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QUALITATIVE AND QUANTITATIVE ASSESSMENT OF PHYTOCHEMICALS, IN VITRO AND IN VIVO STUDIES ON MEDICINAL PLANTS TRIBULUS TERRESTRIS, PIPER NIGRUM AND CICHORIUM INTYBUS

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Abstract:
Arthritic disorders include a variety of over one-hundred (100) autoimmune and chronic degenerative conditions associated with persistent or repeated bouts of pain, inflammation, and physical impairment. The prevalence of arthritic disorders and musculoskeletal pain varies from fourteen to thirty-six (14 to 36) percent in developed nations. Traditionally Tribulus terrestris, Piper nigrum and Cichorium intybus have potential against arthritis, therefore scientific evidence is not available yet therefore, purpose of the current research was the assessment of phytochemicals either qualitative and quantitative, in vitro evaluation of the antioxidant activity of medicinal plants Tribulus terrestris, Piper nigrum and Cichorium intybus, in vivo acute toxicity testing and explore anti-arthritic potential of hydro-ethanolic test samples on male Wistar albino rats. In current study qualitative phytochemical analysis was performed for the identification of phytochemicals in Piper nigrum, Cichorium intybus and Tribulus terrestris hydroethanolic extract further followed by quantitative analysis of these phytochemicals. It has been proven that Piper nigrum, Cichorium intybus and Tribulus terrestris has potential source of radical scavengers. All samples shown high DPPH scavenging potential with IC50 Tribulus terrestris 69.99 ±14.58, Cichorium intybus 68.41 ±14.73 and Piper nigrum 77.81 ±11.8 with p value <0.05 which shown the significance of antioxidant activity. No toxicity signs or deaths were recorded during the 72 hours of treatment by oral route with Cichorium intybus but Piper nigrum and Tribulus terrestris shown 20% mortality rate at the highest dose of 4000mg/kg. In vivo studies shown that the hydro-ethanolic extract of medicinal plants reduced dose dependant anti- arthiritic activity. As noted, there was pronounced percentage inhibition of paw edema at the sixth hour. Cichorium intybus shown the highest 1.73, 2.90, 1.85, 1.35 anti- edematous activity then Piper nigrum 1.76, 2.57, 1.75, 1.55 and Tribulus terrestris 1.73.
2.16, 2.55, 1.55 at the highest concentration at 200 mg/kg at 0, 1, 3 and 6 hours after induction of carrageenan control. The current study estimated Piper nigrum, Cichorium intybus and Tribulus terrestris are harmless and cost effective therapy for the management of arthritis.

Key words: Medicinal plants, Immunomodulatory; Antiarthritic, Mortality rate, Hydro-ethanolic extract.

1. Introduction:

Arthritis is a chronic musculoskeletal illness that affects a large number of people. Arthritis affects people of all ages and comes in a variety of forms, the most common of which being rheumatoid

arthritis and osteoarthritis. Weight-bearing joints (the knee, hips, foot, and spine), synovial joint lining, peri-articular bone, and surrounding supportive connective tissue components are all affected by arthritis (Khare and Shukla 2022). External and internal therapies have been used to treat it. Various genetic and environmental variables create different phenotypes and are linked to the complicated arthritis process. The complement system and immune complex that cause the development of rheumatoid arthritis are maintained by cytokines. Metalloproteinases have an impact on immune complexes. Antigens activate CD4+ T-cells, which then activate macrophages, monocytes, and synovial fibroblasts. TNFa cytokines interlukin-6 and interlukin-1 are generated as a result. Matrix metalloproteinases secretion is boosted by cell surface signaling (Garred, Tenner et al. 2021). Rheumatoid arthritis (RA) and osteoarthritis(OA) are two forms of arthritis. Chronic inflammation is a side effect of RA, which is an autoimmune illness. This form of arthritis is caused by synovial membrane hyperplasia, which results in large-scale bone loss surrounding the joints. With cardiovascular, skeletal, and physiological problems, some symptoms such as pain, stiffness, and limited movement are present. Nonsteroidal anti-inflammatory medicines (NSAIDs) and steroids are two treatments that can help manage RA (Cao, Peng et al. 2021).Osteoarthritis affects more women than males after menopause, and it mostly affects the joints of the hands, hips, and knees. It is a disease that causes cartilage deterioration in the joints and is the leading cause of disability worldwide (Long, Liu et al. 2022).

