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Safety and Anti-inflammatory studies of Nervace ® Tablets

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Abstract: *Anti-oxidant, Anti-inflammatory activity of Nervace tablet was evaluated using various research technologies. Tablets were even analysed for Total Phenolic and Total flavonoid content. Total Phenolic content was found to be in the range of 10mg -12mg per tablet. Nervace Tablets was found to be completely safe upto 5000mg/kg of body weight. It showed very good antioxidant activity with IC_{50} value of 509.52 mcg/ml. It significantly inhibited albumin denaturation showing promising results for the management of Inflammation and arthritis. Thus Nervace tablets can be used safely in the treatment of Inflammation and arthritis.*
Keywords: *Antioxidant, Safety, Nervace, Guggul, Ayurchem, Neuralgia*

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I. INTRODUCTION:

Ayurveda is the system of medicines which is the tradition of India and across Asiatic sub-continent. It believes to treat the disorder or diseases from the root cause. Herbs mentions in ayurvedic texts are been in use in India since ages. Many research papers have been published to verify the authenticity of use of herbs. Reverse pharmacology is the branch which utilizes the traditional knowledge of Ayurveda and finds out the mechanism of action of various herbs and classical formulation. It involves the use of targeted based research studies which are well known for the pathogenesis of the disorder or diseases. Various animals models or in-vitro methods are available which shows the path of drug discovery from the Ayurvedic plants. These models gives us leads molecules from natural resources. Phenolic compounds and flavonoids are the two important functional groups present in ayurvedic plants which have been extensively studied as preventive role in various disorders. Both functional have been reported to have anti-oxidant, anti-inflammatory, anti-cancer, anti-diabetic, anti-TB activities. It has been published about the role of phenolics and flavonoids as phytoestrogen in prevention of post- menopausal disorders.

Nerve inflammation is caused either due to physical injury or due to autoimmune disorder leads to serve pain and inflammation. Inclusions of several mechanisms in the pathogenesis of neuropathic pain have been shown by various researchers. Oxidative stress and formation of ROS (reactive oxygen species) both are acknowledged as the main pathways through which neuropathic pain arises. Pregabalin is the most widely prescribed drug for nerve inflammation. GABA has Pregabalin as its structural analog but not functional.Many studies have stated that pregabalin has anxiolytic and anti-inflammatory activities in rodentsalong with anti-allodynic and anti-hyperalgesic activities in several neuropathic pain models. However drowsiness along with constipation abnormal eye movements dry mouth erectile dysfunction are associated with pregabalin. Hence search for new molecules from natural sources is always in demand. Flavonoids, Total Phenolicsand guggul compounds are promising compounds for treating nerve inflammation. Flavonoids by virtue of their anti-oxidant activityis beneficial in neurodegeneration. Flavonoids causes expression of proteins related to neuronal repair and synaptic plasticity. Many research studies have been reported the beneficial activity of flavonoids in neuroprotection.

Till date no scientific literature is available for the study of anti-inflammatory and anti-oxidant activity of AjmodadiGutika, Visvadiguggul anayurvedic formulation. Ours is the first scientific study to report the same. Nervace Tablet , a polyherbal combination of Ajmodadigutika,VisavadiGuggul along with Nirgundi and Jyotismati.It is widely prescribed for nerve inflammation and neuralgia. This study is planned to assess the safety, anti-inflammatory and anti-oxidant activity of Nervace Tablets.

II. EXPERIMENTAL

MATERIAL AND METHODS

Chemicals - 2,2-diphenyl-1-picrylhydrazyl Powder (DPPH), Bovine serum albumin Fraction- V(BSA) and Aspirin powder from Hi Media. Solvent Methanol(analytical grade) obtained from Merck, Nervace Tablets from Ayurchem Products, Mumbai.

Studies:

1.0 Acute Toxicity Studies of Nervace Tablets:

Acute Toxicity studies on Nervace tablets were conducted as per the OECD guilines 420. The study was conducted at Bombay college of Pharmacy with CPCSEA registration no. 242/PO/RE/S/2000/CPCSEA; 01/08/2000 vide protocol approval no. CPCSEA-BCP-/2017-01/11. Briefly Sprague Dawley rats were grouped into following groups as shown in table no 1. After giving drug all animals were observed for first 30 mins and then every 24 hours till 14 days. Observation such as change in Skin fur, Locomotor activity, autonomic signs and weight, as per OECD guidelines.

