Hemidesmus indicus: Analgesic and anti-inflammatory activity of ethanolic activity of leaves

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Abstract

Hemidesmus indicus is also known as Indian sarsaparilla. It also called as endless root. Hemidesmus indicus is known to have many therapeutic benefits. The present investigation aimed in determining effectiveness of ethanolic extract of Hemidesmus indicus leaves as an analgesic and anti-inflammatory agent. The leaves of Hemidesmusindicus were collected and dried. The extraction was carried out through maceration process. The rats weighing around 150-200g and mice weighing 25-30g were selected. Acute toxicity study exhibited ethanolic extract of Hemidesmus indicus leaves is safe up to 2000mg/kg. The ethanolic extract of Hemidesmus indicus leaves were used in the corresponding doses - 100, 200, 400 mg/kg body weight. Analgesic activity was evaluated based on "Tail immersion Method" and "Acetic acid induced writhing method". The central and peripheral analgesic effect was evaluated. The anti-inflammatroy activity was evaluated based on carrageenan induced rat paw oedema method and in-vitro method like inhibition of albumin denaturation. In both the activities test dose of 200mg, 400mg/kg body weight of extract showed significant activity. Preliminary photochemical analysis of ethanolic extract of Hemidesmus indicus leaves revealed the presence of constituents like tannins, saponins, flavanoids, and glycosides. According to these findings the ethanolic extract of Hemidesmus indicus possesses a significant analgesic and anti-inflammatory activity.

Keywords

Hemidesmus indicus, tannins, Analgesic activity, anti-inflammatory activity, Acetic acid, carrageenan

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Introduction

Traditional medicine is "the sum total of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health, as well as in the prevention, diagnosis, improvement or treatment of physical and mental illnesses". Finished herbal products that contain as active ingredients parts of plants, other plant materials, or combinations, herbs, herbal materials, herbal preparations all come under Herbal medicines. Traditional medicine is a practice which differs from country to regions since it is based on the factors like culture, history, and philosophy. Their mechanism and usage are different from conventional medicine [1-10]. In Asian

countries India has highest number of medicinal plants. There are 17,000 plant species in India out of which 7,500 are recognized for their medicinal importance. This proportion is highest in India when compared to any other countries in the world. Approximately 2000 medicinal plants have been reported by Ayurveda system of medicine and later by Sidha and Unani system of medicine. There is a report of 340 natural medicines and their traditional uses in old herbal text Charaka Samhita. At present around 25% of drugs are of plant origin and remaining are of synthetic moieties extracted from plant origin [2,11-29].

The unavailability of scientific and clinical data is a major obstacle in converting herbal medicines into conventional medicinal practices. Standardization is important to confirm the safety and efficacy of medicinal products and theirpractices. To determine safety and efficacy most of medicinal plants don't have regulatory approval. The herbal medication involves the use of unpurified extract which contains several other constituents. This involves synergistic effect where effect of entire herb is greater than the combined effects of its components. Using entire plant instead of taking isolated constituents toxicity gets reduced. To discover and isolate the pharmacologically active compound the knowledge of folk medicine and traditional healers is required [30-50].

The main area of greater significance is the bioavailability of the active constituents of the plant. It is an area which is not effective for herbal active constituents. There is a requirement to develop traditional medicinal plant importance both in domestic and international perspective due to richness of active constituents present in it and the legacy of ancient healthcare tradition. The current demand for medicinal plants was estimated by world health organization at around 14 billion dollars annually. There is a requirement for medicinal plant dependent raw materials around 15 to 25 percent per annum. The market for medicinal plants is expected to grow over $5 trillion by 2050. People will be better informed regarding the safety and effectiveness of a drug to treat the disorder once the local ethanomedical preparations are scientifically get approved and therapeutically non-toxic thereby health status gets improved. Medicinal herbs have not only been used to treat disease but also used as a medical support all over the world to maintain a good health condition of a both humans as well as a animals [4].