Zingiber officinale, Curcuma longa, Calotropis procera, Camellia sinensis, Ficus benghalensis, Actaea racemose, Piper nigrum, Withania somnifera, Smilax officinalis, Uncaria tomentosa, Persea Americana (Konar, Mukherjee et al. 2022) and many other medicinal plants have anti-arthritic properties but in current research we used three medicinal plants Tribulus terrestris, Piper nigrum, Cichorium intybus belongs to the family of Zygophyllaceae, Piperaceae, Asteraceae are used for gouty arthritis.

Purpose of current research was hydro-ethanolic extract preparation of medicinal plants Tribulus terrestris, Piper nigrum and Cichorium intybus. Phytochemical screening (qualitative and quantitative) of selected medicinal plants. In vitro evaluation of the antioxidant activity of test samples. In vivo acute toxicity testing of plants and evaluation of the anti-arthritic activity of test samples which are used for management of arthritic disorders.

2. Materials and methods:

2.1 Extraction

Three plants were subjected to research study, primarily Piper nigrum, Cichorium intybus, and Tribulus terrestris. After collection and drying, plants were subjected to identification and assignment of voucher numbers. The plants were purchased from the market. One kilogram of the plant sample was thoroughly rinsed and washed twice with freshwater followed by deionized water, to remove dust and other contaminants. After being washed, the sample was kept under shade at room temperature and dried completely. The dried mass of plants was then crushed, using a grinder, into a fine powder, and extracted using the maceration method in the aqueous ethanolic solvent with a ratio of 70:30. The resulting aqueous ethanolic mixture was filtered on muslin cloth.

After 72 hours of soaking, the filtrate was filtered again on Whatman filter paper. This process was replicated once more, three times in total, combining all three filtrates. The solvent (ethanol) was evaporated to concentrate crude extract on the rotary evaporator. This concentrated crude extract was further air dried and the semi-solid mass of crude drugs was obtained. This collected crude extract was processed at 4°C in the fridge for future use in pharmacological activities.

2.2 Phytochemical Analysis:

Analysis for identification of the major phyto-constituents was carried out as follows:

2.2.1 Qualitative analysis: Various phytochemicals including alkaloids, flavonoids, tannins and saponins was detected in plant extract preparation by standard methods (Jain, Jain et al. 2014).

2.2.2 Quantitative estimation of phytochemicals:

2.2.2.1 Total phenolic content (TPC): The Folin-Ciocaletu reagent method was performed to find out the TPC in plant extracts (Jain, Jain et al. 2014).

2.2.2.2 Total flavonoid content (TFC): Total flavonoid content was determined by following the method (Pranuthi, Narendra et al. 2014).

2.3 In vitro Antioxidant Assay:

2.3.1 DPPH free radical scavenging Assay: Using the previously established 1,1-diphenyl-2-picrylhydrazyl (DPPH) stable as a free radical scavenger. Samples (62.5, 125 and 250 µg/mL) were combined with one millilitre of a 90µl DPPH solution a final volume of 4 mL by adding 95% methanol. After an hour, the absorbance of blank and test solutions was recorded at ambient temperature The specimen ascorbic acid was used as a positive control. Every sample was examined in triplicate. DPPH colour changes was measured by employing a spectrophotometer and spectrophotometric measurements at 517. Defeat of free radicals in percentage (%) was calculated by following equation;

$$Scavenging\ activity\ \% = 100 - \frac{Abs.of control - Abs.of\ sample}{Abs.control} \times 100$$

IC_{50} values, that demonstrate the concentration of extracts that caused 50% neutralization of DPPH radicals, were calculated from the plot of inhibition percentage against concentration(Chatha, Hussain et al. 2014).

