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Anti-arthritic, anti-nociceptive and anti-inflammatory potential of *Cassia absus*: An ethanomedicinal plant of *Febaceae* family

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Abstract: Medicinal plants are becoming popular choice for treatment of chronic disease conditions. *Cassia absus* plant parts have been traditionally used to treat inflammatory conditions .This study was designed for evaluating anti-arthritic, anti-nociceptive and anti-inflammatory potential of *Cassia absus* seeds. n- hexane, methanol, chloroform and aqueous extracts were prepared to be appraised for identification and quantitative determination of various phytochemicals. All the extracts were evaluated for anti-arthritic activity via protein denaturation, anti-nociceptive activity by hot plate method and anti-inflammatory potential through Carrageenan induced paw edema. Three doses; 100,200 and 300mg/kg of each extract were given to Wistar rats. The results of the quantitative analysis revealed that Aqueous and n-hexane extracts contained the highest total flavonoid (104.2±0.24mg QE/g) and phenolic contents (187.4±0.65mg GA/g) respectively. All the extracts exhibited decrease in protein denaturation (n-hexane 66.66%, methanol 59.42%, chloroform 65.21% and aqueous extract 89.85%). Significant increase in mean latency time (secs) was observed in n-hexane, methanol and aqueous extract treated rats as compared to normal rats. All four extracts caused significant reduction in paw inflammation as compared to carrageenan control. It is therefore concluded that all the extracts of *Cassia absus* possessed significant anti-arthritic, anti-nociceptive and anti-inflammatory potential.

Keywords: Rheumatoid arthritis, inflammation, protein denaturation, flavonoids, phenolics, anti-nociceptive

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic incendiary rheumatic disease. It is characterized by progressive symmetric inflammation of affected joints which is clinically manifested as swelling, arthralgia, redness, and limitation in the range of motion of joints (Guo *et al*, 2018). RA causes chronic inflammation of synovial membrane that leads to disabling damage to joints as well as systemic complications. Involvement of other organs leads to extra-articular manifestations. These extra-articular manifestations involve vasculitis, pulmonary involvement, and formation of rheumatic nodules (Qorban *et al*, 2018). The higher prevalence of RA is reported in Northern America and Europe than in Asia according to World Health Organization (WHO) and is affecting approximately 0.5-2% adults globally (Singh *et al,* 2020). The global mortality due to RA is three times greater than in the common population (Safiri *et al*, 2019). The adults carry risk for RA disease about 1 in 28 for women (3.6%) and (1 in 59) for men (1.7%) suggesting that the women generally are two to three folds more prone towards chronic polyarthritis than men (Shashi, 2021).

The use of conventional drugs and modern RA Pharmacologic therapies, “non-steroidal anti-inflammatory drugs, steroids, synthetic and biological disease modifying anti-rheumatic drugs” are directed towards preventing joint deterioration while targeting the mediators of inflammation and thereby decreasing the pain and inflammation. These slow the RA progression and improve the patients’ quality of life, but the lifetime use of synthetic drug regimens leads to gastrointestinal and cardiovascular system related adverse effects. The modern day scenario of drug use is turning towards the use of traditional plants due to increased level of interest amid people these days for lesser chances of side effects and better potential compatibility with lasting usage compared to the allopathic medicines (Singh *et al*, 2020).

Cassia absus belongs to Fabaceae family. The genus *Chamaecrista* or Cassia belongs to class named “Cassiinae”. It is an upright annual plant up to 1500 m tall. It is locally called “Chaksu”. The leaves are bitter and sour. It is largely found in Sri Lanka, Paksitan and India’s wastelands. It is distributed in all tropical regions throughout the world (Ahmad *et al*, 2018). For therapeutic purposes, its seeds and leaves are most frequently used. It is traditionally used in the treatment of Inflammation, irritable bowel syndrome, urolithiasis, hypertension, trachoma, bronchitis, dysentery, conjunctivitis, asthma, constipation, cough, tumors, hemorrhoids, leucoderma, venereal ulcer and liver diseases. It also possesses antioxidant and anti diabetic activities (Ahmad *et al*, 2018).

Literature survey gives sufficient evidence that various natural phytochemicals like flavonoids, terpenoids and alkaloids in plants offer a significant protection against inflammatory conditions involving effective mechanisms

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(Kour *et al,* 2022). Use of such plants can slow the RA progression by providing analgesic, anti-inflammatory and anti-oxidant activities. However, the literature review revealed that in-vivo analgesic, anti-inflammatory and anti-arthritic potential of *Cassia absus* seeds has not yet been investigated, so the aim of present research is to evaluate these using different extracts of *Cassia absus*.