Pain is a multifactorial human experience which is associated with trauma, oldtraditional thoughts, and individual mindset. The uncertainty in pain may cause impairments in emotional and social behaviour. The post- operative intensity of pain depends on preoperative anxiety, catastrophic, neuroticism as well as depression. The intensity of pain for post operative pain is depends on the preoperative anxiety and depression. The acute pain can be treated by administering NSAIDs or by using COX-2 inhibitors [5]. Normally to relive pain only analgesics or non-steroidal anti-inflammatory drugs don't provide adequate relief. In such cases antidepressants are used in treating diseases such as fibromyalgia, neuropathic pain, rheumatoid symptoms, low back pain and headache as an auxiliary treatment to manage chronic pain [6]. Inflammatory disorders such as rheumatoid arthritis and osteoarthritis are treated by NSAIDs. The most commonly used NSAID is Aspirin. Aspirin act by reducing inflammation, swelling, pain and fever. The main mechanism involved is inhibiting production of prostaglandins by acting on the enzyme cycloxygenase [7,8,51-64].

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In traditional system of medicine Hemidesmus indicus is used in ethanomedical treatments. It was widely used in treating leucorrhoea and menstrual problems. It was also used as an antipyretic. In India this plant was used in hair and scalp preparations. It also found use in treating diarrhoea, cough, lack of apatite, thirst, respiratory disorders, cardiac disorders, rheumatic disorder. But no researchhas been carried out on the effect of ethanolic extract of Hemidesmus indicus leaves extract as an analgesic and anti-inflammatory agent.The objective of the present study was to authenticate and collect the leaves ofHemidesmusindicus, prepare ethanolic extract of theleaves subject the extract to preliminary qualitative phytochemical screening, conduct acute oral toxicity studies of the extract of and evaluate the analgesic and anti-inflammatory activity.

Methodology

Drugs and Chemicals/Equipments:

Table 1: List of drugs and chemicals used for the study

Sl No	Chemical name
1	Diclofenac sodium injection I.P (Karnataka antibioticsand pharmaceutical Ltd)
2	Pentazocine lactate injection I.P (Ranbaxy Laboratories Ltd)
3	Acetic acid (Loba chemical laboratories)
4	Carrageenan (HI Media Laboratories Ltd)
5	Ethanol (MERCK Ltd)
6	Disodium dihydrogen phosphate (RFCL Ltd)
7	Potassium dihydrogen phosphate (loba chemicals)
8	Sodium chloride (siso research laboratories ltd)
9	Egg albumin

Equipments used in the study:

- Rotex Bod incubator
- IITC 520 Plethysmograph (IITC Life sciences)
- Visible spectrophotometer

Collection and authentication of plant material:

The leaves of *Hemidesmus indicus* were collected from Mangaluru, Karnataka, India in the months of June 2019 and it was authenticatedby Dr Jyothi Miranda, Associate Professor and HOD, Department of Botany, St. Aloysius College (Autonomous), Mangaluru.

Preparation of Extract:

Hemidesmus indicus leaves were sun dried, powdered grossly and macerated. The powdered leaf was macerated with ethanol and held in a closed chamber for seven days with stirring occasionally. After seven days, the whole solvent extract was extracted, and the concentrate evaporated to produce a syrupy consistency in a water bath. For further usage extract was kept in a desiccator.

Preliminary qualitative phytochemical investigation: [53,54]

To investigate the active constituents, present in the ethanolic extract of *Hemidesmus indicus* leaves, the extract was subjected for qualitative phytochemical examination. The chemical tests are as follows

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-Alkaloids

Dragendroff's test

2mg of extract was dissolved in 5ml of distilled water. 1ml of Dragendroff's reagent iwasadded to this solution. The formation of orange or reddish orange precipitate indicates the presence of alkaloids.

Hager's test

To 2mg of extract, 1ml of Hager's reagent was added. The formation of yellow precipitate confirms the presence of alkaloids.

Wagner's test

To make the solution acidic to 2mg of extract 1.55%v/v of hydrochloric acid and 1ml ofWagners reagent was added. The resulting yellow or brown coloured precipitate indicates the existence of alkaloids.

Mayer's test

To 2mg of extract 1ml of Mayers reagent was added. The formation of white or pale-yellow precipitate indicates the existence of alkaloids.

-Reducing sugar tests (Carbohydrates)

Molisch test

To 2mg of extract 20% of alcholic α-napthol solution was added. Concentrated sulphuric acid was added to the mixure through the sides of test tube. The violet ring shows carbohydrates are present. Adding more alkaline substance results in the disappear of violet ring.