Table No 1: Grouping of animals for acute toxicity studies:

Group No	Medicine	Dose
I	Vehicle Control	-
II	Nervace Tablets, Low dose	8.80 mg / kg
III	Nervace Tablets, Medium Dose	88.0 mg /kg
IV	Nervace tablets, High Dose	880.0 mg / kg

2.0 Antioxidant activity of Nervace tablets using Determination of DPPH free radical scavenging Activity:[25]

Free radical scavenging Capacity of Nervacetablets was determined using based on2,2-diphenyl-1-picrylhydrazyl. The ability of the antioxidants present in theNervace tablet , as well as individual ingredients Ajmodadigutika and Visvadiguggul.The formulation use to decolorize the DPPH radical. DPPH radicals absorbed maximum at 514 nm, which disappears with reduction by an antioxidant compound. Briefly about 0.5 gm Nervace tablet , Ajmodadigutika and Visvadiguggulwas measured using an Analytical balance (Citizen CY 220) and was added to 5 ml of distilled water separately. The solution was mixed well using a vortex. Boil on water bath for 10 min.Serial dilution from 100 to 1000 µg/ml was performed using methanol for Nervace tablet , Ajmodadigutika and Visvadiguggul.Test solution (0.5 ml) of different concentrations (100 to 1000mcg/ml)and Control solution were mixed with 2.5ml DPPH solution (100µM). Then the samples were incubated at Room temperature in dark for 20min.Absorbance of reaction mixture was measured for each concentration at 514 nm using UV-Visible spectrophotometer (Shimadzu UV-1800) Each test was repeated thrice and the mean absorbance was recorded. The percentage of inhibition of Radical scavenging activity was determined on a percentage basis with respect to control using the following formula:Inhibition of DPPH (%) = (AC - AT/ AC) X 100Where,AC - Absorbance of control solution and AT - Absorbance of Test solution.

3.0 Anti-inflammatory activity of Nervace tablets using Bovine serum albumin (BSA) denaturation method:[26]

About 0.2gm of Aspirin,Nervace tablet , Ajmodadigutika and Visvadiguggulwas measured using an Analytical balance (Citizen CY 220) and was added to 20 ml of distilled water Separately. The solution was mixed well using a vortex.Serial dilution from 1000µg/ml to 0.01µg/ml was performed for Nervace tablet , Ajmodadigutika and Visvadiguggulland for reference Drug (Aspirin).Test solution (0.05ml) of different concentrations from 0.01 microgram per ml – 1000 microgram per ml and standard drug Aspirin (0.05ml) of different concentrations 0.01 ,0.1,1,10,100,1000 µg/ml) were mixed with 0.5% w/v aqueous solution of BSA (0.45ml). Then the samples were incubated at 37ºC for 20min followed by incubation at 57ºC for 3min. 2.5ml of phosphate buffer (pH 6.4) was added to all the above samples after cooling. Absorbance of reaction mixture was measured for each concentration at 255nm using UV-Visible spectrophotometer (Shimadzu UV-1800) Each test was repeated thrice and the mean absorbance was recorded. The percentage of inhibition of protein was determined on a percentage basis with respect to control using the following formula:Percentage inhibition (%) = 100- [(ATS - APC)/ ATC] x100 whereas: ATS - absorbance of the test solution, APC - absorbance of the Product control ATC - absorbance of the test control solution.

4.0 Estimation of Total Phenolics and Flavonoids inNervace Tablet, Ajmodadigutika and Visvadiguggul:

Total phenolics were determined using Folin-Ciocalteau reagent. The sample (200 µl) was mixed with 200 µl of Folin-Ciocalteau reagent (previously diluted 1:1 with distilled water) and allowed to stand at room temperature for 5 min. A 2000 µl sodium bicarbonate solution (7% w/v) was added to the mixture. After 90 min at room temperature, absorbance was measured at 700 nm using a UV/Vis spectrophotometer. Total phenolics

were quantified by calibration curve obtained from measuring the absorbance of a known concentration of gallic acid standard. The concentrations are expressed as milligrams of gallic acid equivalents (GAE) per ml of sample.

