Evaluation of poly herbal extract for treatment of osteoarthritis

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ABSTRACT:

Osteoarthritis causes degenerative changes in the joints i.e. “wear and tear” resulting in pain and impaired function.Pathological cartilage loss occurs due to imbalance in the catabolic& anabolic mechanisms of cartilage remodeling.Ethanolic extract of Cissus*quadrangularis* and*Nigella sativa* in different combination was prepared such as *Cissus quadrangularis* and *Nigella sativa* (50:50, 20:80 and 80:20).Anti-inflammatory evaluation of extract was done by using carrageenan induced paw edema model ; analgesic activity evaluation of extract was done by using acetic acid-induced writhing test andosteoarthritis evaluation of extract was done activity by using collagenase type II-induced osteoarthritis (CIOA) rat model was carried out.Though the combination ethanolic extract *Cissus quadrangularis* and *Nigella sativa* (20:80)shows better anti-inflammatory and analgesic activity; ethanolic extract of *Cissus quadrangularis* and *Nigella sativa* (80:20) was found to be more efficient in treating osteoarthritis.

Keywords: Osteoarthritis, Cissus*quadrangularis* and *Nigella sativa*

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Introduction:

Osteoarthritis : The most prevalent type of arthritic illness osteoarthritis causes degenerative changes in the joints as a result of everyday "wear and tear” resulting in discomfort pain and impaired function.[1]Pathological cartilage loss caused by a mismatch between the anabolic and catabolic mechanisms of cartilage remodeling, which are triggered by oxidative and inflammatory alterations in the tissues that surround it, mainly the synovium and subchondral bone.[2-4]Despite the fact that OA is the most common musculoskeletal syndrome in the world, with significant physical, economic, and societal consequences, specialists were unable to pinpoint its exact origin. Overuse overloading and misalignment of the limbs as well as genetic abnormalities and metabolic disturbances obesity inflammatory responses and diabetes are all key contributors to the onset and development of OA[5]Nonsteroidal anti-inflammatory drugs (NSAIDs) the most commonly prescribed therapeutic approaches for osteoarthritis are nonsteroidal anti-inflammatory drugs (NSAIDs) and analgesics. While these medications might improve the symptoms, they aren't the best treatment options. Peptic ulcers and (less usually) renal or hepatic failure are significant side effects of NSAIDs. Furthermore, none of these medications help to reduce or prevent progression of OA. Furthermore, there is proof that NSAID administration may hasten joint degeneration in both animals and humans with experimental OA. [6- 8]The cartilage of joint breaks down as a result of inflammatory OA A slippery tissue that envelopes the ends of the bones in a joints is called cartilage. The trauma of movements is absorbed by healthy cartilage. Bones rub together when cartilage is lost. This friction might cause lasting injury to the joint over time[9]

Broadly OA is categorized into two different forms:

Primary or Idiopathic OA: It depends on gene or aging related condition primary OA has quite a stronger genetic component because of its polygenic nature.[10-12]

Secondary or Post-traumatic OA: Secondary OA occurs after a any traumatic incidence It is commonly associated along with micro traumas earlier knee surgery as well as other multiple factors which includes Endocrinology (Diabetes, thyroid problems hyperparathyroidism) Metabolic activity (hemochromatosis, ochronosis, Marfan syndromes Ehler Danlos syndromes) genetically inherited weight gain and so on In today's world lifestyles diets overweight and a lack of physical activity are all significant contributors to the onset of OA [12]