2.4 In Vivo Studies:

2.4.1 Experimental animals: The investigation was utilizing either male Wister rats. Wister rats was weigh between 145 and 250 grams. The animals were kept in a typical environment (23°C, 40–70% humidity, and a 12h/12h light/dark cycle). The animals were fed a regular pelleted diet (CHO 60%, protein 20%, fat 5%, fiber 5%), while having constant access to water. One week prior to the start of the trial, the animals were integrated. The Animal Ethical Committee of the Islamia University of Bahawalpur was approve the study protocol.

2.4.2 Experimental design Dose calculation: For the sub-acute toxicity study, high dosages were chosen. Before the beginning of the study, the dose of every rat in all groups were designed according to their body weights.

2.4.3 Acute toxicity study test

The assay of acute toxicity was performed with respect to the Organization for Economic Cooperation and Development (OECD) guideline. The mice were starved for 24 hours prior to the start of the investigation. A total of ten mice of both sexes were used. There was strict observation. The animals were monitored for the first half-hour after medication, then for the next 24 hours and 72 hours. Weekly, changes in autonomic effects, central nervous system, and animal weight were monitored, as well as behavioral and morphological alterations (Iserhienrhien 2022).

2.4.4 In Vivo Anti-Arthritic Activity:

2.4.4.1 Carrageenan-induced paw edema: In this experiment, male Wistar albino rats (n = 6) were used. Before the trial, the animals were fasted overnight with constant access to water. A digital plethysmometer was used to record baseline paw volumes in centimeters on the day of the trial (Ugo Basile 7140). Following that, the group was given 2 ml/kg of 1% gum acacia suspension (vehicle control), indomethacin (3 mg/kg), and drug samples to test via gavage. Subcutaneous delivery of 0.1 ml of 1 percent carrageenan (freshly constituted in normal saline) into the sub-plantar surface of the animal's left hind paw would produce paw inflammation thirty minutes after administration of the vehicle/drug. At one, three, and six hours after carrageenan administration, the volume of the paws were measured (Nair, Kumar et al. 2012).

3. Results:

3.1 Phytochemical Screening:

3.1.1 Qualitative assessment of Phytochemicals shown in table no. 1.

3.1.2 Quantitative estimation shown in table no. 2 and 3.

3.2 Antioxidant activity:

DPPH Radical Scavenging Activity shown in table no. 4.

3.3 In Vivo Activities:

3.3.1 Toxicity studies shown in table no. 5

3.4 Anti-arthritic activity:

Effect of Medicinal plants on carrageenan induced paw edema in male Wister albino rats shown in table no. 6.

4. Discussion:

It can be concluded from the results of the present study that the medicinal plants Piper nigrum, Cichorium intybus and Tribulus terrestris are potential sources of radical scavengers. The result of radical scavenging activity shown highest antioxidant activity of Cichorium intybus 82.1%, 65.65%, 49.2% then the Piper nigrum 75.2%, 61.7%, 48.2% and Tribulus terrestris 79.7%,62.95%, 46.2% at the concentration of 250, 125, 62.5µg/ml. All samples show high DPPH scavenging