MATERIALS AND METHODS

Preparation of extracts

Seeds of *Cassia absus* plant were procured from local market of Lahore and identification was done by a taxonomist from Department of Botany, Government College University (GCU)- Lahore, Pakistan. The subject seeds were authenticated vide voucher number GC. Herb. Bot. 3362, dated 14.12.2016 for future reference. The seeds (200g) were ground to fine powder and soaked in n-hexane, methanol, chloroform (Sigma Aldrich) and distilled water separately (1:5) for double maceration. Semiliquid n- hexane and chloroform extracts of *Cassia absus* (HECA & CECA) whereas, semisolid mass of methanol and aqueous extracts of CA (MECA & AECA) were obtained upon evaporation at 40°C in a rotary evaporator under reduced pressure.

Experimental animals

Wistar rats from both sexes weighing approximately 170-220g previously acclimatized were randomly distributed into diverse study groups and kept in the animal house of Punjab University College of Pharmacy (PUCP), University of the Punjab, Lahore, Pakistan. Adequate arrangement of standard laboratory conditions i.e., 28±2°C Temperature, 12 h light/dark cycles and 55±5% relative Humidity was made sure. All animals were provided with a standard pellet diet along with water ad libitum. The approval for conduct of animal studies was obtained from the Institutional Animal Ethical Committee No. D/ 241/ FIMS. The experiment protocol followed international rules and regulation about laboratory animal ethics given in NIH publication no. 85-23 (revised in 2002).

Qualitative and quantitative phytochemical analysis

Preliminary phytochemical studies were conducted on all plant extracts for the identification of various primary and secondary metabolites like alkaloids, saponins, terpenoids, phenols, glycosides, flavonoids, proteins, sterols and carbohydrates according to the previous methods (Shaikh and Patil, 2020).

Total flavonoid content (TFC) and total phenol content (TPC)

TFC was determined by a colorimetric method using aluminum chloride and Total Phenolic Content (TPC) was estimated in the plant extracts by Folin's Ciocalteu's method, respectively (Nancy & Ashlesha, 2015). The standards used for TFC and TPC determination were Quercetin and Gallic acid, respectively.

Total flavonoid content

The calibration curve for determination of TFC was prepared by using Quercetin as standard. Five concentrations ranging from 50 to 250mcg/ml were prepared from stock solution of standard Quercetin in ethanol (1mg/ml). Reaction mixture was prepared by mixing 0.5ml of each of these concentrations with 1.5ml ethanol, 0.1ml of 10% $ALCL_3$, 0.1ml of 1M potassium acetate and 2.8ml of distilled water and then incubated for 30 mins at room temperature. The absorbance was measured at 415 nm with a double beam spectrophotometer (T80 + PG instruments, UK). For blank, the same amount of distilled water substituted 10% $ALCL_3$. Likewise, 0.5ml of both the plant extracts (1 mg/ml) was used for determination of TFC.

Total phenol content

The total phenolic content in all extracts was determined in triplicate by employing Folin Ciocalteu's method. The reference standard used was Gallic acid. Different Gallic acid concentrations were prepared in methanol as 10,20,30,40 and 50µg/ml. From each concentration, 0.5ml was mixed with 2.5ml of dilute FC reagent and 2ml of 7.5% Na_2CO_3 solution. The reaction mixture was kept for 30 min at the standard room temperature and absorbance was measured with spectrophotometer at 765nm. Similarly, the absorbance of all the extracts was measured by mixing 0.5ml of each extract (1mg/ml) as described above.

In vitro anti- arthritic activity

Protein Denaturation Inhibition Assay

The assay for determination of protein denaturation inhibition was carried out following the previous method (by Bashir and Niazi, 2020) with slight modifications. Test control solution comprised of 0.45 ml of BSA (5% w/v) and 0.05ml of DW. Test solution consisted of 0.45 ml BSA and 0.05ml of various test concentrations of extracts; 100, 250 and 500 mcg/ml. Similarly, the product control contained 0.45 ml of DW along with 0.05 ml of different extract concentrations. The Standard solution consisted of BSA (0.45mL) and different concentrations of Diclofenac sodium (0.05ml). All the above-mentioned solutions were incubated for a period of 20 mins at 37°C, followed by an increase in temperature to 57°C for 3 minutes. Subsequently, 2.5 ml of Phosphate buffer with pH: 6.3 is added to all solutions after cooling. At 660 nm wavelength, the absorbance of both control and test solutions was recorded. The assay procedure was performed in triplicate to find the percentage inhibition as follows:

$$\text{Percentage inhibition} = \frac{\text{Absorbance of test solution} - \text{Absorbance of product control}}{\text{Absorbance of control}} \times 100$$

