Analgesic and anti-arthritic effect of Corallocarpus epigaeus

Efecto analgésico y antiartrítico de *Corallocarpus epigaeus*

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Summary

Rheumatoid arthritis is a chronic inflammatory joint disease associated with the development of oxidative stress and inflammation. The safety and efficacy profile of 85% methanolic extract of Corallocarpus epigaeus *(CE) was evaluated in the present study. In safety profile LD_{50} value was determined by carrying out an acute toxicity study. In efficacy profile, the analgesic activity was evaluated by both hot plate and tail immersion tests. The anti-inflammatory activity was assessed by carrageenan-induced paw edema and anti-arthritic effect by complete Freund's adjuvant induced arthritis. Phytochemical screening of different CE extracts and quantitative analysis of both raw herb and 85% methanolic extract have been also carried out. The methanolic extract displayed analgesic activity by increasing the response time in both hot plate and tail immersion method. Extract exhibited 23.19% of anti-inflammatory activity and 33.59% of anti-arthritic effect in complete Freund's adjuvant induced paw edema. The CE extract increased the antioxidant level, along with a decrease of the in oxidative stress developed by complete Freund's adjuvant induced arthritis. In conclusion, CE is a rich source of phytochemicals with analgesic, anti-inflammatory and antioxidant activities.*

Key words: **Corallocarpus epigaeus** **** analgesic* anti-inflammatory* antioxidant properties* hot plate* tail immersion* carrageenan* complete Freund's adjuvant***

Resumen

La artritis reumatoidea es una enfermedad inflamatoria crónica de las articulaciones que se encuentra asociada con el desarrollo de estrés oxidativo e inflamación. En el presente estudio se evalúa el perfil de seguridad y de eficacia de un extracto metanólico al 85% de *Corallocarpus epigaeus* (CE). En el perfil de seguridad se determinó el valor de DL_{50} llevando a cabo un estudio de toxicidad aguda. En el perfil de eficacia, la actividad analgésica se

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evaluó tanto por el método de plato caliente como por medio de la prueba de inmersión de la cola. Se evaluó la actividad antiinflamatoria por edema de pata inducido por carragenina y el efecto antiartrítico mediante artritis inducida por adyuvante completo de Freund. También se han llevado a cabo el análisis fitoquímico de las familias de compuestos presentes en diferentes extractos de CE y el análisis cuantitativo de la hierba cruda y del extracto metanólico al 85%. El extracto metanólico presentó actividad analgésica al incrementar el tiempo de respuesta tanto en el método del plato caliente como en la prueba de inmersión de la cola. El extracto exhibió 23,19% de actividad antiinflamatoria y 33,59% de efecto antiartrítico en edema de pata inducido por adyuvante completo de Freund. El extracto de CE aumentó el nivel antioxidante al tiempo que disminuyó el estrés oxidativo desarrollado por la artritis inducida por el adyuvante completo de Freund. En conclusión, CE es una fuente rica de compuestos fitoquímicos con actividades analgésicas, antiinflamatorias y antioxidantes.

Palabras clave: *Corallocarpus epigaeus* * propiedades analgésicas * antiinflamatorias* antioxidantes * método de plato caliente * prueba de inmersión de la cola * carragenina* adyuvante completo de Freund

Resumo

A artrite eumatóide é uma doença inflamatória crônica das articulações que se encontra associada ao desenvolvimento de estresse oxidativo e inflamação. No presente estudo é avaliado o perfil de segurança e de eficácia de um extrato metanólico a 85% de Corallocarpus epigaeus *(CE). No perfil de segurança foi determinado o valor de DL_{50} levando a cabo um estudo de toxicidade aguda. No perfil de eficácia, a atividade analgésica foi avaliada tanto pelo método de prato quente como por meio do teste de imersão da cauda. Foi avaliada a atividade antiinflamatória por edema de pata induzido por carragenina e o efeito antiartrítico mediante artrite induzida por adjuvante completo de Freund. Também se têm levado a cabo a análise fitoquímica das famílias de compostos presentes em diferentes extratos de CE e a análise quantitativa da erva crua e do extrato metanólico a 85%. O extrato metanólico apresentou atividade analgésica ao aumentar o tempo de resposta tanto no método do prato quente como no teste de imersão da cauda. O extrato exibiu 23,19% de atividade antiinflamatória e 33,59% de efeito antiartrítico em edema de pata induzido por adjuvante completo de Freund. O extrato de CE aumentou o nível antioxidante, ao mesmo tempo que diminuiu o estresse oxidativo desenvolvido pela artrite induzida pelo adjuvante completo de Freund. Em conclusão, CE é uma fonte rica de compostos fitoquímicos com atividades analgésicas, antiinflamatórias e antioxidantes.*