potential shown in table 4 with IC50 Tribulus terrestris 69.99 ±14.58, Cichorium intybus 68.41 ±14.73 and Piper nigrum 77.81 ±11.8. Table 1 shows the qualitative phytochemicals which are present in Piper nigrum, Cichorium intybus and Tribulus terrestris medicinal plants. Protein, alkaloids and tannins are present in Piper nigrum and Cichorium intybus but absent in Tribulus terrestris. Carbohydrates, saponins and fixed oil was present in Piper nigrum, Cichorium intybus and Tribulus terrestris medicinal plants. Phenols and flavonides was present in Piper nigrum and Tribulus terrestris. Glycosides was present in Cichorium intybus and Tribulus terrestris but absent in Piper nigrum. Quantitative analysis shows that Piper nigrum, Cichorium intybus and Tribulus terrestris has total phenolic contents 238.7, 250.9 and 300.6(mg/g) GAE with IC50 95.5 ±3.16, 80.8 ±2.37, 45.9 ±4.89 µg/ml shown in Table 2 and total flavonoids contents 97.5, 85.2, 90.7(QE /g of extract) with IC50 72.8 ±3.85, 79.6 ±1.91, 75.3 ± 1.23µg/ml shown in table 3. The value of p was <0.05 which shows the significance of antioxidant activity.

No toxicity signs or deaths were recorded during the 72 hours of treatment by oral route with Piper nigrum and Tribulus terrestris at doses of 2000 mg/kg but at the dose of 4000mg/kg the mortality rate was 20% shown in table 5. The medicinal plant Cichorium intybus was non-toxic or it cause no death even at the dose of 4000mg/kg and the mortality rate was 0%. The hydro-ethanolic extract of Piper nigrum, Cichorium intybus and Tribulus terrestris fundamentally restrained the edema development in both the primary and second stages. In the result of present study, the anti-edematous action of medicinal plant persevered in the second stage with the maximal impact found at 6h. As noted, there was pronounced percentage inhibition of paw edema at the Sixth hour shown in table 6. Cichorium intybus shown the highest 1.73, 2.90, 1.85, 1.35 anti- edematous activity then Piper nigrum 1.76, 2.57, 1.75, 1.55 and Tribulus terrestris 1.73. 2.16, 2.55, 1.55 at the highest concentration at 200 mg/kg at 0, 1, 3 and 6 hours after induction of Carrageenan control.

5. Conclusion:

These findings demonstrate that medicinal plant Piper nigrum, Cichorium intybus and Tribulus terrestris shown the value of p was <0.05 which shown the significance of activities. Therefore, further studies are needed to evaluate the effects of these medicinal plant on clinical trials.

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7. Conflict of Interests Declaration

In relation to the research, authorship, and/or publication of this work, the authors disclosed no conflicts of interest.

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Table No 1. Phytochemical screening of medicinal plants

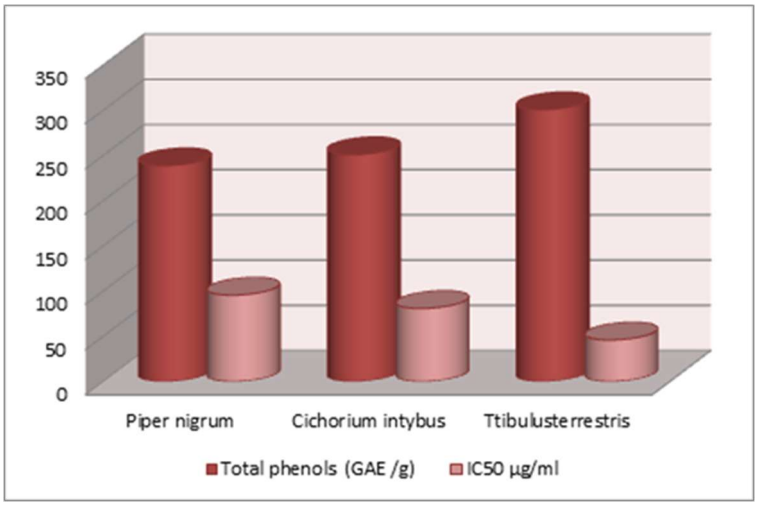
Primary Metabolites	**Tests performed**	*Piper nigrum*	*Cichorium intybus*	*Tribulus terrestris*
Protein	Ninhydrin Test	+	+	-
Carbohydrate	Iodine Test	+	+	+
Tannins	$Fecl_3$ Test	+	+	-
Phenoles	$Fecl_3$ Test	+	-	+