Benedict's test

To 2mg of extract 5ml of Benedict's reagent was added. The reaction mixture was boiled in a water bath for 5 minutes. The appearance of brick red coloured precipitate shows the presence of carbohydrates.

Fehling's test

To 2mg of extract equal parts of Fehling's A solution and Fehling's B solution was added. The reaction mixture is boiled for 5minutes in a water bath. The appearance of red or red brick coloured precipitate shows the presence of carbohydrates.

Tollen's test

2mg of extract was shaken with 5ml of distilled water. To this solution 1ml of Tollen's reagent was added. The formation of black precipitate indicates the presence of carbohydrates.

-Flavonoids

Shinoda test

To 2mg of extract 5ml of ethanol was added. The mixture was dissolved. To this10drops of hydrochloric chloric sas added followed by the addition of small piece of magnesium. The resulting pink or reddish brown colour indicates the presence of flavanoids.

-Saponins

2mg of extract was dissolved in a water and shaken vigorusly then kept aside for some time. The formation of a honeycomb like a froth indicates the presence of a saponins.

-Tannins

To 2mg of extract 1% ferric chloride solution was added. The formation of brownish green colour indicates the presence of tannins.

-Steroids

Libermann-Burchard's test

To 2mg of extract 2ml of acetic anhydride was added. Then the mixture was kept for boiling in water bath. Mixture was cooled.Through the sides of the test tube sides concentrated sulphuric acid was added. The green colour development suggests steroid presence.

Salkowski Test

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To 2mg of extract, 2ml of chloroform was added. To this mixture small amount of chloroform was added along the sides of the test tube. Appearance of red colour indicates the presence of steroids.

-Triterpenoids
Libermann-Burchard'stest
To 2mg of extract 2ml of acetic anhydride was added. Then the mixture was kept for boiling in water bath. After cooling 1ml of concentrated sulphuric acid was added along the sides of the test tube. The formation of green colour indicates the presence of triterpenoids.

-Glycosides
Molisch test
To 2mg of extract 20% of alcholic α-napthol solution was added. To this mixture concentrated sulphuric acid was added along the sides of the test tube. The resulting violet ring indicates the presence of glycosides. The excess addition of alkaline substance results in the disappear of violet ring

Resins
To 2mg of extract acetone is added and mixed very well. Then the solution is poured into a distilled water. The formation of turbidity indicates the existence of resins.

Selection of animals:
The animals used in the study are Wistar ratsweighing 150-200g and Swiss albino mice weighing 25-30g of either sex. The animals were procured from animal house of NGSM Institute ofPharmaceuticalSciences, Deralakatte, Mangaluru. The animals were maintained under a standard temperature of 22±2°c and humidity 55±5°c followed by 12h day and night cycle. They were housed in sanitized cages. They were allowed to access dry pellet diet and water ad libitum. The protocol was approved by Institutional Animal Ethical Committee (IAEC). According to the CPCSEA guidelines animal experiment was carried out.

Selection of dose:
Based on the acute oral toxicity study 1/10th of the maximal dose as X i.e. the middle dose, 50% of the X as the minimum dose, two times the dose of X as the maximum dose were selected for the extract. Three dose levels were selected to determine the analgesic and anti- inflammatory activity of ethanolic extract of Hemidesmus indicus. The mid-dose (200mg/kg) in acute toxicity studies was approximately 1/10th. The low dose (100mg/kg) was 50% of 1/10th and high dose (400 mg/kg) was two times the 1/10th [55].

Evaluation of analgesic activity:
-Tail immersion test [56]
Method: Wistar rats weighing 170-210 g were used in this experiment. They were divided into 5 groups with 6 animal species in each group.

Table 2: Experimental design of Tail immersion method

Group	Treatment
GroupI	Normalcontrol(Normalsaline)p.o
Group II	Standarddrug(Pentazocine,10mg/kg) i.p
Group III	LeafextractofH.indicus(100mg/kg) p.o
Group IV	LeafextractofH.indicus(200 mg/kg) p.o
Group V	Leaf extractofH.indicus(400mg/kg) p.o

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They were kept in individual cages and the tail was freely left out. The mark was made at lower 5 cm portion of the tail. Fresh water was filled in the thermostatic controlled organ bath by maintaining temperature of 55±1°C and for sensitivity test animals were screened by immersing tail of rat in it. The withdrawal of tail by the rats within 3- 5 seconds was taken into consideration. The tail was thoroughly dried after every observation. After administration of drug the basal reaction time towards thermal heat was determined. The reaction time was observed for following time intervals 0.5, 1, 2, 3, 4 and 6 hour. Consequently, the time exceeding 6 second in withdrawing a tail will be considered as significant outcome.