Palavras chave: Corallocarpus epigaeus ** propriedades analgésicas * antiinflamatórias* antioxidantes * método de prato quente * teste de imersão da cauda * carragenina* adjuvante completo de Freund*

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory joint disease characterized by the proliferation of synovial cells and infiltration of the joints by a variety of inflammatory cells (1). Despite extensive research, the etiopathogenesis of RA still remains obscure. The main symptom of rheumatoid arthritis is inflammation.

Inflammatory responses are of major significance in human health since many of the common debilitating diseases such as RA, spondylolysis, arthalgia, ankylosis, etc., are biological manifestations of an impaired or exaggerated inflammatory response. Inflammation is the basic way in which the body reacts to infection, irritation and other tissue injury, the key features being redness, warmth, swelling, accumulation of leucocytes and pain. Moreover, Kamanli (2) has reported that increasing clinical data provided compelling evidence for the involvement of free radicals in RA. The inflammation and oxidative stress related diseases like RA have made pharmaceutical industries to think for drugs with anti-inflammatory properties combined with analgesic and antioxidant activity. Various herbs are evaluated for all these activities (3). However, *Corallocarpus epigaeus* has not been studied so far.

Corallocarpus epigaeus (Rottler) C. B. Clarke (CE) belongs to the family *Cucurbitaceae* and is commonly used in folk medicine in the treatment of various ailments, including, dysentery, enteritis, laxative, rheumatism and syphilis (4). The 90% ethanolic extract of CE has been reported to possess anti-steroidogenic activity (5). This study was undertaken to evaluate scientifically the anti-arthritic and analgesic activity of CE along with the phytochemical evaluation.

Materials and Methods

PLANT MATERIAL

The roots of *Corallocarpus epigaeus* (Rottler) C. B. Clarke (CE) were collected from Madurai region, Tamilnadu, India. The plant was identified and authenticated by a Botanist at Division of Pharmacognosy, Centre for Advanced Research in Indian System of Medicine (CARISM), SASTRA University, Thanjavur, Tamilnadu, India. A voucher specimen was deposited in the Herbarium of the same department.

EXTRACTION

The plant material was dried under shade. One kg of crushed root of the plant was soaked separately with seven liters of different solvents like *n*-hexane, chloroform, ethyl acetate and 85% methanol for 7 days. The extract was filtered and concentrated by distillation. The final traces of solvent were dried under reduced pressure at 50 °C. The yields of the extracts were as follows: *n*-hexane 0.72%, chloroform 0.42%, ethyl acetate 0.66% and 85% methanol 0.78%.

PHYTOCHEMICAL SCREENING OF CE EXTRACTED WITH DIFFERENT SOLVENTS

The extracts were tested for the presence of alkaloids, flavonoids, glycosides, phenols, resins, saponins, tannins, volatile oils, carbohydrates and amino acids using standard procedures (6).

QUANTITATIVE ESTIMATION OF PHYTOCHEMICAL COMPOUNDS

Various phytochemical compounds of the raw herb like phenolics (7), tannins (7), carbohydrates (8), vitamin C (9) and vitamin E (10) of CE were estimated using UV-spectrophotometer (Lambda 25).

ACUTE TOXICITY STUDY

Ten groups of animals were selected, and each group consisted of 8 mice of both sexes. Each group of animals was treated orally with the CE extract at the dose of 100, 200, 300, 500, 700, 900, 1100, 2000, 3000 and 4000 mg/kg body weight (bw) as mentioned in the Table III. The animals were monitored for 24 h and mortality was noted. The LD_{50} was determined according to the previously reported method (11).