Anti-nociceptive activity

Hot Plate Test

The anti-nociceptive activity was assessed using the hot plate method previously described by Inaltekin and Kivrak, 2021 (with slight modifications). Fourteen groups of rats (n=4 each) were used for the experiment. Group 1 received distilled water PO and served as Control, Group 2 rats were given 10mg/kg b.wt. of Diclofenac Na IP to serve as a positive control, Group 3, 4 and 5 received n-hexane extract of *Cassia absus* (HECA), Group 6, 7 and 8 received Methanol extract of C*assia absus* (MECA), Group9, 10 and 11 were given Chloroform extract (CECA) and Group 12, 13 and 14 were treated with Aqueous extract (AECA) at doses, 100mg, 200 mg and 300 mg/kg b.wt. separately. Rats were placed on a hot plate (Panlab, Spain) maintained at temperature 55±2ºC. The response to pain stimulus was recorded as latency time in seconds (jump response or paw licking following withdrawal of one or both paws after exposure to hot plate). Latency time to pain response was recorded at interval of 0, 15, 30, 45 and 60 minutes following drug administration. A cutoff time of 45 sec was kept to elude tissue damage to rats' paw.

In-vivo anti-inflammatory activity

Carrageenan induced paw edema

The anti-inflammatory potential of *Cassia absus* was evaluated by employing Carrageenan-induced rat paw edema model (Ijaz *et al.,* 2021). Concisely, 84 rats were classified randomly into different treatment groups (n=6 each) and the rat paw size was measured in mm using Digital vernier caliper. 1 hr before injection of 0.1 ml of 1 % freshly made carrageenan solution (w/v) in the right hind paw of rats, Group 1 rats were administered Distilled water PO; Diclofenac Na (10mg/kg) b.wt. was given to Group 2 rats intraperitoneally, Group 3-5 rats were given HECA, Group 6-8 received MECA, Group 9-11 were treated with CECA and Group 12-14 received AECA at dose levels of 100-300mg/kg b.wt. separately, respectively. The paw size was subsequently measured at 0, 1, 2, 3, 4 and 5 hours post carrageenan injection. The percentage inhibition of paw edema was calculated according to formula:

$$\%\text{ paw edema inhibition} = \frac{(\text{Et - Eo})_{\text{control group}} - (\text{Et - Eo})_{\text{treated group}}}{(\text{Et - Eo})_{\text{control group}}} \times 100$$

Where; Eo represents the paw size at zero hr before carrageenan injection, and Et represents the paw size at the corresponding time, and (Et-Eo) shows the paw edema

STATISTICAL ANALYSIS

Graph pad prism version 8.0.1 was used for the statistical analysis of results. For comparison between different study groups, one-way ANOVA, followed by post hoc Dunnett's test was used. All the data were expressed as Mean ± Standard Error of Mean (mean ± SEM) and $p < 0.05$ was considered as statistically significant.

RESULTS

The Percentage yield of all extracts was calculated. Percentage yields of *Cassia absus* n-hexane, methanol, chloroform and aqueous extracts were 2.72, 8.77, 4 and 9.2% respectively.

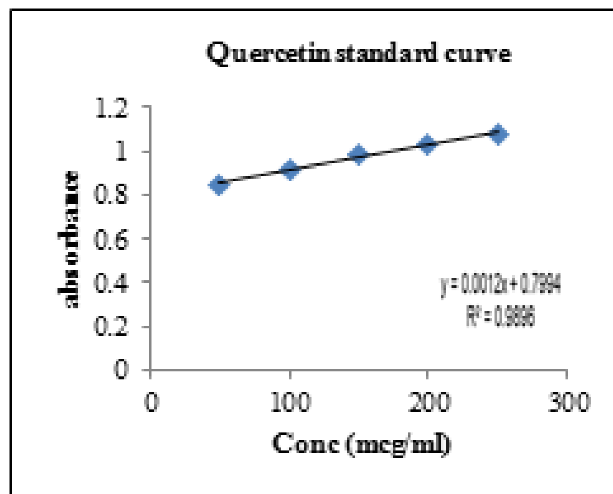

Fig. 1a: Quercetin standard calibration curve for the estimation of flavonoids

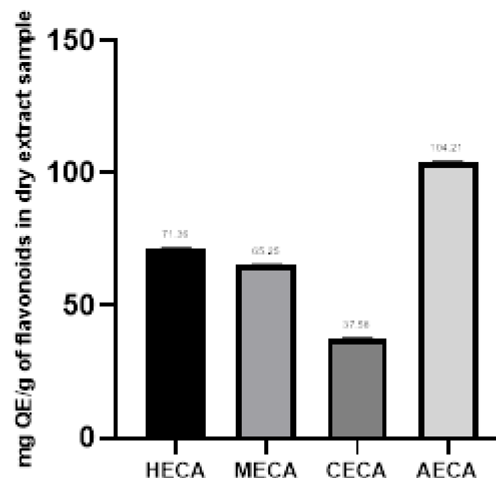

Fig. 1b: Total flavonoid content of the extracts Values are Mean ± SEM for 3 determinations

Preliminary phytochemical study

The presence of primary and secondary plant metabolites in all extracts was evaluated by performing various qualitative analyses. It was found that alkaloids, flavonoids, phenolics and sterols were detected in all extracts whereas proteins were only found in n-hexane and aqueous extracts. Similarly, Saponins were identified in only Chloroform extract and carbohydrates were present in n-hexane and chloroform extracts. Results of the analysis are shown in table 1.