Evaluation

After administering a test and standard drug, for each group animals individually for every 30min interval reaction time is measured up to 90min. ED50 values and time response curves (onset, peak and effect duration) of eachcompound was determined. The percentage increase in reaction time was calculated as follows

% increase in reaction time = ($RT_{at}/RT_{bt}-1$)X 100

RT_{at} = Reaction time after treatment

RT_{bt}= Reaction time before treatment

-Acetic acid induced writhing method [57]

Pain was induced in the peritoneal cavity of mice by injections of irritants. Writhing is a feature in animals which involves a stretching of lower abdomen. The test was used to detect analgesic activity. Intraperitoneally an irritant agent, such as acetic acid was administered to the mouse, to determine the stretching reaction.

Procedure

Albino mice of weight between 20 and 25 g of either sex were used. Five groups of 6 mice in each group were used for the study. They were categorized as follows:

Table 3: Experimental design of acetic acid induced writhing method

Group	Treatment
I	Normal control (Acetic acid 0.6%v/v) i.p
II	Standard drug (Diclofenac sodium, 10mg/kg) i.p
III	Leaf extract of *H.indicus*(100 mg/kg) p.o
IV	Leaf extract of *H.indicus*(200 mg/kg) p.o
V	Leaf extract of *H.indicus*(400 mg/kg) p.o

Test drug was given to animals at different pre-treatment times beforeadministering acetic acid. The mouse was individually put in glass beakers and allowed in it for some time. The writhings of each animal was counted. The observation of writhingwas continued for 30min. The writhing was determined by extending the abdomen with at least one hind limb stretching simultaneously. The percentage inhibition was calculated as follows:

%inhibition = $(1-R_t/R_c) \times 100$

R_t = In treated group mean number of writhes

R_c =In control group mean number of writhes

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Evaluation

To evaluate the peak time of drug activity the dose range is calculated. Peak time is the time period which having maximum amount of inhibition. The dose which has less than 70% of inhibition will be having minimal activity.

Evaluation of Anti-inflammatory effect:

-Carrageenan induced rat paw edema method [58]

It is a method employed to evaluate the edema formed after administering an irritating agent such as carrageenan into the left hind paw. The drug treated animals were compared with the control group animals. Paw volume before treating an irritant and after treatment was recorded. The intensity of inflammation depends on the type of irritants being used.

Procedure

The rats of either sex male or female having a body weight of 150- 200g were used. Overnight the animals were starved. The rats were allowed to consume water to get a proper hydration. The rats were categorized with 6 animal species in 5 categories. Following is their classification:

Table 4: Experimental design of Carrageenan rat paw oedema method

Group	**Treatment**
I	Normal control (Normal saline) i.p
II	Standard drug (Diclofenac sodium, 10mg/kg) i.p
III	Leaf extract of *H.indicus*(100 mg/kg) p.o
IV	Leaf extract of *H.indicus*(200 mg/kg) p.o
V	Leaf extract of *H.indicus*(400 mg/kg) p.o

The lateral malleolus the paw was marked with a marker. In subsequent reading the paw was dipped up to this mark and using Plethysmograph paw volume was measured. 1% of carrageenan in normal saline was injected into the sub plantar region of left hind paw 30miuntes after administration of drug substances. The volume was measured for 30min,60min, 120min, and 180min.

Evaluation

The increase in paw edema volume after 30min, 60min, 120min and 180min was calculated as percentage inhibition and compared to the volume measured immediately after receiving an irritant. Animals thatare treated effectively exhibits a less amount of edema. For each treated and control groups the difference of average values calculated for each time intervals and statistic calculation was carried out. The percentage inhibition of edemawas calculated as follows:

V_t = In drug treated group volume of paw edema
V_c =In control group volume of paw volume

Anti-inflammatory activity assessment using in-vitro method:

-Inhibition of albumin denaturation [59]

The reaction combination (5 ml) contained 0.2 ml of fresh hen's egg albumin, 2.8 ml phosphate buffer saline with pH 6.4 and 2 ml of EEHI at varying concentrations. Distilled water serving as

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control had same amount as the test volume. Incubated at 37±2°C for 15 minutes in BOD incubator, the two mixtures were then later heated to a temperature of 70°C for around 5 minutes. When cooled, the absorbance was measured by a blank vehicle at 660 nm (SHIMADZU, UV 3600). At concentrations of 78.125, 156.25, 312.5, 625, 1250, 2500 µg/ml, the reference drug Diclofenac sodium was similarly tested, and absorptionwas identified.