EXPERIMENTAL ANIMALS

In this study, 96 adult Wistar albino rats of either sex weighing 180–210 g, obtained from the Central Animal House, Centre for Advanced Research in Indian System of Medicine (CARISM), SASTRA University, Tamilnadu, India were used. The rats were fed with standard laboratory chow (Nutrilab Petcare Division, Tetragon Chemie Pvt ltd, Bangalore, India) and sterile water before the experiment. The animal laboratory was equipped with automatic temperature (22 ± 1 °C) and lighting controls (12 h light/12 h dark). All the animal experiments were performed after getting clearance from Animal Ethical Committee (Clearence No. 11/SASTRA/IAEC/RPP).

EXPERIMENTAL PROTOCOL FOR HOT PLATE METHOD

In the Hotplate test (12) rats were divided into different groups as follows. Each group consisted of six animals. Group 1 animals were treated with 5% Tween 80 in water, Group 2 animals were treated with 50 mg/kg bw CE extract, Group 3 animals were treated with 75 mg/kg bw extract, Group 4 animals were treated with 100 mg/kg bw extract. All animals were treated orally. In this test, animals were individually placed on a hot plate maintained at a constant temperature (55 ± 0.3 °C). The latency to first sign of hind paw licking or jump response to avoid heat nociception was taken as an index of nociceptive threshold with cut off time of 15 sec. The nociceptive threshold was observed every 60 min up to 4 hours after the drug administration.

EXPERIMENTAL PROTOCOL FOR TAIL IMMERSION METHOD

In the Tail immersion method (13) rats were divided into different groups as follows. Each group consisted of six animals. Group 1 animals were treated with 5% Tween 80 in water, Group 2 animals were treated orally with 50 mg/kg bw extract, Group 3 animals were treated with 75 mg/kg bw extract. Group 4 animals were treated with 100 mg/kg bw extract. The antinociceptive effect was determined in rats using the tail-flick analgesiometer. The responses were elicited every 15 min up to 60 min after treatment, with CE and the vehicle.

EXPERIMENTAL PROTOCOL FOR CARRAGEENAN INDUCED INFLAMMATION

Anti-inflammatory effects of the 85% methanolic extract of CE were investigated, after inflammation being induced by carrageenan (14, 15).

The animals were divided into the following groups. Group 1 animals were treated with 5% Tween 80 in water, Group 2 animals were treated with 10 mg/kg bw indomethacin, Group 3 animals were treated with 50 mg/kg bw extract, Group 4 animals were treated with 75 mg/kg bw extract, Group 5 animals were treated with 100 mg/kg bw extract. The test sample was administered 1 h before administration of an intradermal injection of carrageenan (0.1 mL of a 1% solution in 0.9% saline solution) into the plantar region of the right hind paw. The contralateral paw was injected with 0.1 mL saline solution. The paw volume was measured before the injection and each hour after, for a period of 4 h by means of volume displacement methods. The difference between the left and right paw volumes indicated

the degree of inflammation. The average increase in paw volume of each group was calculated and compared with the control (saline) and the indomethacin groups.

EXPERIMENTAL PROTOCOL FOR FREUND'S ADJUVANT INDUCED ARTHRITIS

Animals were divided into three groups as follows. Each group consisted of six animals. Group 1 animals were treated with 5% Tween 80 in water, Group 2 animals were administered with Complete Freund's adjuvant (CFA), Groups 3 animals were treated with 50 mg/kg bw CE extract along with CFA. The right foot pad of each rat was injected subcutaneously with 0.05 mL of CFA (16). The animals were treated with CE extract and standard drug for 45 days. On 46th day all the animals were sacrificed by cervical dislocation under ether anesthesia. Liver and kidney were excised and washed with saline. 10% tissue homogenate was prepared in Tris buffer and various biochemical parameters like Thiobarbituric acid Reactive Substances (TBARS) (17), Reduced Glutathione (GSH) (18), Glutathione peroxidase (GPx) (19) and Catalase (20) were estimated.