Flavonoids	Shinoda Test	+	-	+
Saponins	Foaming Test	+	+	+
Glycosides	Salkowski's Test	-	+	+
Steroid		-	-	+
Alkaloids	Mayer's Test	+	+	-
Fixed oil	Spot Test	+	+	+
Gum and Mucilages		-	-	-
Quinones		-	-	+

The symbols+ appreciable amounts and – refer to absent amounts, respectively.

Table No 2. Total phenolic contents

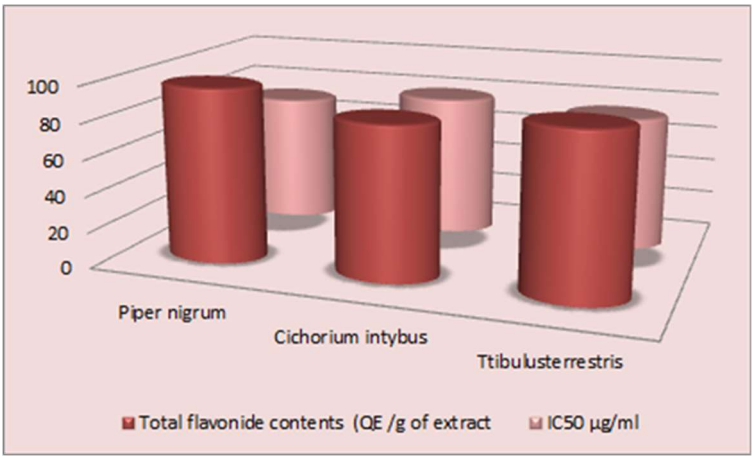
Plant Extracts	Total phenolic contents (mg/g) GAE	IC_{50} µg/ml + S.D
Piper nigrum	238.7	95.5 ±3.16
Cichorium intybus	250.9	80.8 ±2.37
Tribulus terrestris	300.6	45.9 ±4.89



Graph 1. Total phenolic contents calculations

Table No 3. Total flavonoids contents

Plant Extracts	Total flavonoids contents (QE/g of extract)	IC_{50} µg/ml + S.D
Piper nigrum	97.5	72.8 ±3.85
Cichorium intybus	85.2	79.6 ±1.91
Tribulus terrestris	90.7	75.3 ± 1.23

