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Pharmacological Evaluation of Skeletal Muscle Relaxant Activity from the Leaves of Folk Medicinal Plant *Ichnocarpus Frutescens*

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Abstract: ***In the present study the test samples of leaf extract of Ichnocarpus frutescens belonging to the family Apocyanaceae were tested for skeletal muscle relaxant activity. In the present study skeletal muscle relaxant activity was evaluated by rota-rod model and inclined screening test. The objective of this study was to investigate in-depth the skeletal muscle relaxant activity of the ethanol and aqueous extract of Ichnocarpus frutescens leaves. The present results showed that the ethanolic extract of Ichnocarpus frutescens leaves possess a significant skeletal muscle relaxant activity in experimental Swiss albino mice. At dose of 200 mg/kg and 400mg/kg it showed highly significant skeletal muscle relaxant activity at 30, 60, 90,120 min of duration. Preliminary phytochemical screening reveals the presence of glycosides, carbohydrates, flavonoids, tannins and proteins in the plant extract.***

Keywords: skeletal muscle relaxant**,** ethanol,aqueous, Ichnocarpus frutescens

1. Introduction

The word “herb” has been derived from the Latin word, “herb” and an old French word “herba”. Nowadays, herb refers to any part of the plant like fruit, seed, stem, bark, flower, leaf, stigma or a root, as well as a non-woody plant. Earlier, the term “herb” was only applied to non-woody plants, including those that come from trees and shrubs. These medicinal plants are also used as food, flavonoid, medicine or perfume and also in certain spiritual activities [1]. According to WHO, around 21,000 plant species have the potential for being used as medicinal plant, there are at least 121 major plant drugs of known structure, but none of them currently produced through synthetic means.For developing phytomedicines as a major area of concern, it would be essential to adopt a holistic interdisciplinary approach, have a scientific basis of the understanding of the plant system, new innovations and their conservation for utilisation in further on a suitable basis[1].

Ichnocarpus Frutescens is one such medicinal plant reported to have medicinalproperties and was used to cure many disorders. In this contest an attempt is made to screen the skeletal muscle relaxants activity of aerial parts of the plant *Ichnocarpus Frutescens*

1.1 Morphology

A large much-branched twining shrub; young branches finely fulvous-tomentose.Leaves 4.5-7.5 by 2-3.8cm. Elliptic–oblong, acute or acuminate, glabrous above glabrousor slightly pubescent and pale beneath,base usually rounded; main nerves 5-7 pairs, with finely reticulate venation between; petioles 3-6mm.long, flowers greenish white, numerous, in axillary and terminal rusty-pubescent trichotomous pediculate cymes; pedicels 3-4mm.long, often three together, rusty-pubescent, calyx fulvous hairy, divided ½ waydown; lobes ovate acute, without glands inside ,corolla-tube2.-5.3mm long with the narrow portion below about 0.85mm.Long themiddle portion of the tube much inflated (almost globular) over the stamens, the upper portion constricted below the lobes; lobes 5 mm. long, pubescent on the upper side with white hairs, broad and oblong at the base, produced at the apex in to a long falcate slender twisted acumen which is deflexed in bud and flower. Disk of 5 erect linear lobes, longer than the hairy ovary. Follicles 10-15cm.by 4mm. straight or slightly curved. Very slender, cylindric, rusty-pubescent at first,afterwards glabrous.seed 1.3-2cm. long, linear, black, not beaked; coma as long as the seed, scanty, white.

The root is sweetish, cooling, and aphrodisiac; cures “kapha”, thirst, vomiting, fever, biliousness “vata”,and diseases of the blood; in other respect it behaves like the root of *Hemidesmus indicus.*

The stalk and leaves are used in the form of decoction in fevers [2].

1.2 Chemical constituents

Studies on chemical constituents of the plant reveals the presence of phenylpropanoids, phenolic acids, coumarines, flavonoids, sterols and pentacyclic triterpenoids i.e. 1 2-dehydrolupanyl-3β-palmitate, lupeol acetate, friedelin, friedelinol, 12-dehydrolupeol, oleanolic acid, nonane, 5-hydroxyoctacosan-25-one, dotriacontanoic acid, sitosterol and sitosterolpalmitate [3].

Stem contains α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-α-amyrin**,** 6, 8, 8-trimethylpentacosan-7-one[4], α-amyrin and its acetates, lupeol and its acetates, friedelin, epi-friedelinol and β-sitosterol[5], n-butyl oleate, n-octyltetracontane, tetratriacontadiene, n-nonadecanyl benzoate, benzocosanylarachidate[6].