STATISTICAL ANALYSIS

Results were expressed as Mean ± S.D. Statistical significance was calculated by using One Way Analysis of Variance (ANOVA) by SPSS software version 12.0. $P < 0.05$ was considered as significant. Values bearing different letters as superscripts showed significant differences ($p < 0.05$). Significant difference ($p < 0.05$) for paw volume in Freund's adjuvant induced arthritis was only carried out using Student's't' test.

Results

The results of Table I show that the *n-hexane* extract of CE although having a lot of non polar constituents, is devoid of the main compound families indicated in the table, whereas, the chloroform extract contained alkaloids and phytosterols and the ethyl acetate extract was a rich source of phenolic compounds and phytosterols. Likewise, the 85% methanolic extract showed to be a rich source of phenolic compounds, phytosterols, carbohydrates and amino acids. Since 85% methanolic extract of CE was a rich source of most of the phytochemicals, this extract was selected for further study.

Various phytochemical compounds like phenolics, tannins, carbohydrates, vitamin C and vitamin E were estimated in both raw herb and 85% methanolic extract (Table II). The raw herb contained higher concentration of carbohydrates, whereas 85% methanolic fraction possessed higher concentration of tannins followed by carbohydrates and vitamin C.

The acute toxicity study of the 85% methanolic extract showed that at a dose of 500 mg/kg bw of extract, 50% of the animals died. The experimental LD_{50} value was 500 mg/kg bw. The experimental LD_{100} value was 4000 mg/kg bw of extract (Table III).

Results of Table IV reveal that a lower dose of CE extract exhibited a maximum activity at the 2nd hour. When the dose was slightly increased (75 mg/ kg bw) a significant difference and maximum result were observed at the 1st hour. When the dose was enhanced to 100 mg/kg bw the maximum effect was observed at the 1st hour. Moreover, higher activity was observed at a higher dose of extract (100 mg/kg bw).

Table I. Phytochemical screening of the different extracts from *Corallocarpus epigaeus*

Phyto constituents	*Hexane*	*Chloroform*	*Ethyl acetate*	*85% Methanol*
Alkaloids	Absent	Present	Absent	Absent
Flavonoids	Absent	Absent	Absent	Absent
Polyphenolics	Absent	Absent	Present	Present
Phytosterols	Absent	Present	Present	Present
Saponins	Absent	Absent	Absent	Absent
Fixed oils and fats	Absent	Absent	Absent	Absent
Carbohydrates	Absent	Absent	Absent	Present
Amino acids and proteins	Absent	Absent	Absent	Present

Table II. Quantitative estimation of various phytochemical compounds in raw and 85% methanolic extract of *Corallocarpus epigaeus*

S.No	*Phytochemical compounds*	*% in raw herb*	*% in 85% methanolic extract*
1	Phenolics	1.87 ± 0.46	0.0033±0.0002
2	Tannins	2.23 ± 0.45	0.0124±0.0001
3	Carbohydrates	8.0 ± 1.23	0.0084±0.0003
4	Vitamin C	1.27 ± 0.98	0.0062±0.0005
5	Vitamin E	1.45 ± 0.08	0.0013±0.0003

Table III. Acute toxicity data in mice for the 85% methanolic extract of *Corallocarpus epigaeus*

Group	*Dose (mg/kg)*	*Log dose (mg/kg)*	*Dead/ Total*	*Dead (%)*	*Corrected (%)*	*Probit*
1.	100	2.0000	0/8	–	3.12	3.19
2.	200	2.3010	0/8	–	3.12	3.19
3.	300	2.4770	3/8	37.50	37.50	4.68
4.	500	2.6980	4/8	50.00	50.00	5.00
5.	700	2.8450	4/8	50.00	50.00	5.00
6.	900	2.9540	4/8	50.00	50.00	5.00
7.	1100	3.0410	4/8	50.00	50.00	5.00
8	2000	3.3010	5/8	62.50	62.50	5.32
9.	3000	3.4771	6/8	75.00	75.00	5.67
10.	4000	3.6021	8/8	100.00	96.88	6.82