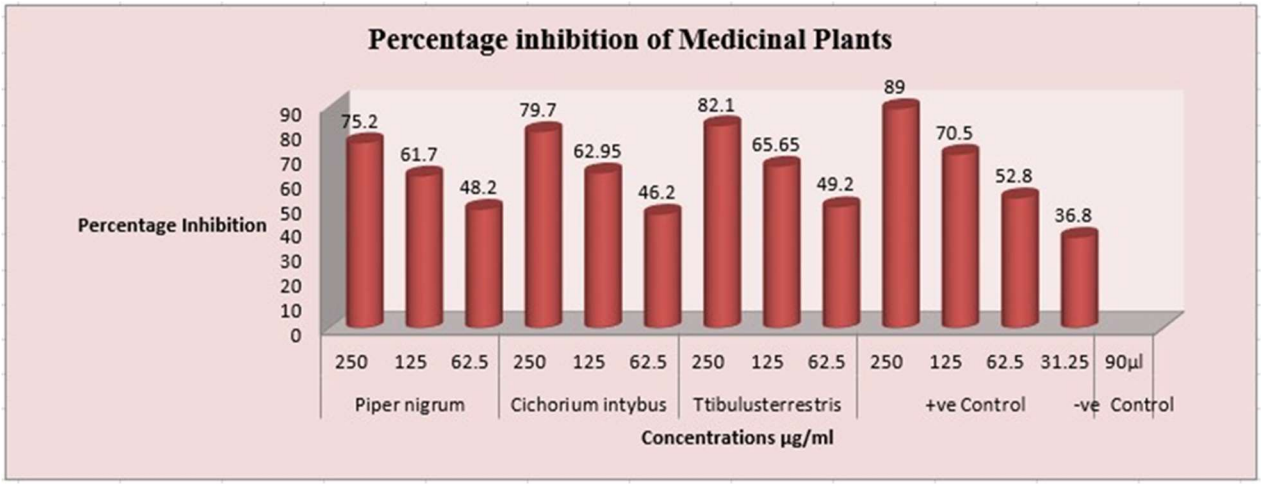


Graph 2. Total flavonoid contents calculations

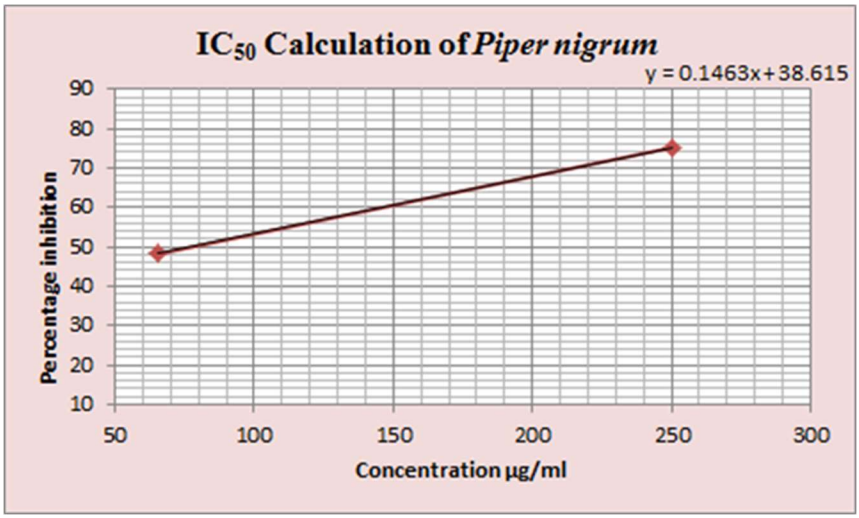
Table No 4. Antioxidant Activity of Medicinal Plants

Sr. #	Samples	Dose Conc. µg/ml	Percentage inhibition	IC_{50} µg/ml + S.D
1	Piper nigrum	250	75.2	77.81 ±11.8
2		125	61.7	
3		62.5	48.2	
4	Cichorium intybus	250	79.7	68.41 ±14.73
5		125	62.95	
6		62.5	46.2	
7	Tribulus terrestris	250	82.1	69.99 ±14.58

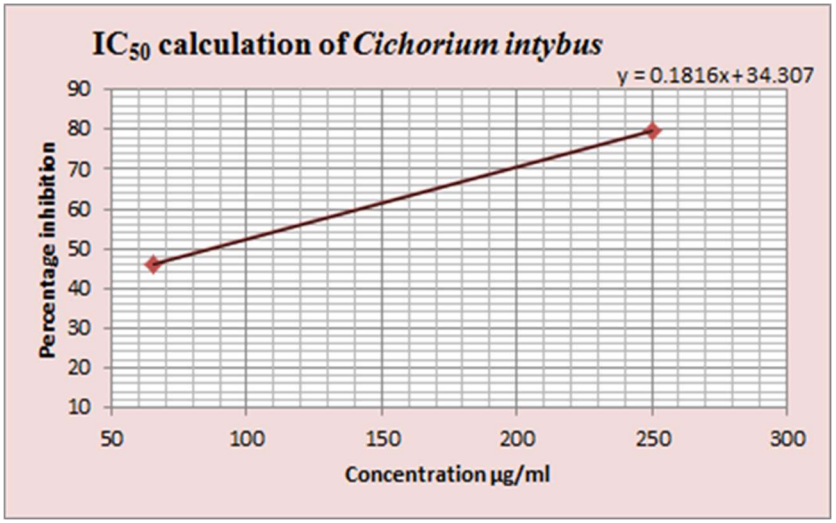
8		125	65.65	
9		62.5	49.2	
19	+ve Control	250	89.0	62.67 ±15.94
20		125	70.5	
21		62.5	52.8	
22		31.25	36.8	
23	-ve Control	90µl		