Hadjod: It consist of dried or fresh plant of Cissus*quadrangularis* belonging to family Vitaceae[13] Hadjod (Had means bone, Jod means setter), bone setter and Adamant creeper, Square stalked vine, veldt grape, devil's backbone, adamant creeper, nalleru, sannalam, vajravelli, mangara valli, asthisamharaka, hadjod as well as pirandai.[14] Surface appears smooth, glabrous, buff in colour with a greenish tint, with a red - brown angular section; there is no flavour or odour. Simple leaves are 2.5-5 cm long, widely oblong as well as reniform, often 3-7 lobed, smooth, denticulate, cordate, spherical, truncate or cuneate just at base; petioles are 6-12 mm in length; stipules are tiny, broadly ovate, and obtuse. Short peduncle cymes and spreading umbellate branches characterise the flowers. Cup-shaped, tapered, or lobed in a somewhat vague way calyx is visible. Petals are four in number, ovate-oblong, short, and stout. Berries have a long apiculate form and are obovoid or globose in shape. When ripe, the berries are crimson in hue and have one (rarely two) seeds[15,16]Phytoconstituents present inHadjod are Triterpenes - α- and β- amyrine, β- sitosterol, phenol, tannins, carotene and vitamin C. Unsymmetric tetracyclic triterpenoids- d-amyrin, and 3,3',4,4'-tetra hydroxy biphenyl. Flavonoids- quercetin and kaempferol, and stilbene derivatives, quadrangularins A,B,C ,resveratrol, pallidol, and phytosterols . Stem extract is abundant in calcium ions as well as phosphorus, both of which are necessary for bone formation. Hadjod possess antibacterial activity, antioxidant and free radical scavenging potential,central nervous system activity,antiulcer activity, anthelmintic activity, bone healing Activity[17]

Kalonji : Synonym of Kalonji is Black cumins, Love-in-a-mist. It consist of dried seeds of *Nigella sativa* belonging to familyRaunculaceae . Phytoconstituents present in kalonji are fixedoil, protein, alkaloids, saponin, as well as 0.4 - 2.5 percent essential oil are all found in *N. sativa* seeds. Unsaturated fatty acids such aseicosadienoic, arachidonic, linolenic acid, and linoleic acid make up the majority of the fixed oil. Palmitic, myristic and stearic acids are the saturated fatty acids found in the oil. Many components were characterized but the pharmacologically active constituent of volatile oil are thymoquinone, dithymoquinone, thymol and thymohydroquinone Dithymoquinone is the dimerised form of Thymoquinone.Pharmacological properties of *N. Sativa* seed are asantioxidant activity, analgesic and anti-inflammatory activity, antidiabetic activity, anti-cancer activity, wound healing properties, nephroprotective activity, and effect on nervous system.[18,19]

Material Collection and Authentication: The plant Cissus quadrangularis and Nigella sativa were distributed throughout the India, It has been collected it from the Local Market. The plant was authentified by Dr. Rashmi Mishra ; Botanist ; Bilwal Medchem and Research Laboratory Pvt Ltd, Jaipur , Rajasthan on 15/01/2022 (Letter no:- BMRL/PA/2022-12)

Extraction of Plants:

Extractions of Cissus quadrangularis[20]: The powder of Cissus quadrangularis was subjected to ethanolic solvent extraction using sohxlet apparatus. Then extract was concentrated and subjected to phytochemical screening, Pharmacological Screening as well as used in formulation.

Extractions of Nigella sativa[21]: The powder was subjected for extraction by using ethanol and Soxhlet extractor. The extract was filtered and the solvent (ethanol) evaporated and subjected to phytochemical screening, pharmacological Screening as well as used in formulation.

Animals: The study was carried out on Albino wistar rats and mice weighing between 100 – 300 g. They are obtained from animal house of Bilwal medchem and Research laboratory Pvt. Ltd, Jaipur, Rajasthan (Reg. No.-2005/PO/RcBT/S/18/CPCSEA). Experimental protocol is approved by IAEC of Bilwal medchem and Research laboratory Pvt. Ltd. (IAEC approval number- BMRL/IAEC/2022-18 Dated: 24/02/2022).Rats were fed with Amrut rat feed manufactured by Pranavagro Ltd. Sangali, Maharashtra. RO purified water is available for the drinking purpose for the animals. Clean sterilized husk is used as a bedding material for the animals. Animals were placed in clear propylene cages under 12:12 light dark lighting schedule. Animal house is well ventilated with appropriate humidity and temperature.

Polyherbal Extract of Test Drug: Ethanolic extract of Cissus quadrangularis and Nigella sativa was mixed in following ratio

Test sample A: Ethanolic extract of Cissus quadrangularis and Nigella sativa (50:50)

Test sample B: Ethanolic extract of Cissus quadrangularis and Nigella sativa (80:20)

Test sample C: Ethanolic extract of Cissus quadrangularis and Nigella sativa (20:80)

In-vivo animal experimental study:

1)Acute oral toxicity study[22] : Acute oral toxicity study will be conducted according to OECD guideline 423 ANNEX 2c.

