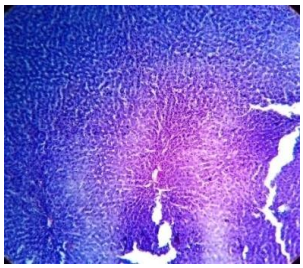
Control group



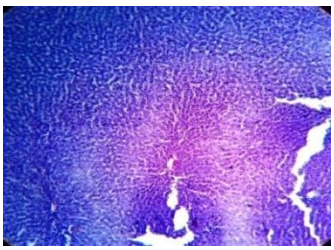
Vincristine treated group



Pregabalin treated group

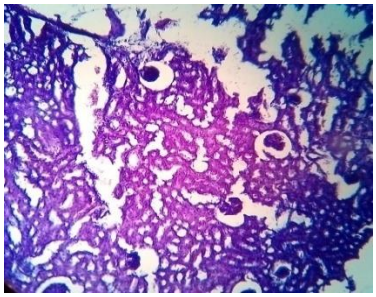


HAEBS2 00mg/kg

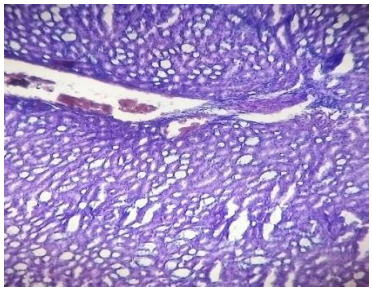


HAEBS 400mg/kg

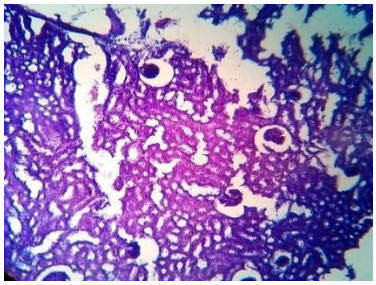
Fig 6: Effect of HAEBS in Vincristine induced histopathological change in kidney



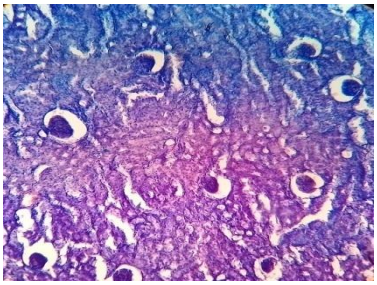
Control group



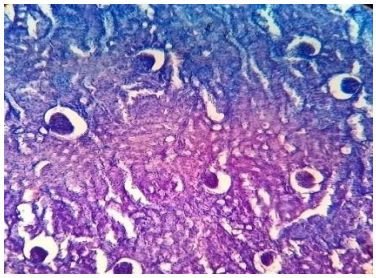
Vincristine treated group



Pregabalin treated group

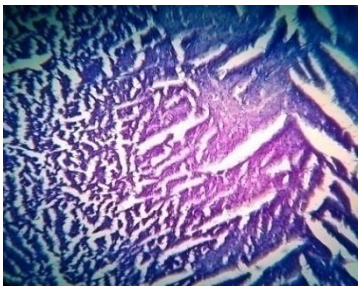


HAEBS-200mg/kg

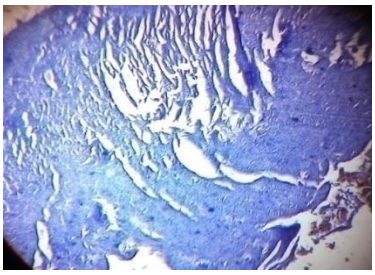


HAEBS-400mg/kg

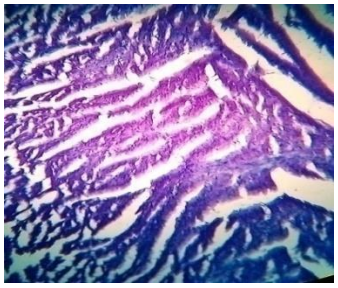
Fig 7: Effect of HAEBS in vincristine induced histopathological change in heart



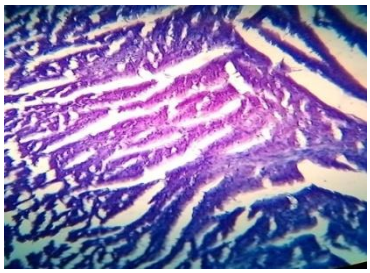
Control group



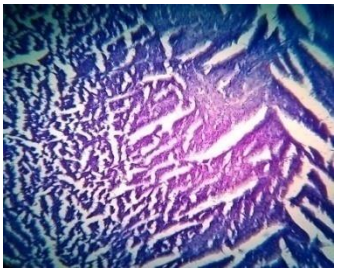
vincristine treated group



Pregabalin treated group



HAEBS-200mg kg



HAEBS-400mg/kg

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 and is shown in figure 4 **.**

Effect of HAEBS in vincristine induced histopathological change in liver

Histopathological observation of the liver showed degenaration of central vein and vascular zadion, inflammation (over activation of kubffer cells), sinusoidal dilation and heptocytes in vincristine treated group while the extract treated remains normal when compared with pregabalin. 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 HAEBS 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 pregabalin. 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 HAEBS in vincristine induced histopathological change in heart

Histopathological observation of the heart showed acute myocardial necrosis, increased mitotic rate and neutrophilia in vincristine treated group while the extract treated remains normal when compared with pregablin. It is found that extract treated group showed protective effects of vincristine induced histopathological alterations when compared with pregabalin group while control group remains normal and is shown in figure **7**.

DISCUSSION

In the present study, the results of antineuropathic activity suggest that HAEBS at 200 and 400mg/kg b.w, *p.o,* alleviates neuropathic pain by virtue of its antihyperalgesic activity and antiallodynic activity. Treatment of the extract also augumented the level of GSH and decreases the elevated TBARS and calcium levels. Both dose of *Bougainvillea spectabilis* (HAEBS) showed doubled the levels of total proteins when compared with pregabalin and control. Lower dose of *Bougainvillea spectabilis* (HAEBS) decreased the total calcium levels while the higher dose did not show any changes in total calcium levels. Both dose of *Bougainvillea spectabilis* (HAEBS) increased the reduced glutathione levels, lower dose of (HAEBS) increased levels of lipid peroxidation levels whereas higher dose decreased levels of lipid peroxidation levels, similar effects were produced as that of pregablin. In this study lower dose of HAEBS and vincristine treated group showed increased levels of reduced glutathione levels implicated the anti-oxidant potential effect of *Bougainvillea spectabilis.*

Both dose of HAEBS showed increased the levels of proteins and lipid peroxidation when compared with control and standard which indirectly indicates the phytoconstituents alters the basic metabolism to correct its illness at the tissue levels. It implicates 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 practice of plants have been used throughout the world for the treatment of neuropathic pain. This research will bring the importance of phytochemicals on neuroprotective function and, in particular their mechanism of action and therapeutic potential[28].

CONCLUSION

This study revealed that in vivo antioxidant effect of hydroalocoholic 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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