Table 1: Phytochemical analysis of Cassia absus extracts.

Extract	Alkaloids	Saponins	Terpenoides	Phenols	Flavonoids	Protein	Sterol	Carbohydrates
n-hexane	+	-	+	+	+	-	+	+
Methanol	+	-	-	+	+	+	+	-
Chloroform	+	+	+	+	+	-	+	+
Aqueous	+	-	+	+	+	+	+	-

Where (+) = Present and (-) = absent

Table 2: *In vivo* anti- nociceptive activity of various extracts of *Cassia absus* extract[a] Mean Latency time (secs)

Groups	0 min	15 mins	30 mins	45 mins	60 mins
Normal control	4.15 ± 0.50	3.26 ± 0.54	4.09 ± 0.83	4.30 ± 0.45	4.15 ± 0.56
Diclofenac Na (10mg/kg)	5.10 ± 0.06	6.19 ± 0.83**	7.74 ± 0.10**	8.98 ± 1.19**	10.97 ± 1.12***
HECA (100mg/kg)	5.77± 0.31	5.62 ± 0.23*	8.33 ± 0.88**	6.76 ± 1.10	6.22 ± 0.51
HECA (200 mg/kg)	4.15 ± 0.44	5.02 ± 0.37	6.89 ± 0.75*	7.51 ± 0.84**	8.51 ± 1.21**
HECA (300mg/kg)	4.10 ± 0.22	5.09 ± 0.67	7.14 ± 0.70*	7.46 ± 0.36***	7.39 ± 0.40*
MECA (100mg/kg)	5.25 ± 0.42	5.96 ± 0.72	7.12 ± 0.27*	7.20 ± 0.68	6.01 ± 0.73
MECA (200 mg/kg)	5.87 ± 0.59	7.70 ± 1.10**	8.61 ± 0.67***	7.20 ± 0.93	7.46 ± 1.28
MECA (300mg/kg)	5.22 ± 0.37	9.19 ± 0.73***	6.37 ± 0.55	6.60 ± 1.11	6.37 ± 0.74
CECA (100mg/kg)	4.28 ± 0.19	5.41 ± 0.68	4.72 ± 0.29	5.60 ± 0.39	4.54 ± 0.80
CECA (200 mg/kg)	5.18 ± 0.23	4.34 ± 0.54	4.61 ± 0.76	4.33 ± 0.47	6.00 ± 0.35
CECA (300mg/kg)	4.63 ± 0.21	4.70 ± 0.47	5.83 ± 0.39	6.00 ± 0.50	6.36 ± 1.58
AECA (100mg/kg)	5.70 ± 0.21	8.06 ± 0.82*	10.36±0.85***	8.13 ± 0.72*	8.33 ± 0.56**
AECA (200mg/kg)	5.36 ± 0.40	10.15 ± 1.94**	10.70±0.46***	7.82 ± 1.18*	12.04 ± 1.09***
AECA (300mg/kg)	5.19 ± 0.09	6.01 ± 0.51	9.71 ± 0.77***	8.07 ± 0.69*	11.14 ± 2.00**

[a] Comparison of Normal control group with all treatment groups using one way ANOVA, followed by Dunnett's test. Values are expressed as Mean ± SEM (n = 4). *p < 0.05, **p < 0.01 and *** p < 0.001 represent a significant Increase in the Latency time as compared to the Normal control group.

Table 3: *In vivo* acute anti-inflammatory activity of various extracts of *Cassia absus*[b] Paw size (mm) (% inhibition)