Study design: Three animals was selected for each group. Three polyherbal formulations are prepared by the ethanolic extract of Cissus quadrangularis and Nigella sativa.

The dose level used as follows

Group 1: Administered dose was 2000 mg/kg test sample A.

Group 2: Administered dose was 2000 mg/kg test sample B.

Group 3: Administered dose was 2000 mg/kg test sample C.

Administration of doses: The test substance was administered in a single dose by gavages using an oral feeding needle. Animals was kept fasting prior to dosing. Following the period of fasting, the weight of each animal was measured and the test substance was administered. After the administration of test substance food may be withheld for a further 3-4 hours in rats.

Observations: Animals was observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention was provided during the first 4 hours and daily thereafter, for a total of 14 days. All observations was systematically record for each animal. Observation includes-1.Behavioral changes; 2. Biochemical changes; 3. Histopathological changes

2)Evaluation of analgesic activity (Acetic acid-induced writhing test in mice)[23]: Female Swiss albino mice (25 – 30 g) were treated according to the method described by Koster et al, 1959. Mice were pre-treated orally with test samples and acetylsalicylic acid, 60 min before administration of acetic acid solution at a dose of 10 ml/kg (0.6%, I.P.). The number of abdominal constrictions (full extension of both hind paws) was cumulatively counted over a period of 15 min.

The mice were divided into five groups of six mice each.

Group 1: - Vehicle control (2% Tween 80)

Group 2: - Standard (Acetylsalicylic acid 100 mg/kg p.o.)

Group 3: Test sample A (200 mg/kg)

Group 4: Test sample B (200 mg/kg)

Group 5: Test sample C (200 mg/kg)

The percent inhibition of writhing was calculated as follows:

% Inhibition = (VC-VT/VC) x 100; Where, VT, number of writhes in drug treated mice; VC, number of writhes in control group mice.

3) Evaluation of anti-inflammatory activity (Carrageenan induced paw edema in rats)[24]: Female Wistar rats (180 – 220 g) were treated according to the method described by Winter et al, 1962. Inflammation was produced by

injecting 0.1ml of 1% lambda carrageenan in sterile normal saline into the sub plantar region of the right hind paw of the rat. Rats were pre-treated orally with test samples and diclofenac 1h before the carrageenan injection. The paw volume was measured from 0-6 h, at an hourly interval using plethysmometer. The mean changes in injected paw volume with respect to initial paw volume were calculated.

Female Wistar rats were divided into five groups of six rats each.

Group 1: Carrageenan control (2% Tween 80).

Group 2: Standard (Diclofenac 10 mg/kg p.o.).

Group 3: Test sample A (200 mg/kg)

Group 4: Test sample B (200 mg/kg)

Group 5: Test sample C (200 mg/kg)

4)Evaluation of anti-Osteoarthritis activity (Collagenase type II-induced osteoarthritis (CIOA) rat model)[25.26]: Induction of OA Rats were anesthetized with diethyl ether (Merck, India). The shaved right knee joints of Group 1 were injected with 50 µl of normal saline solution which served as healthy control. Animals from the Group 2 were injected with collagenase type II (from Clostridium histolyticum, obtained from Sigma Aldrich, USA). Collagenase was dissolved in saline and 50 µl (50 units) was injected intra-articularly into the right knee joint. The injection was given twice, on days 1st and 4th of the experiment. Injections were given using 31- gauge 0.25 X 8 mm needle.

Experimental group design

Group 1: Normal saline (50 µl)

Group 2: Collagenase (50 µl)

Group 3: Collagenase + Indomethacin (3 mg/kg)

Group 4: Collagenase + Test sample A (200 mg/kg)

Group 5: Collagenase + Test sample B (200 mg/kg)

Group 6: Collagenase + Test sample C (200 mg/kg)

Body weight, Knee diameter and Paw volume measurement : Changes in body weight and knee diameter were measured on days 0th , 5th , 10th , 15th, 20th , 25th and 30th . Knee diameter was measured using digital Vernier caliper. Mean changes in body weight and joint swelling after treatment were calculated. Paw volume was measured once in a week using plethysmometer.