Leaves contain flavones viz. apigenin and luteolin, glycoflavones i.e. vitexin and isovitexin, proanthocyanidin and phenolic acids, vanillic, syringic and synapic acid, protocatechuic acid [7]. Ursolic acid acetate, kaemferol, kaemferol-3-galactoside (trifolin) and Mannitols were also identified from leaves [8].

Roots reported to consist of β-sitosterol[9] and 2-hydroxy-4-methoxybenzaldehyde[10]. Flowers contain quercetin and quercetin-3-O-β-D-glucopyranoside [11].

1.3 Medicinal uses

Whole plant is used as tribal medicine in atrophy, bleeding gums, convulsions, cough, delirium, dysentery, glossitis, heamaturia, measles, night blindness, relieves pain due to insect bites, splenomegaly and tuberculosis. Plant is also used in abdominal and glandular tumour.

Roots are used as a substitute for Indian Sarsaparilla (Hemidesmus indicus) as alterative, antidysentric, antipyretic, demulcent, diaphoretic, diuretic, hypoglycaemic and tonic; beneficial in anorexia, leucorrhoea, skin diseases, syphilis and urinary calculi. Warm leaves are applied by the tribes of Rajasthan, on the swelling to cure guinea worm infection. Decoction of leaves and stems is used in fever and skin eruption [12]-[14].

2. Extraction

The extraction is done through soxhlet apparatus5-6 made from thick. The sample (powder of *Ichnocarpus FrutescensR.Br*. 40 gm.) was weighed and placed in the thimble filter paper, which was then loaded into the main chamber of the Soxhlet extractor [15] solvent. The extractor was then placed onto a flask containing the extraction (Ethanol and aqueous).The Soxhlet was then equipped with a condenser. The solvent was heated to reflux. Thechamber containing the solid material was slowly filled with warm solvent to dissolve some of the desired compound [16] siphon. When the Soxhlet chamber wasalmost full, the chamber was automatically emptied by a side arm, with the solvent running back down to the distillation flask. This cycle was allowed to repeat many times, over 36 hrs. During each cycle, a portion of the non-volatile compound dissolved in the solvent. The extract was passed through a filter paper. The filtrates were concentrated with a vacuum pump at 40°C, giving a yield of 7.93%, which was stored in universal bottles and refrigerated at 4°C prior to use.

Figure 1: Photograph showingIchnocarpus Frutescens plant and powder

3. Materials and Methods

3.1 Collection and authentication of plant material

Table 1: The Collection of Plant Details

Plant	*Ichnocarpus Frutescens*R.Br.
Family	Apocyanaceae
Collected	Kattehakkalu Village,Thirthahalli Taluk
District	Shimoga District
State	Karnataka
Identified and Authenticated	Dr.Rudrappa, HOD, S.R.N.M National College Of Applied Science Balraj-Urs, Road, Shivamogga, Karnataka
Herbarium	(NCP/Herbarium No:7)

4. Phytochemical Screening

Table 2: Qualitative chemical investigations of extracts of Ichnocarpus frutescens

S. No	*Phytoconstituents*	*Ethanol Extract*	*Aqueous Extract*
1	Carbohydrates	+	+
2	Proteins	+	__
3	Saponins	+	+
4	Triterpenoids	+	
5	Flavonoids	+	+
6	Steroids	_	_
7	Glycosides	+	+
8	Alkaloids	__	__
9	Tannins	__	__

5. Animals used

According to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and Institutional Animal Ethical Committee (Ref No .NCP/IAEC/CL/08/2015- 16). All the Animals were procuredfrom Central Animal House National College of Pharmacy Shivamogga.

Animal	Young Swiss-albino mice
Age	4-5 weeks
Average Weight	25-30gm
Purchased	S.S medical College, Davanagere.
Condition	They were kept under standard environmental condition for one week for adaptation and was fed mice formulated rodent food and water.

6. Acute toxicity studies

Toxicity test studies conducted as per internationally accepted protocol drawn under OECD guidelines. 425. (OECD guidelines. 425 modified, adopted March 23, 2006) in Swiss albino mice[17].

7. Pharmacological Activity

In present study the topical preparation of root extracts of *Ichnocarpus frutescens (*L.) R.br wasexamined for Skeletal Muscle Relaxant by Rota rod apparatus in rats and inclined screen test in Young Swiss-albino mice respectively.