Statistical analysis:

The results were shown as a mean ± SEM and evaluated using one-way analysis of variance (ANOVA), then Dunnette's test using the version 5 of the Graph Pad prism program. A statistically significant value of p is less than 0.05.

Results

Preparation of extract:

Percentage yield of ethanolic extract of Hemidesmus indicus leaves is as follows. (Figure 1)

Extract: Ethanolic extract
Color: Dark green
Consistency: Semisolid
Percentage yield: 8.6%

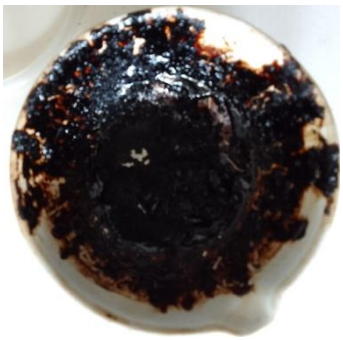

Figure 1: Ethanolic leaf extract of Hemidesmus indicus

Preliminary phytochemical analysis of ethanolic extract of Hemidesmus indicus leaves:

To determine the chemical constituents, present in the leaf of Hemidesmus indicus the ethanolic extract of the leaves were subjected for preliminary phytochemical analysis. The results obtained are as follows.

Table 5: Results of preliminary phytochemical analysis of Hemidesmus indicus leaves

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Sl No	Test	Result
1	Alkaloids	
	a. Dragendroffs	- ve
	b. Hager's test	- ve
	c. Wagner's test	- ve
	d. Mayer's test	- ve
2	Carbohydrates	
	a. Molisch's test	- ve
	b. Benedict's test	- ve
	c. Fehling's test	- ve
3	Flavanoids	
	Shinoda test	+ ve
4	Saponins	+ ve
5	Tannins	+ ve
6	Steroids	
	a. Libermann-Burchard test	- ve
	b. Salkowaski test	- ve
7	Tri terpenoids	
	Libermann- Burchard test	- ve
8	Glycosides	
	Molisch's test	- ve
9	Resins	- ve

Evaluation of analgesic activity:
-Tail immersion method

The increase in dose of ethanolic extract of *Hemidesmus indicus* leaves was found to increase in tail withdrawal reflex time. The obtained result of EEHI leaves at doses of 200mg/kg and 400mg/kg was statistically significant ($p < 0.05$). The result is summarized as follows.

Table 6: Effect of EEHI leaves on tail immersion method in rat

Group	Treatment	Dose mg/kg	Reaction time in seconds			
			Before treatment	30 min	60 min	90 min
I	Control	2	1.79 ± 0.05	1.84 ± 0.08	2.01 ± 0.18	2.06 ± 0.20
II	Standard	10	2.11 ± 0.17	5.67 ± 0.25***	7.16±0.16***	8.65 ± 0.33***
III	Ethanloic extract	100	1.66 ± 0.14	1.76 ± 0.14	2.23 ± 0.70	2.73 ± 0.16
IV	Ethanolic extract	200	2.05 ± 0.61	2.83 ± 0.22**	3.41 ± 0.24**	4.38 ± 0.39**
V	Ethanolic extract	400	1.99 ± 0.85	3.57 ± 0.42**	5.25 ± 0.43**	6.92 ± 0.11**

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All values are expressed as Mean ± SEM. N=06 animals in each group.***p<0.05 = highly significant, **p<0.05 =Significant.

Treated groups are compared with the control group, using one way ANOVA followed by Dunett's test.

-Acetic acid induced writhing method

In a dose dependent manner orally administered EEHI leaves at doses of 200mg/kg and 400mg/kg significantly (p<0.05) inhibited writhing response induced by acetic acid. The result obtained is summarized as follows.