Glycosaminoglycans (GAG) release[27]: Blood was taken from rats before and after the treatment through retro-orbital vein puncture and serum was separated. Extracellular matrix of cartilage contains proteoglycans, which consists of a core protein to which glycosaminoglycans (GAGs) chains are covalently attached. The ability of the cartilage to stand compressive forces is aided by the sulfated GAGs such as chondroitin sulfate GAG release from explants into the surrounding fluid, is a proven marker of cartilage matrix damage. GAG content in serum was measured by 1, 9-dimethyl methylene blue (DMMB) dye binding assay

Result :

Extraction :

Table No: 1

Sr. No	Name of the Extract	Percentage Yield (%)w/w	Nature	Colour	Odour	Taste
1.	Ethanolic extract of *Cissus quadrangularis*	7.8	Sticky Semi-solid	Green	None	Bitter
2.	Ethanolic extract of *Nigella sativa*	6.3	Semisolid	Blackish	None	Bitter

In-vivo animal experimental study:

1) Acute oral toxicity study: All observations was systematically record for each animal. No significant changes in behavioral changes, biochemical changes, histopathological changes was observed

2) Acetic acid-induced writhing test in mice:

Table No: 2

Groups	Treatment	Number of writhing (Mean ± SD)	Percentage inhibition (%)
Group 1	Vehicle control (2% Tween 80)	64.16 ± 2.85	-
Group 2	Acetylsalicylic acid 100 mg/kg p.o.	25.33 ± 2.94	60.52
Group 3	Test sample A (200 mg/kg)	43.33 ± 3.88	32.46
Group 4	Test sample B (200 mg/kg)	51.33 ± 3.44	19.99
Group 5	**Test sample C (200 mg/kg)**	**34.16 ± 2.31**	**46.75**

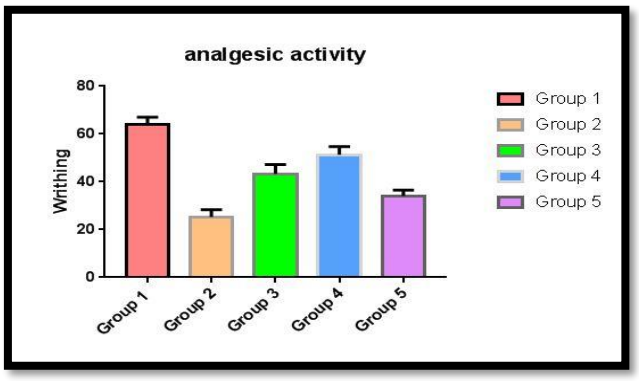

Figure No1: Effect of ethanolic extract in appropriate proportion on Acetic acid-induced writhing test in mice

3) Evaluation of Anti-inflammatory activity

Table No : 3

Groups	Treatment	Change in paw volume (ml)		
		1 Hour	3 Hour	5 Hour
Group 1	Carrageenan control 2% Tween 80	1.40 ± 0.03	2.53 ± 0.03	2.81 ± 0.02
Group 2	Diclofenac 10 mg/kg p.o.	1.16 ± 0.30	1.25 ± 0.02	1.35 ± 0.02
Group 3	Test sample A (200 mg/kg)	1.34 ± 0.02	1.41 ± 0.03	1.62 ± 0.02
Group 4	Test sample B (200 mg/kg)	1.78 ± 0.02	2.13 ± 0.02	2.54 ± 0.02
Group 5	**Test sample C (200 mg/kg)**	**1.27 ± 0.02**	**1.36 ± 0.03**	**1.48 ± 0.02**