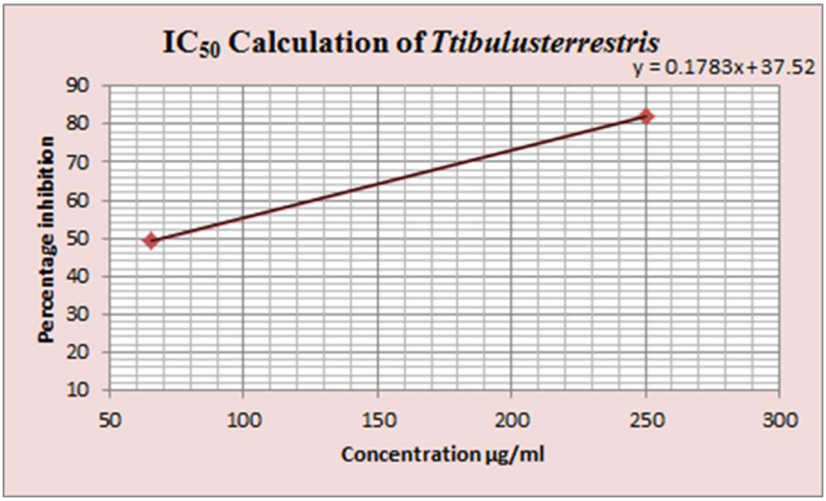
Graph 3. Graphical representation of Percentage inhibition



Graph 4. IC_{50} Calculation of Piper nigrum



Graph 4. IC_{50} Calculation of Cichorium intybus



Graph 5. IC_{50} Calculation of Tribulus terrestris

Table No 5. Oral acute toxicity test of medicinal plants

Groups	Samples	Dosage mg/kg	Animal Number	Death Number	Mortality rate
1	Control group		5	0	0
2	*Piper nigrum*	1000	5	0	0%
		2000	5	0	0%
		4000	5	1	20%
3	*Cichorium intybus*	1000	5	0	0%
		2000	5	0	0%
		4000	5	0	0%
4	*Tribulus terrestris*	1000	5	0	0%
		2000	5	0	0%
		4000	5	1	20%

Table No. 6 Effect of Medicinal plants on carrageenan induced paw edema in male Wister albino rats.

Treatments	Dose mg/kg	0 h	1 h	3h	6h
Carrageenan control		1.77	3.75	3.80	3.90
Negative Control		1.76	1.70	1.70	1.70
Indomethacin	3	1.75± 0.374	2.80±0.168	2.65±0.987	1.95±0.54
Piper nigrum	50	1.75± 1.275	3.75±0.989	2.90±0.873	2.03±0.75
	100	1.77± 0.988	3.13±0.756	2.55±0.434	1.90±0.67
	200	1.76±0.567	2.57±0.678	1.75±0.87	1.55±0.54
Cichorium intybus	50	1.69±0.675	3.32±1.87	2.80±0.235	2.01±0.006
	100	1.72±0.874	3.80±0.456	2.95±0.54	1.85±0.05
	200	1.73±1.32	2.90±0.87	1.85±0.87	1.35±0.09
Tribulus terrestris	50	1.76±0.02	3.71±1.933	2.55±0.76	2.00±0.654
	100	1.77±0.113	2.90±0.076	2.30±0.654	1.80±0.9

	200	1.73±0.015	2.16±0.76	1.55±0.00	1.45±0.87