Total flavonoids were estimated using $AlCl_3$ method. Sample solutions were prepared in 80% methanol. To prepare $AlCl_3$ reagent, 133 mg crystalline aluminium chloride and 400 mg crystalline sodium acetate was dissolved in 100 ml of 80% methanol. For flavonoid estimation, to 2 ml of sample, 400 µl of water and 1 ml of $AlCl_3$ reagent was added. Absorbance was recorded at 430 nm against blank containing no $AlCl_3$ reagent. Stock solution of quercetin (1 mg/ml) was prepared in 80% methanol. Various dilutions of quercetin (5-25 µg/ml) were prepared in methanol and a standard curve was plotted. The amount of flavonoids was calculated as quercetin equivalent from the calibration curve of quercetin (5-25 µg/ml).

Statistical analysis::

All data were analyzed statistically using UV spectrophotometer (Shimadzu UV-1800). The descriptive data were expressed as mean ± standard error of mean. Linear regression analysis was performed to find out correlation coefficient. The percentage of inhibition rate between different groups were analyzed by independent sample t-test.

III. RESULTS:

1.0 Acute Toxicity of Nervace Tablets:

Acute toxicity studies gives knowledge about the potential of a chemical, mixture or formulation to be hazardous to health. The maximum dose tried in the experimental study was 5 times higher than the normal human dose. Nervace Tablets did not show any signs of locomotors, autonomic signs like salivation, urination, convulsions, tremors was observed. There were no signs of pain was observed. All the animals which were weighed before and after the study were normal and did not show any signs of toxicity, mortality. Infact natural and normal increase in the weight was observed in all animals.

2.0 Antioxidant activity of Nervace tablet, AjmodadiGutika and Visvadiguggul:

Free radical scavenging potential (DPPH) of the polyherbal Formulation Nervace tablet , Ajmodadigutika and Visvadiguggulat different concentrations is represented below. The free radical scavenging activity increases with increase in the concentration of the sample which was reflected at the decrease in the absorbance. The ability of Nervace tablet , Ajmodadigutika and Visvadiguggulto scavenge DPPH free radical was calculated as percentage inhibition and inhibitory concentration at 50%. TheIC_{50} value of Nervace tablet , Ajmodadigutika and Visvadiguggulwas found to be 509.52 µg/ml, 352.12 µg/ml, 525.78 µg/mlrespectively as shown in Table no. 2.

Table No. 2: Percentage inhibition of Free radicals using DPPH

Conc (µg/ml)	**Percentage inhibition of Free radicals**		
	Nervace Tablets	**Ajmodadigutika**	**Visvadiguggul**
100	20.96	23.99	22.51
200	27.84	39.11	34.54
300	34.61	43.64	39.86
400	41.93	54.43	42.65
500	48.36	63.20	46.85
600	54.02	71.37	54.54
700	62.34	85.68	63.35
800	70.91	95.16	65.31
900	81.51	95.36	68.53
1000	87.48	96.27	74.96
IC_{50}	**509.52 mcg**	**352.12 mcg**	**525.78 mcg**

3.0 Anti-inflammatory Activity of Nervace tablet , Ajmodadigutika and Visvadiguggulusing bovine serum denaturation:

Anti-inflammatory activity of Nervace tablet , Ajmodadigutika and Visvadiguggulwas evaluated against BSA denaturation method. Inhibition of protein denaturation increased with increase in the concentration. The value of IC 50 of Nervace tablet , Ajmodadigutika and Visvadiguggulwas 3.35 mg/ml, 10.62 mg/ml, 20.41 mg/ml and 4.80mg/ml, respectively. In addition to above the of IC 50 of Standard Aspirin was 8.87 mg/ml.

Table No. 3: Anti-inflammatory activity using Albumin denaturation:

Conc**(µg/ml)**	Nervace Tablets	Ajmodadigutika	Visvadiguggul	Aspirin
0.01	8.95± 1.15	2.10± 0.05	12.07 ± 0.90	12.02 ± 2.80

0.1	9.81± 1.57	2.64± 0.19	13.43 ± 1.25	12.19 ± 3.00
1	10.06± 1.50	3.29± 0.36	15.51 ± 1.18	12.50 ± 0.01
10	11.67± 1.27	5.02± 0.32	17.88 ± 1.13	15.70 ± 1.71
100	12.90± 0.35	6.03± 0.32	19.73 ± 1.13	16.30 ± 0.93
1000	15.06± 1.91	6.98± 0.18	22.20 ± 0.44	17.43 ± 0.02
IC 50	**8.39 mg/ml**	**13.22 mg/ml**	**4.85 mg/ml**	**8.87 mg/ml**

Results are shown as mean ± SEM. SEM: Standard error of the mean.