Table IV. Evaluation of analgesic activity of *Corallocarpus epigaeus* on rats by hot plate method

Groups (n=6)	*Dose*	*Response time (sec)*				
	Reaction time (h)	*0 h*	*1 h*	*2 h*	*3 h*	*4 h*
Group 1	-	3.1 ± 0.90	3.1 ± 1.50	3.8 ± 0.96	3.1 ± 0 25	3.8 ± 0.50
Group 2	MLE 50 mg/kg	2.8 ±0.30[a]	6.3 ± 0.96[b]	6.9 ± 3.70[c]	5.6 ± 0.50[b]	6.4 ± 1.50[b]
Group 3	MLE 75 mg/kg	2.1 ± 0.30[a]	5.8 ± 2.40[c]	4.5 ± 1.20[b]	5.1 ± 0.50[b]	4.4 ± 0.60[b]
Group 4	MLE 100 mg/kg	2.4 0.30[a]	8.5 ± 1.30[d]	6.3 ± 2.30[c]	6.9 ± 1.03[c]	4.3 ± 0.50[b]

Results are expressed as Mean ± SD. Values bearing different letters as superscripts showed significant differences ($p < 0.05$) using One-Way ANOVA, Duncan multiple range test.

In tail immersion method the response time was increased significantly ($p<0.05$, Table V), the maximum response being observed after 30 minutes of drug administration. The response time was not dose-dependently increased.

In carrageenan-induced paw inflammation, animals treated with 50 mg/kg bw of extract did not exhibit any significant result. The response was significantly increased with 75 and 100 mg/kg bw of extract. Moreover, the CE extract at a dose of 100 mg/kg bw of extract exhibited only 23.19% of activity, whereas, indomethacin showed 50.20% activity (Table VI).

On treating animals with CE for 45 days, the paw volume was noted to be decreased. The significant difference was observed from the 15th day onwards and the significant difference was noted up to 45th day of treatment. On 45th day the CE treatment exhibited 33.59% of activity (Table VII).

Table V. Evaluation of analgesic activity of *Corallocarpus epigaeus* on rats by tail immersion method

Groups (n=6)	*Dose*	*Response time (sec)*				
	Reaction time (h)	*0 h*	*1 h*	*2 h*	*3 h*	*4 h*
Group 1	-	4.3 ± 0.50	4.0 ± 0.80	3.8 ± 0.50	5.3 ± 0.96	4.0 ± 0.80
Group 2	MLE 50 mg/kg	2.0 ± 0.01 [a]	3.3 ± 0.50 [a]	3.6 ± 0.80 [b]	3.4 ± 0.50 [b]	3.6 ± 0.50 [b]
Group 3	MLE 75 mg/kg	2.8 ± 0.30 [a]	3.6 ± 0.50 [b]	3.9 ± 0.30 [b]	3.4 ± 0.30 [b]	3.0 ± 0.80 [a]
Group 4	MLE 100 mg/kg	2.5 ± 0.60 [a]	2.9 ± 0.30 [a]	3.1 ± 0.30 [b]	3.8 ± 0.60 [b]	3.4 ± 0.50 [b]

Results are expressed as Mean ± SD. Values bearing different letters as superscripts showed significant differences ($p < 0.05$) using One-Way ANOVA, Duncan multiple range test.

TBARS level was found to increase in liver of arthritic animals, as shown in Table VIII. Animals treated with CE showed a significant decrease of the TBARS level ($p<0.05$, Table VIII). The antioxidants like catalase, reduced glutathione, glutathione peroxidase decreased in the liver of the diseased animals, whereas, all these antioxidants were found to be increased upon CE treatment.

Catalase and reduced glutathione were decreasing in kidney tissues to protect other organs from damage caused by CFA. The CE treatment significantly increased catalase ($p<0.05$, Table VIII), whereas the reduced glutathione level kept decreasing. This might be due to the release of glutathione immediately into the circulation to protect the entire body from damage caused by CFA.