Groups	0 hour	1st hour	2nd hour	3rd hour	4th hour	5th hour
Carrageenan Control	3.05 ± 0.08	5.02 ± 0.30	5.08 ± 0.14	5.12 ± 0.14	5.15±0.13	5.19 ± 0.16
Diclofenac Na (10mg/kg)	3.52 ± 0.07	4.08±0.14** (71.78)	3.88±0.11**** (82.21)	3.95±0.14*** (79.34)	4.05±0.16** (75)	4.01±0.14** (77.27)
HECA (100mg/kg)	2.82 ± 0.07	3.70±0.23*** (56)	3.45±0.20**** (70)	3.42±0.27**** (71)	3.40±0.26**** (72.60)	3.73±0.28*** (58.60)
HECA (200 mg/kg)	3.25 ± 0.10	3.73±0.07*** (76.23)	3.87±0.11**** (69.23)	3.93±0.17*** (68)	4.16 ±0.20* (66.7)	4.05±0.25* (64)
HECA (300mg/kg)	2.95±0.08	3.87±0.27** (55)	3.68±0.30**** (65)	3.84±0.32*** (58)	3.88±0.24** (57.5)	3.83±0.25*** (52)
MECA (100mg/kg)	3.32 ± 0.11	4.60 ± 0.20 (36)	4.48 ± 0.14 (38)	4.36 ± 0.19 (45)	4.41 ± 0.24 (49.5)	4.53 ± 0.17 (45)
MECA (200 mg/kg)	3.20 ± 0.18	4.42 ± 0.15 (39)	3.90±0.11**** (66)	4.22 ± 0.15* (53)	4.60 ± 0.31 (34.7)	5.02 ± 0.24 (16.8)
MECA (300mg/kg)	3.48 ± 0.14	4.57 ± 0.11 (46)	4.32 ± 0.11* (49)	4.67 ± 0.12 (44)	4.66 ± 0.28 (45)	4.70 ± 0.15 (44)
CECA (100mg/kg)	3.97 ±0.09	4.23 ± 0.15 (74)	4.52 ± 0.14 (73)	4.57 ± 0.22 (72)	5.05 ± 0.23 (49)	5.09 ± 0.27 (48)
CECA (200 mg/kg)	3.53 ± 0.08	3.90 ± 0.16** (79)	4.00 ± 0.18*** (77.8)	4.53 ± 0.21 (53)	4.69 ± 0.22 (46)	4.85 ± 0.33 (40)
CECA (300mg/kg)	3.46 ± 0.12	4.12 ± 0.22** (60)	4.02 ± 0.03*** (74)	4.41 ± 0.12 (55)	4.76 ± 0.14 (42.6)	4.92 ± 0.13 (33)
AECA (100mg/kg)	3.16 ± 0.14	3.56±0.14**** (79.2)	3.71±0.09**** (73.07)	3.78±0.11**** (70.89)	3.97 ± 0.17** (62.03)	3.95 ± 0.18** (64.09)
AECA (200mg/kg)	3.35 ± 0.06	3.95 ± 0.15** (70.2)	3.78±0.13**** (79.8)	3.96 ± 0.15*** (71.83)	3.99 ± 0.19** (70.8)	3.74± 0.15*** (82.2)
AECA (300mg/kg)	3.32 ± 0.07	4.04 ± 0.19** (64.35)	4.01 ± 0.16*** (66.34)	4.06 ± 0.10 ** (64.78)	4.00 ± 0.20 ** (68.51)	4.18 ± 0.23* (60.45)

[b] Comparison of Carrageenan control group with all treatment groups using one way ANOVA, followed by Dunnett's test. Values are expressed as Mean ± SEM (n = 6). *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001 represents a significant decrease in the paw size as compared to the Carrageenan control group.

Total flavonoid content estimation

The extracts were evaluated for total flavonoid content. The calibration curve of Quercetin is given as fig. 1a, and fig. 1b represents the total flavonoid contents. The flavonoid content was expressed in mg quercetin equivalents/g. The n-hexane (71.36±0.50 mg QE /g) and aqueous (104.2±0.24 mg QE /g) extracts contained the maximum Total Flavonoid Content.

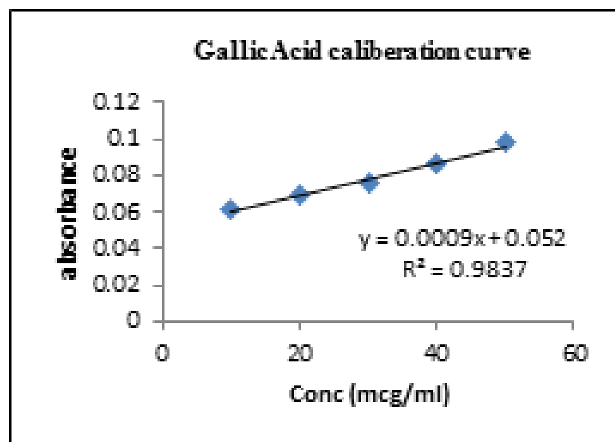

Fig. 2a: Gallic acid standard calibration curve for the estimation of phenols

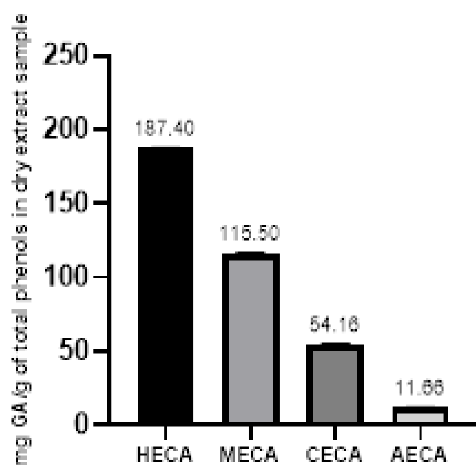

Fig. 2b: Total Phenol content of the extracts Values are Mean ± SEM for 3 determinations

Total phenolic content estimation

The Folin Ciocalteu method was used to estimate Total Phenolic content. The calibration curve with Gallic acid is represented as fig. 2a and fig. 2b shows the phenolic contents of extracts. The maximum amount of Total Phenolics was estimated to be present in the n-hexane extract (187.4±0.65 GA/g).