4.0 Estimation of Total Phenolics and Flavonoids inNervace Tablet, Ajmodadigutika and Visvadiguggul:

Total Phenolics in Nervace Tablet, AjmodadiGutika and Visvadiguggul was found to be 1.98%, 2.30% and 1.18% respectively. Total Flavonoids in Nervace Tablet, AjmodadiGutika and Visvadiguggul was found to be 0.06%, 0.03% and 0.03 % respectively.

IV. DISCUSSION:

Ayurvedic "pathophysiology" links *vata*, *pitta*, and *kapha* to the process of joint motion, metabolic and secretory functions (synovial fluid), and lubrication and preservation (structure and homeostasis), respectively. Inappropriate food habits are thought to weaken digestion and metabolism, causing an accumulation of impurities, which build up in the blood. Circulating throughout the body, these impurities are blocked in the structural curvature of the joints and theorized to remain lodged there.

AjmodadiGutikais a Ayurvedic medicine traditionally used in sciatica.As per the ayurvedic text this formulation is more beneficial for Rheumatoid Arthritis, Swelling, Gout, Sciatica, Backache, and other pain disorders.Similarly Guggulu formulation is widely being accepted as pain killer and anti-inflammatory compounds. Visvadiguggul is the combination of various spices with shudhaguggul. Nervace Tablets is the combination of AjmodadiGutika, Visvadiguggul along with bhavnadravya of Nirgundi and Jyotismati. Concept of this formulation was derived on the following basis as to reduce nerve inflammation, improves nerve conduction, reduce inflammation and analgesic activity.

Nervace Tablets a polyherbal formulation is the combination of Standardized aqueous extract of RasnaerandadiQuath Extract, PunernavadiQuath Extract and Kaishoreguggul etc. Nervace Tablet was found to be completely safe for human consumption as observed in toxicity studies. LD50 value of Nervace Tablet is more than 5000 mg/kg and can be classified as Class 5 category as per the OECD guidelines.

It is well known reported fact that free radicals are the major cause for majority of the autoimmune disorder. In Ayurveda free radicals are termed as ‘aam’ (toxins). Hence scavenging free radicals is of prime importance for the treatment of nerve inflammation. Ingredients of Nervace tablets have shown very good free radical scavenging activity. Anti-oxidant activity of Nervace tablet can be attributed to presence of Phenolics and flavonoids compounds. Phenolics and Flavonoid compounds have already know for anti-oxidant activity.

Protein Denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of proteins is a well-documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, ability of plant extract to inhibit protein denaturation was studied.Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly prescribed medications in the world because of their verified effectiveness in reducing pain and inflammation. NSAIDs has accounted for prevention of the protein denaturation, which acts as antigens and prompts autoimmune diseases. These drugs contain several adverse effects, particularly gastric irritation prompting the development of gastric ulcers.

Various ayurvedic text mentions the role of AjmodadiGutika, Visvadiguggul along with bhavnadravya of Nirgundi and Jyotismatiin pain and inflammation. No scientific report is available till date to study the anti-inflammatory activity of AjmodadiGutika, Visvadiguggul and Nervace Tablets . Ours is the first study to report the same. It was observed that all the three ingredients showed good anti-inflammatory. The results are highly significant and comparable to the standard drug used to assess the activity. Since standard drugs like Aspirin has many side effects whereas the use of AjmodadiGutika and Visvadiguggulmakes it more beneficial. Similarly results were obtained when Nervace Tablets (polyherbal combination of AjmodadiGutika, Visvadiguggul along with bhavnadravya of Nirgundi and Jyotismati) was used in inhibition of protein denaturation. The mechanism may be attributed due to following factors like antioxidant activity, while scavenging aami.e toxins generated in the body due to faulty metabolism and incompatible food habits, anti-inflammatory activity there by reducing the protein damage.

V. CONCLUSION:

Nervace tablets was found to be safe in human consumption. It showed good antioxidant, anti-inflammatory activity.

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