Discussion

The CE extract significantly increased both hot plate reaction and tail flick response time in rats. It is known that centrally acting analgesic drugs elevated the pain threshold of rats towards heat. The present findings reveal that CE is centrally acting. Flavonoids have been earlier re-

Table VI. Effect of methanolic extract of *Corallocarpus epigaeus* on carrageenan induced paw edema in rats

Groups	*Dose (mg/kg bw)*	*Mean Increase in Paw edema (mm)*	*% Inhibition of Paw edema*
Group 1	5% Tween 80	2.00 ± 0.14[a]	–
Group 2	10	0.99 ± 0.10 [c]	50.20
Group 3	50	1.79 ± 0.15[a]	10.72
Group 4	75	1.69 ± 0.21[ab]	15.33
Group 5	100	1.54 ± 0.14[b]	23.19

Results are expressed as Mean ± SD. Values bearing different letters as superscripts showed significant differences ($p < 0.05$) using One-Way ANOVA, Duncan multiple range test.

Table VII. Effect of *Corallocarpus epigaeus* on Freund's complete adjuvant induced inflammation

Days	*Control*	*Corallocarpus epigaeus*	
	P.V	*P.V*	*P.I*
1	4.1 ± 0.04	4.05 ± 0.13	
8	6.3 ± 0.05	6.05 ± 0.02	3.97
15	6.7 ± 0.12	5.87* ± 0.16	12.39
22	7.1 ± 0.15	5.77* ± 0.1	18.73
29	6.5 ± 0.62	5.1* ± 0.12	21.54
36	6.5 ± 0.61	4.48* ± 0.11	31.08
43	6.4 ± 0.15	4.25* ± 0.05	33.59

Note: P.V – Paw volume in mm; P.I – Percentage Inhibition as compared to Control; Mean ± S.D, * - $p < 0.05$. Significance was calculated using Student's 't' test.

Table VIII. Effect of *Corallocarpus epigaeus* on Freund's complete adjuvant induced oxidative stress

Biochemical parameters	*Organs*	*Group 1 (n=6)*	*Group 2 (n=6)*	*Group 3 (n=6)*
TBARS (nmoles/mg protein)	Liver	1.19 ± 0.08[a]	1.57 ± 0.18[b]	1.13 ± 0.02[a]
	Kidney	1.18 ± 0.05[a]	1.23 ± 0.13[a]	1.19 ± 0.08[a]
Catalase (mM of H_2O_2 consumed/ min/mg protein)	Liver	19.28 ± 1.41[b]	13.84 ± 1.42[a]	17.02 ± 1.49[ab]
	Kidney	19.34 ± 1.38[b]	15.61 ± 1.04[a]	17.94 ± 1.0 [ab]
Reduced Glutathione (µg/mg protein)	Liver	38.03 ± 7.49[b]	23.27 ± 1.24[a]	33.46 ± 4.08[ab]
	Kidney	40.99 ± 3.30 [b]	35.36 ± 20.40[ab]	27.88 ± 2.58[a]
Glutathione peroxidase (?g of GSH utilized/min/ mg protein)	Liver	0.1532 ± 0.0157[b]	0.1043 ± 0.0009[a]	0.1565 ± 0.0001 [b]
	Kidney	0.1091 ± 0.0011[a]	0.1087 ± 0.0002[a]	0.1095 ± 0.0002[a]

Results are expressed as Mean ± SD. Values bearing different letters as superscripts showed significant differences ($p < 0.05$) using One-Way ANOVA, Duncan multiple range test.

ported to have analgesic activity through inhibition of the enzyme prostaglandin-synthetase, more specifically the endoperoxidase (21).

The anti-inflammatory activity of a drug was measured under *in vivo* conditions by noting the reduction in edema produced by injecting a small amount of solution or suspension of edemogens like carrageenan (22) into the plantar tissues of the hind paw of the rats. The most widely used assay in this category is the carrageenan-induced edema (23). The amount of swelling is measured by the thickness of the paw, its weight or amount of water or mercury (24) that it displaces. Carrageenan is a mixture of polysaccharides composed of sulphated galactose units and is derived from Irish tea moss *Chondrus crispus* (25). A modification of edema assays involved the measurement of leakage of a protein-bound marker from the circulation into the tissues.