7.1 Rota rod apparatus

Swiss albino mice were divided into four groups consisting of six animals each.
Group I: Control group treated with 1/10th saline water.
Group II: Received reference standard Diazepam at a dose.
Group III: Received Ethanol leaf extracts of *Ichnocarpus Frutescens* 200mg/kg.
Group IV: Received Ethanol leaf extracts of *Ichnocarpus Frutescens* 400mg/kg.
Group V: Received aqueous leaf extract of *Ichnocarpus Frutescens* 200mg/kg.
Group VI: Received aqueous leaf extract of *Ichnocarpus Frutescens* 400mg/kg.
Animals remaining on Rota-Rod (22 rpm) 2 min or more in low successive trials after the administration of test material or control vehicle the same test of 30 min for 2 hr. The fall off time from the rotating rod was noted. The difference in the fall off time from the rotating rod between the control and treated rats was taken as an index of muscle relaxation [18], [19].

Table 7.2: Table showing the effect of Ethanol & Aqueous extract of plant leaves of Ichnocarpus frutescens on skeletal muscle relaxant activity by Rota rod Model

Group	Fall Of Time (in seconds)			
	30min	60min	90min	120min
G- I	204±21.94	206±16.04	203±24.03	206.6±16.6
G- II	39.50±7.95	28.1±7.19	19.3±4.57	10.33±2.47
G-III 200 mg/kg	107.5±16.1*	104.5±20.5**	101.6±21.0**	90.00±18.0***
400 mg/kg	90.0±17.1**	84.1±19.5**	74.1±15.7***	60.0±12.3***
G-IV 200mg/kg	127.1±24.0	123.3±24.5*	121.6±12.4*	113.3±15.2**
400 mg/kg	111.8±18.6*	106.6±20.7**	100.8±13.5**	92.5±20.1***

Note: Data was analysed using one way ANOVA followed by pairwise comparison. Values are expressed as mean ± S.E.M. n=6, ***P < 0.001 is considered as highly significant.

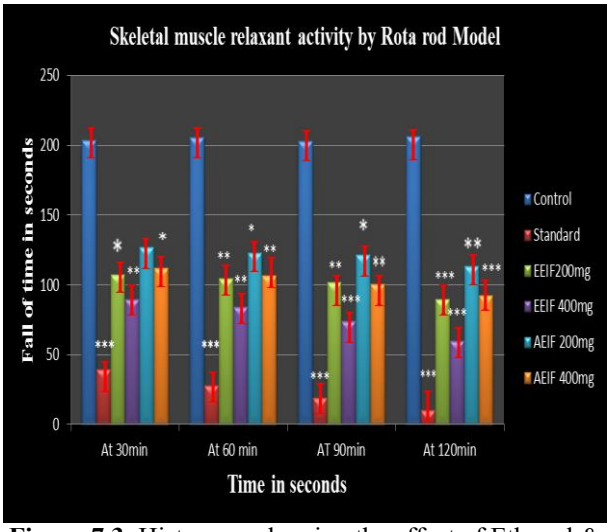

Figure 7.3: Histogram showing the effect of Ethanol & Aqueous extract of plant leaves of Ichnocarpus frutescens on skeletal muscle relaxant activity by Rota rod Model.

Inclined screen test

Each group of rats (n=6) were left for 1hr on a flat, slippery, rectangular glass (42cm × 37cm) inclined at 30º to the horizontal, 30 min after the administration of *I. frutescens* (200 mg/kg and 400 mg/kg oral), Diazepam (4 mg/kg, i.p.), Acacia (1% oral) to observe for a paralyzing effect severe enough to cause the rats to slide off the screen [20].

Mice were divided into six groups consisting of six animals (n=6) each.

Group I: Control group treated with 1/10th saline water.
Group II: Received reference standard Diazepam at a dose4 mg/kg.
Group III: Received Ethanol leaf extracts of *Ichnocarpus frutescens*200mg/kg.
Group IV: Received Ethanol leaf extracts of *Ichnocarpus frutescens*400mg/kg.
Group V: Received aqueous leaf extract of Ichnocarpus *frutescens200mg/kg.*
Group VI: Received aqueous leaf extract of*Ichnocarpus frutescens*400mg/kg.