Inhibition of protein denaturation (Bovine serum albumin)

The maximum %age inhibition of protein denaturation was exhibited by n-hexane (75.36%) and aqueous extracts (75.36%) of *Cassia absus* followed by chloroform (65.21%) and methanol extracts (66.66%) when compared to the standard Diclofenac sodium (89.85%) (fig. 3).

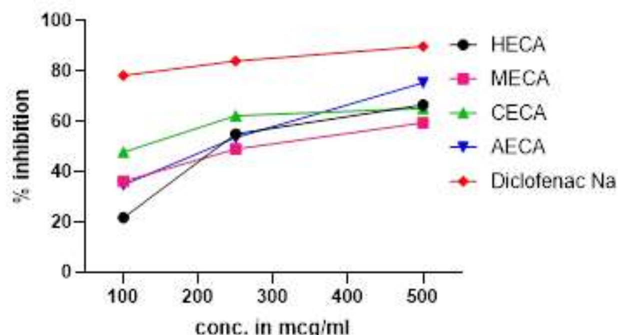

Fig. 3: Effect of *Cassia absus* extracts on Bovine serum albumin denaturation.

Anti-nociceptive activity of cassia absus

In hot plate test, after 30 up to 60 mins, both n- hexane and Aqueous extracts treated rats showed maximum significant increase in latency time (8.51±1.21 and 12.04±1.09 secs) with 200mg/kg when compared to un treated normal control animals as shown in table 2. While, 8.61±0.67 secs was recorded as significant increase in LT with MECA 200 mg/kg for up to 30 minutes and 9.19 ± 0.73 secs with 300 mg/kg at 15 mins. CECA at all dose levels was found to have non significant effect (table 2).

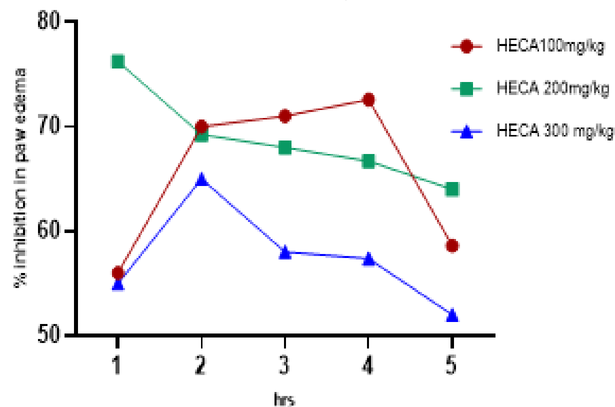

Fig. 4: Effect of n- Hexane extract of *Cassia absus* on % inhibition in paw edema

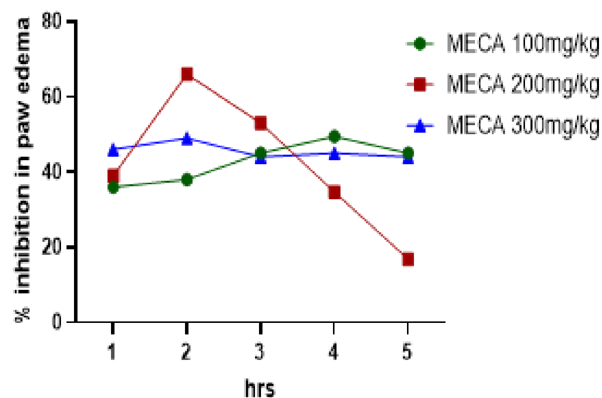

Fig. 5: Effect of Methanol extract of *Cassia absus* on % inhibition in paw edema.

Effects of cassia absus on rat paw edema

The n- hexane and aqueous extracts of the plant exerted the maximum percentage inhibition of paw edema (68.83 ±2.04 and 74.97±2.50%) respectively at 200mg/kg dose and was comparatively higher than that of Chloroform and methanol extracts (59.16±8.12 and 42.70±2.48%) at equivalent dose level (fig. 4-7). The increase in paw thickness and the edema was notably reduced by pretreatment with Diclofenac Na (10 mg/kg) and all plant extracts at various dose levels (table 3).