Table 7: Effect of EEHI leaves on acetic acid induced writhing method in albino mice

Group	Treatment	Dose mg/kg	Number of writhings Mean± SEM
I	Control (saline)	0.1 ml	89.83 ± 2.12
II	Standard (Diclofenac)	10	25. 66 ± 1.05***
III	Ethanolic extract	100	84.16 ± 2.32
IV	Ethanolic extract	200	65.66 ± 1.14**
V	Ethanolic extract	400	46.33 ± 1.42**

All values are expressed as Mean ± SEM. N=06 animals in each group.
***p<0.05 = highly significant, **p<0.05 =Significant

Treated groups are compared with the control group, using one way ANOVA followed by Dunett's test.

Evaluation of anti-inflammatory activity:
-Carrageenan induced rat paw edema method
In a dose dependent manner orally administered EEHI leaves at doses of 200mg/kg and 400mg/kg significant (p<0.05) to inhibit the oedema formed. The result obtained is summarized as follows.

Table 8: Effect of EEHI leaves on carrageenan induced rat paw edema method in rats

Group	Treatment	Dose(mg/Kg)	30 min	60 min	120 min	180 min
I	Control	2ml/kg	0.51 ± 0.027	0.53 ± 0.023	0.54 ± 0.021	0.55 ± 0.021
II	Standard	10mg/kg	0.18± 0.018***	0.21±0.018***	0.19±0.010***	0.18±0.009***
III	Ethanolic Extract	100mg/kg	0.57 ± 0.052*	0.56 ± 0.019*	0.55 ± 0.018	0.54 ± 0.021
IV	Ethanolic Extract	200mg/kg	0.42 ± 0.24**	0.42 ± 0.015**	0.42 ± 0.014**	0.41 ± 0.013**
V	Ethanolic Extract	400mg/kg	0.34 ± 0.26**	0.34 ± 0.007**	0.34 ± 0.008**	0.31± 0.015**

All values are expressed as Mean ± SEM. N=06 animals in each group.
***p<0.05 = highly significant, **p<0.05 =Significant

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Treated groups are compared with the control group, using one way ANOVA followed by Dunett's test.

-Albumin denaturation method

The ethanolic extract of Hemidesmus indicus leaves exhibited a concentration dependent inhibition of protein denaturation. The concentration range was 100µg/ml to 400µg/ml. The results are summarized as follows.

Table 9: Absorbance of EEHI leaves at 660nm

Group	Concentration (µg/ml)	Absorbance	% Inhibition
	Control	0.023	-
1	31.25	0.381	34.32
2	62.50	0.463	42
3	125	0.518	53.43
4	250	0.604	66.57
5	500	0.6816	93.64
6	1000	1.076	101.98

Table 10: Absorbance of Standard (Diclofenac sodium)

Sl No	Concentration	Absorbance	% Inhibition
	Control	-	-
1	78.125	0.114	11.04
2	156.25	0.554	54.59
3	312.5	0.68	78.12
4	625	1.58	167.02
5	1250	1.67	172.12
6	2500	1.74	184.64

Discussion

This research was undertaken for determining analgesic and anti-inflammatory effect of ethanolic extract of Hemidesmus indicus leaves in experimental animals. A preliminary phytochemical analysis stated the presence of flavanoids, saponins, tannins, and coumarin olignoids. Due to the presence of various phytochemical constituents, it is revealed that Hemidesmus indicus leaves possesses a potential source of crude drug. Acute toxicity studies found that extract is safe up to 2000mg/kg of body weight. The methods used to evaluate analgesic activity are tail immersion method, Acetic acid induced writhing method. Anti-inflammatory activity is evaluated using in-vivo method like carrageenan induced rat paw oedema method and invitro method like inhibition of albumin denaturation. It was found that ethanolic extract of Hemidesmus indicus leaves exhibits a significant analgesic and anti-inflammatory activity in a dose dependent manner.