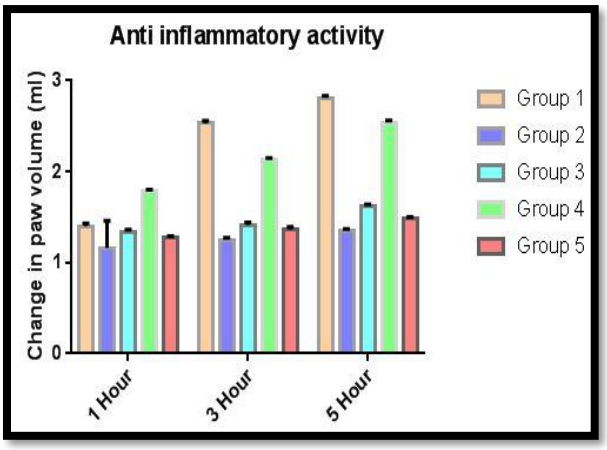

Figure No 2: Effect of ethanolic extract in appropriate proportion on Anti-inflammatory activity in mice

3)Evaluation of anti osteo arthritis activity (Collagenase type II-induced osteoarthritis (CIOA) rat model)

1. Effect on body weight

Table No: 4

Groups	**Treatment**	**Change in body weight (gm) (Mean ± SD)**
Group 1	Normal saline (50 µl)	20.33 ± 4.03
Group 2	Collagenase (50 µl)	5.66 ± 1.86
Group 3	Collagenase + Indomethacin (3 mg/kg)	4.83 ± 1.47
Group 4	Collagenase + Test sample A (200 mg/kg)	20.83 ± 2.85
Group 5	**Collagenase + Test sample B (200 mg/kg)**	**14.5 ± 1.87**
Group 6	Collagenase + Test sample C (200 mg/kg)	22.5 ± 3.08

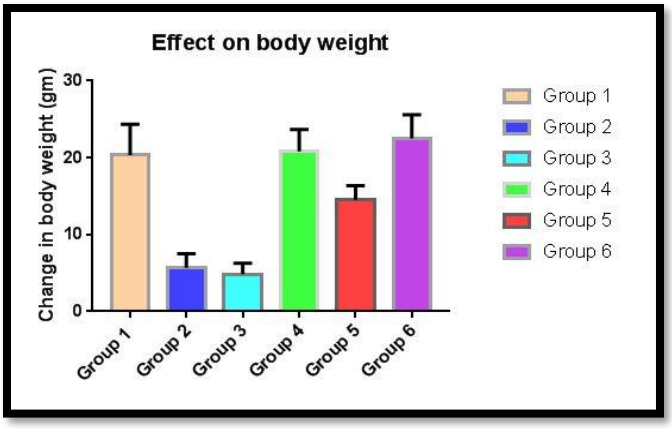

Figure No 3: Effect of ethanolic extract in appropriate proportion on body weight

2. Effect on knee diameter

Table No: 5

Groups	**Treatment**	**Knee swelling (mm) (Mean ± SD)**
Group 1	Collagenase (50 µl)	0.78 ± 0.11
Group 2	Collagenase + Indomethacin (3 mg/kg)	0.2 ± 0.08
Group 3	Collagenase + Test sample A (200 mg/kg)	0.41 ± 0.07
Group 4	**Collagenase + Test sample B (200 mg/kg)**	**0.32 ± 0.07**
Group 5	Collagenase + Test sample C (200 mg/kg)	0.58 ± 0.07

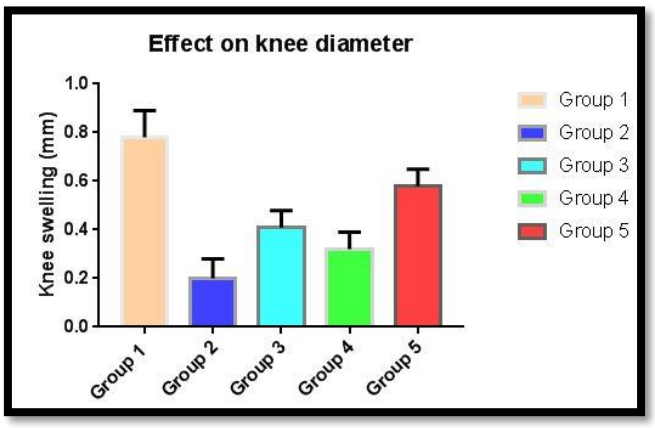

Figure No 4: Effect of ethanolic extract in appropriate proportion on Knee Diameter