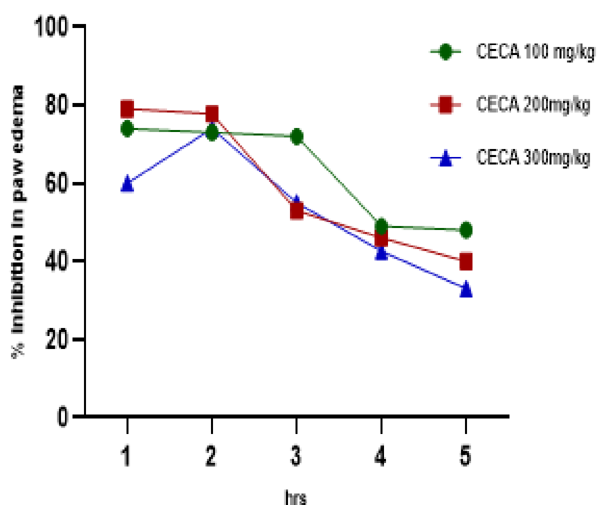

Fig. 6: Effect of Chloroform extract of *Cassia absus* on % inhibition in paw edema

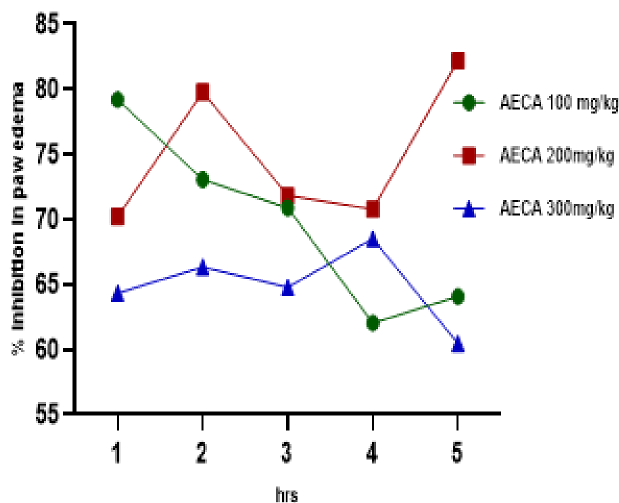

Fig. 7: Effect of aqueous extract of *Cassia absus* on % inhibition in paw edema

DISCUSSION

Various environmental factors including chemical exposure, stress etc cause denaturation of proteins leading to the production of auto-antigens linked with RA. The current study has demonstrated the *in vitro* anti- arthritic and *in vivo* anti nociceptive, anti-inflammatory properties of various extracts derived from *Cassia absus*. Preliminary analysis to qualitatively determine phytochemicals along with quantitative estimation of the former were carried out.

Quantitative evaluation of the plant extracts revealed that maximum TFC and TPC were found in AECA and HECA respectively. Both the Phenolics and flavonoids compounds are responsible for antioxidant potential of various plants. Oxidative stress due to free radical generation is one of the remarkable hallmarks of chronic polyarthritis. Owing to the anti-oxidant nature of polyphenols and flavonoids, significant reduction in oxidative stress linked with chronic inflammatory states like arthritis occurs. (Akhtar *et al*, 2019).

For appraisal of anti-arthritic activity of *Cassia absus*, in vitro assay for inhibition of albumin denaturation was carried out. In arthritis, tissue protein denaturation occurs which is related to production of auto antigens. All the extracts significantly reduced bovine serum albumin denaturation *in vitro*. However, maximum inhibition was showed by the n-hexane and Aqueous extracts. This effect is relatable to the similar activity of NSAIDS against inflammation in arthritis (Thakur *et al*, 2018).

Pain is a multifaceted process mediated by various physiological mediators such as prostaglandins, substance-P, bradykinins etc. (Nguyen *et al*, 2020). In the present study, *Cassia absus* extracts were investigated for antinociceptive activity using hotplate test. The increase in the response latency time by n- hexane and Aqueous extracts in hot plate test at doses of 100-300mg/kg shows anti nociceptive effect.

The carrageenan induced paw edema rat model was used to investigate the anti-inflammatory activity of different extracts of *Cassia absus*. The result indicated that the inflammatory response (indicated by paw swelling) following single carrageenan injection and *Cassia absus* showed a significant inhibitory effect on edema with maximum inhibition caused by n- hexane and aqueous extracts. It is widely reported that carrageenan induced paw edema occurs in two stages. Stage I (0-2h post stimulation) is associated with release of histamine, 5-HT and the Stage II (2-6 h post stimulation) is related to the production of inflammatory mediators like bradykinin, COX-2 and prostaglandins ((Nguyen *et al*, 2020). Hence, the inhibition of carrageenan-induced inflammation by *Cassia absus* extracts is believed to be caused by the inhibition of the histamine, 5-HT along with cyclooxygenases and consequent inhibition of prostaglandin synthesis.