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The peripheral analgesic activity is evaluated by using acetic acid induced writhing method. The elevated level of peritoneal fluid of PGE2 and PGF2 is indicative of writhing response. Due to the peritoneal injection of acetic acid, there will be increase lipoxygenase products. The release of endogenous substances such as serotonin, bradykinins, and histamine occur, and this results in stimulation of nerve endings. The inhibition of prostaglandin synthesis by EEHI leaves occurs due to its action on visceral receptors which are sensitive to acetic acid thereby it shows anti nociceptive effect [60]. Central analgesic activity is evaluated by using tail immersion method by causing thermal pain. The action via supra spinal and spinal receptors like μ, ð, k is exhibited by drugs acting like morphine. The release of endogenous peptides such as endorphin,encephalin is due to action of drugs like morphine which is because of the activation of the peri aquaductal area in midbrain. In the synapse of dorsal horn, the endogenous peptides act as inhibitors of impulse transmission by descending into spinal cord. The relevant mechanism attributing ethanolic extract of Hemidesmus indicus exerts its action on central opioid receptors this may be possible mechanism, or it may cause release of endogenous opioid peptides. In the current study ethanolic extract of Hemidesmus indicus at a dose of 200mg/kg body weight and 400mg/kg body weight were significant. In tail immersion method at 90min the percentage increase in reaction time was maximum. Therefore, the maximum latency for thermal condition and reduction in writhing response are the important factors in tail immersion and acetic acid induced writhing method for evaluating analgesic activity [61].

Acute inflammation is evaluated by using standard experimental model such as carrageenan induced rat paw oedema method. Carrageenan is used as an inducing agent, and it produces a biphasic response. The initial phase i.e., up to 2.5 hour after administering a inducing agent, involves a release of histamine, serotonin and Kinin and in the next phase release of prostaglandin and slow reacting substance occurs which will be high at 3 hours. This results in the production of free radicals and cycloxygenase. The cycloxygenase inhibitors as well as lipoxygenase inhibitors are sensitive to carrageenan induced rat paw oedema method which involves inhibition of prostaglandin synthesis by regulating enzyme cycloxygenase [62]. Ethanolic extract of Hemidesmus indicus leaves possess a significant inhibition of rat paw oedema. However, the inhibition is less when compared to standard drug Diclofenac sodium. From above data it is found the 200mg/kg and 400mg/kg body weight of ethanolic extract of Hemidesmus indicuspossess an effective inhibition of rat paw oedema. From the obtained data it is correlated that the inhibition of enzyme cycloxygenase and thereby inhibiting prostaglandin synthesis is the main mechanism through which ethanolic extract of Hemidesmus indicus possesses an anti-inflammatory activity [63].

To study the anti-inflammatory activity of EEHI leaves protein denaturation bioassay is used as an invitro method. In inflammation, denaturation of tissue protein is a main factor. The drugs which are capable to prevent protein denaturation are chosen in drug development process for inflammation. In this investigation the EEHI leaves showed increase in absorbance as compared to control. Hence EEHI has anti-inflammatory effect [64].

The results obtained in the present study revealed that the experimental animals like mice and rats treated with ethanolic leaves extract of Hemidesmus indicus produced a significant analgesic and anti-inflammatory

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activity in all the animal models as well as in in-vitro study when compared to the control.

Conclusion

The acute toxicity study on Hemidesmus indicus leaves proved that it is safe up to 2000mg/kg of body weight. The preliminary phytochemical analysis of Hemidesmus indicus leaves revealed the presence of saponins, tannins, flavanoids and coumarin olignoids as a pytoconstituents.

The ethanolic extract of Hemidesmus indicus leaves was evaluated in three different dose levels such as 100mg, 200mg, 400mg/body of the animal. The analgesic activity was evaluated for both steroidal and non-steroidal type of an activity. Chemical induced writhing method is used for evaluating peripheral analgesic activity and tail immersion method is used for evaluating central acting analgesic activity. In both the methods the extract at doses of 200mg and 400mg/ body weight of animal was significant when compared to control.

The anti-inflammatory activity was evaluated by carrageenan induced rat paw oedema method for evaluating acute inflammation. In this method the test drug dose at 200mg and 400mg/kg of body weight was significant. The invitro method such a protein denaturation is used to evaluate the ability of extract to stabilize the albumin. The ethanolic extract of Hemidesmus indicus had ability to prevent denaturation for heat treated protein.

Therefore, from all these above findings it can be concluded that ethanolic extract of Hemidesmus indicus leaves possess an analgesic and anti-inflammatory activity. Tounderstand the exact mechanism involved by the phytoconstituents present in the Hemidesmus indicus leaves, further studies on isolation and phytoconstituents mechanism of action need to be carried out.

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