Table 8.1: Table showing the effect of Ethanol & Aqueous extract of plant leaves of Ichnocarpus frutescens on skeletal muscle relaxant activity by inclined phase model

Groups	**Fall Of Time (in seconds)**		
	30min	**60min**	**90min**
Control 0.5 ml	91.6±3.33	90.83±5.44	90.50±4.78
Diazepam 4 mg/kg	30.0±2.88	23.33±2.10	11.66±2.02
EEIF 200mg/kg	73.3±2.10	63.33±7.26**	57.83±3.32 ***
400mg/kg	59.1±3.51**	44.16±2.38***	31.00±5.29***
AEIF 200mg/kg	83.3±7.14	78.00±4.39	70.00±5.77*
400mg/kg	84.1±9.16	71.66±1.66*	62.83±7.70**

Note: Data was analysed using one way ANOVA followed by pairwise comparison. Values are expressed as mean ± S.E.M. n=6, ***P < 0.001 is considered as highly significant.

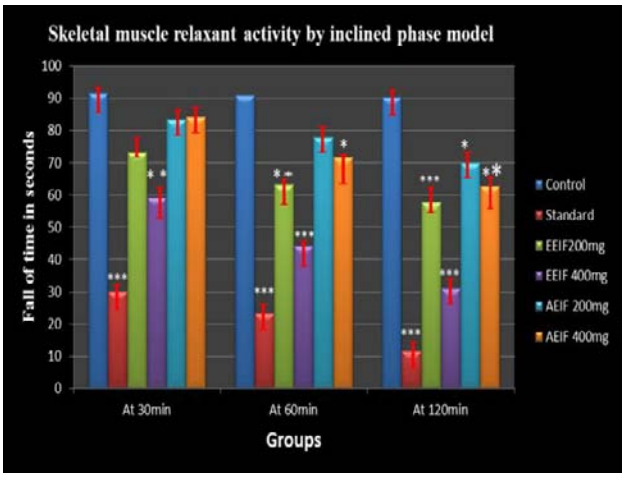

Figure 8.2: Histogram showing the effect of Ethanol & Aqueous extract of plant leaves of Ichnocarpus frutescens on skeletal muscle relaxant activity by inclined phase model.

8. Statistical Analysis

All the values were statistically analyzed by one-way analysis of variance (ANOVA) followed by Dunnett multiple comparison test. Data from distilled water treated animals were used as the control and data from diazepam treated animals were used as standard values. All values are expressed as Mean ± S.E.M. Results were regarded as significant at P< 0.05[21], [22].

9. Discussion

In the present study the test samples of leaf extract of *Ichnocarpus frutescens* belongs to the family Apocyanaceae were tested for skeletal muscle relaxant activity. Several reports are available on many plant species belonging to the presently studied family Apocyanaceae with skeletal muscle relaxant activity. In the present study skeletal muscle relaxant activity was evaluated by rota-rod model and inclined phase model.

The objective of this study was to investigate in-depth the skeletal muscle relaxant activity of the ethanol and aqueous extract of *Ichnocarpus frutescens* leaves. The present results showed that the ethanolic extract of *Ichnocarpus frutescens* leaves possess a significant skeletal muscle relaxant activity in experimental Swiss albino mice. At dose of 200 mg/kg and 400mg/kg it showed highly significant skeletal muscle relaxant activity at 30, 60, 90,120 min of duration. Preliminary phytochemical screening reveals the presence of glycosides,carbohydrates, flavonoids, tannins and proteins in the plant extract. Therefore, the observed skeletal muscle relaxant activity may be attributed to these compounds. Further studies are in progress to isolate the active constituents responsible for this activity. Since the pharmacological profile of the present investigation of the ethanol and aqueous of *Ichnocarpus frutescens* was similar to that of benzodiazepines, it is also possible that they might interact with benzodiazepine receptor located adjacent to the GABA receptor. Therefore, the use of ethanol and aqueous of *Ichnocarpus frutescens* leaves in folkloric medicine may be due to its CNS action.

10. Conclusion

Skeletal muscle relaxant activity was performed by rota-rod model and inclined phase model. In the present study all the test samples (Ethanol and aqueous leaf extracts exhibited significant (P < 0.001) skeletal muscle relaxant activity. Among these test samples ethanol leaf extract exhibited more skeletal muscle relaxant action when compare to control.

It can be concluded that active constituents responsible for skeletal muscle relaxant activity might be present in the leaf extracts. However, further studies are necessary to find the exact mechanism of skeletal muscle relaxant effect and to isolate the active compound(s) responsible for this pharmacological activity.

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