CONCLUSION

The present findings establish that all *Cassia absus* seed extracts showed anti-arthritic, anti- nociceptive and anti-inflammatory activities. However, n-hexane and aqueous

extracts have more such potential as compared to methanol and chloroform extracts. This is relatable to the suppression of various pro-inflammatory mediators and validates their use in the management of polyarthritis. Further studies are necessary to appraise the in-vivo anti-arthritic potential of most active extracts.

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REFERENCES

Ahmad S, Hassan A, Abbasi WM and Rehman T (2018). Phytochemistry and pharmacological potential of Cassia absus-a review. *J. Pharm. Pharmacol*., **70**(1): 27-41.

Akhtar MF, Saleem A and Saleem M (2019). A comprehensive review on ethnomedicinal, pharmacological and phytochemical basis of anticancer medicinal plants of Pakistan. *Curr. Cancer Drug Targets.*, **19**(2): 120-151.

Bashir S and Niazi ZR (2020). Appraisal of *in vitro*, in vivo and multi-targeted molecular docking analysis of atorvastatin to elucidate its anti-arthritic potential. *Pak. J. Pharm. Sci.*, **33**(3): 1183-1190.

Guo Q, Wang Y, Xu D, Nossent J, Pavlos NJ and Xu J (2018). Rheumatoid arthritis: Pathological mechanisms and modern pharmacologic therapies. *Bone Res.*, **6**(1): 1-14.

Ijaz M, Fatima M, Anwar R and Uroos M (2021). Green synthesis of gold nanoparticles from *Manilkara zapota* L. extract and the evaluation of its intrinsic *in vivo* antiarthritic potential. *RSC Advances.*, **11**(44): 27092-27106.

Inaltekin A and Kivrak Y (2021). Evaluation of the effect of vortioxetine on pain threshold by hot-plate test in mice. *Arch Neuropsychiatry,* **58**(4): 274.

Kour G, Choudhary R, Anjum S, Bhagat A, Bajaj BK and Ahmed Z (2022). Phytochemicals targeting JAK/STAT pathway in the treatment of rheumatoid arthritis: Is there a future? *Biochem. Pharmacol.*, p.114929.

Kumar V, Verma A, Ahmed D, Sachan NK, Anwar F and Mujeeb M (2013). Fostered antiarthritic upshot of *Moringa oleifera* Lam. stem bark extract in diversely induced arthritis in wistar rats with plausible mechanism. *Int. J. Pharm. Sci***.**, **4**(10): 3894.

Nancy P and Ashlesha V (2015). Pharmacognostic and phytochemical studies of *Cassia absus* seeds extract. *Int. J. Pharm. Pharm. Sci.*, **8**: 325-332.

Nguyen T, Chen X, Chai J, Li R, Han X, Chen X, Liu S, Chen M and Xu X (2020). Antipyretic, anti-inflammatory and analgesic activities of *Periplaneta americana* extract and underlying mechanisms. *Biomed. Pharmacother.,* **123**: 109753.

Qorban GNM, Badghaish MMO, Albaqami AS, Nemer AA, Alali AJ, Yaqoub A, Hassan RF, Alshamrani HA, Badahman OH, Ansaif RA and Alasmari MA (2018). Rheumatoid arthritis, pathophysiology and management. *Egypt J. Hosp. Med*., **70**(11): 1898-1903.

Safiri S, Kolahi AA, Hoy D, Smith E, Bettampadi D, Mansournia MA, Almasi-Hashiani A, Ashrafi-Asgarabad A, Moradi-Lakeh M, Qorbani M and Collins G (2019). Global, regional and national burden of rheumatoid arthritis 1990-2017: A systematic analysis of the global burden of disease study 2017. *Ann. Rheum. Dis.*, **78**(11): 1463-1471.

Sasongko H, Kusumastuti NI, Alifa GR and Rahmawati AA (2019). *In-vivo* analgesic and anti-inflammatory effects of Eel (*Anguilla bicolor* bicolor) oil. *Pharmaciana.*, **9**(1): 59-70.

Shaikh JR and Patil MK (2020). Qualitative tests for preliminary phytochemical screening: An overview. *Int. J. Chem. Sci.*, **8**(2): 603-608.

Shashi K Agarwal (2021). Healthy Lifestyles and their Beneficial Effect on Arthritis. *East African Scholars J. Med. Sci.*, **4**(10): 221-230.

Singh S, Singh TG, Mahajan K and Dhiman S (2020). Medicinal plants used against various inflammatory biomarkers for the management of rheumatoid arthritis. *J. Pharm. Pharmacol*., **72**(10): 1306-1327.

Thakur S, Riyaz B, Patil A, Kaur A, Kapoor B and Mishra V (2018). Novel drug delivery systems for NSAIDs in management of rheumatoid arthritis: An overview. *Biomed. Pharmacother*., **106**: 1011-1023.