3. Effect on paw volume:

Table No: 6

Group	**Treatment**	**Paw oedema volume (ml)**				
		0 Day	**7 Day**	**14 Day**	**21 Day**	**28 Day**
Group 1	Collagenase (50 µl)	2.40 ± 0.08	3.17 ± 0.01	3.38 ± 0.09	3.47 ± 0.07	3.65 ± 0.05
Group 2	Collagenase + Indomethacin (3 mg/kg)	2.28 ± 0.06	3.02 ± 0.05	3.10 ± 0.04	2.60 ± 0.06	2.53 ± 0.04
Group 3	Collagenase + Test sample A (200 mg/kg)	2.49 ± 0.04	3.11 ± 0.04	3.19 ± 0.06	3.34 ± 0.04	3.42 ± 0.08
Group 4	**Collagenase + Test sample B (200 mg/kg)**	**2.36 ± 0.04**	**3.32 ± 0.03**	**3.51 ± 0.07**	**2.74 ± 0.06**	**2.81 ± 0.05**
Group 5	Collagenase + Test sample C (200 mg/kg)	2.53 ± 0.03	3.44 ± 0.05	3.64 ± 0.02	3.42 ± 0.03	3.48 ± 0.03

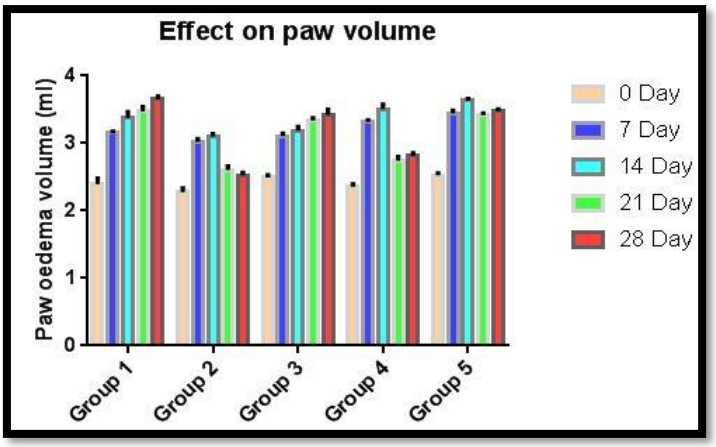

Figure No 5: Effect of ethanolic extract in appropriate proportion on Paw Volume

4. Effect on Glycosaminoglycans (GAG) release

Table No: 7

Groups	**Treatment**	**GAG (µg/ml serum) (Mean ± SD)**
Group 1	Normal saline (50 µl)	118 ± 9.89
Group 2	Collagenase (50 µl)	516 ± 14.54
Group 3	Collagenase + Indomethacin (3 mg/kg)	804 ± 9.24
Group 4	Collagenase + Test sample A (200 mg/kg)	329 ± 5.62
Group 5	**Collagenase + Test sample B (200 mg/kg)**	**648 ± 8.84**
Group 6	Collagenase + Test sample C (200 mg/kg)	462 ± 12.91

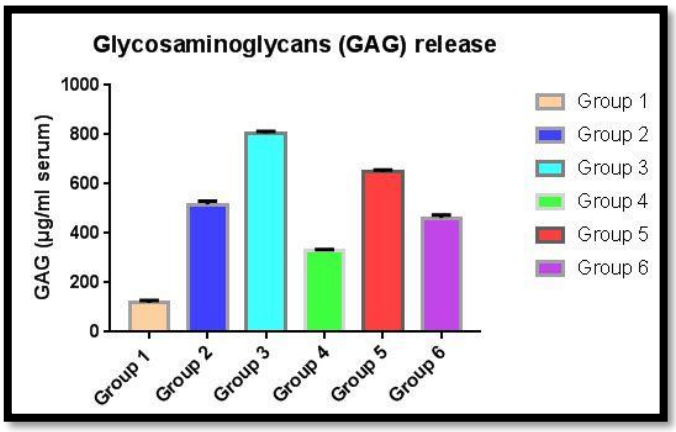

Figure No 6 : Effect of ethanolic extract in appropriate proportion on Glycosaminoglycans (GAG) release

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