CE is able to suppress edema and this effect may be due to the inhibitory effects on the release of histamine, 5-hydroxytryptamine and kinin-like substances which are reported to release from mast cell degradation during the first hour of carrageenan- induced artificial paw edema (26). Compounds like bioflavonoids present in the extract may be responsible for the anti-inflammatory action because of decreasing capillary permeability (27). The flavonoids have been reported to produce several anti-inflammatory effects (28).

The Reactive Oxygen Species (ROS) are produced continually in most tissues and are part of normal cell functions, and their generation may increase in vascular disease, such as atherosclerosis, when enhanced formation of ROS may be pathogenic (29). The main source of ROS *in vivo* is aerobic respiration in mitochondria, peroxisomal oxidation of fatty acids, microsomal cytochrome P_{450} metabolism of xenobiotic compounds, stimulation of phagocytosis by pathogens or lipopolysaccharides, arginine metabolism, and tissue-specific enzymes (30). Donation of a single electron to molecular oxygen results in the formation of the superoxide radical ($O_2^{\bullet-}$). Donation of a second electron yields peroxide, which then, undergoes protonation to yield hydrogen peroxide (H_2O_2). Donation of a third electron, such as occurs in the Fenton reaction ($Fe^{2+} + H_2O_2$? $Fe^{3+} + {}^{\bullet}OH + HO^{-}$), results in the production of the highly reactive hydroxyl radical (•OH). Finally, donation of a fourth electron yields water. Singlet oxygen ($^1O_2$), a very short-lived and reactive form of molecular oxygen in which the outer electrons are raised to a higher energy state, can be formed by a variety of mechanisms, including the Haber-Weiss reaction.

Neutrophils play a key role in the pathogenesis of inflammation. These neutrophils are recruited and activated in rheumatoid arthritis joints by pro-inflammatory cytokines and cause damage to the joints by releasing granules containing collagenase and elastase and by generating ROS (32). Also, ROS produced by macrophages, lymphocytes and endothelial cells contribute to the destruction of cartilage (33). This might be the reason for the increased level of TBARS observed in different organs like liver, kidney of rats.

The increased level of antioxidants observed in the present study might be due to either an increase of the synthesis of antioxidant enzymes or to the occurrence of some phyto-constituents in the extract, which may behave as scavengers of free radicals.

The decrease of lipid peroxidation in CE treated rats could be attributed to the synergistic antioxidant potential of the combination of phenols, flavonoids and tannins against free radical mediated injury. Therefore, CE has shown antioxidant activity and has the potential to inhibit lipid peroxidation, ultimately resulting in decreased lipid peroxidative products. Ochoa (34) has previously reported that the polyphenolic diet prevents lipid peroxidation and protects the mitochondrial antioxidant enzymes from oxidative stress.

The hydroxyl and phenoxyl groups present in the phenolic compounds behave in the scavenging reaction of free radicals (35). Moreover, phenolic nucleus and unsaturated side chain containing compounds, present in the extract readily forms a resonance-stabilized phenoxy radical which accounts for its potent antioxidant activity (36). Condensed tannins and hydrolyzable tannins are known as powerful antioxidant agents (37) because they possess a great number of hydroxyl groups, especially many *ortho*-dihydroxy and/or galloyl groups.

Conclusions

Corallocarpus epigaeus is a rich source of phytochemical compounds with high nutritive value. LD_{50} value of 85% methanolic extract of *Corallocarpus epigaeus* was 500 mg/kg bw. The CE extract exhibited analgesic activity by significantly increasing the response time in both hot plate and tail immersion method. CE extract dose of 100 mg/kg bw inhibited the carrageenan-induced edema. Likewise, in CFA administered animals, the tested sample decreased the paw volume and inhibited the oxidative stress in both liver and kidney of diseased rats by increasing the level of antioxidants. Thus, the tested sample showed to be a potent analgesic, anti-inflammatory and antioxidant extract.

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