International Journal of Biochemistry Research & Review

18(3): 1-7, 2017; Article no.IJBCRR.35071
ISSN: 2231-086X, NLM ID: 101654445

Anti-inflammatory Effect of *Entandrophragma angolense* Bark Extracts on Acute Edema of the Rat's Paw Induced by Carrageenan

Yenon Achiè Aurélie[1*], Koffi Akissi Jeanne[1], Bedou Kouassi Denis[1], Yapi Houphouet Félix[1], Nguessan Jean David[1] and Djaman Allico Joseph[1,2]

[1]Biochemical Pharmacodynamy Laboratory, Department of Biosciences, University of Félix Houphouët-Boigny, 22 BP 1679 Abidjan 22, Côte d'Ivoire.
[2]Department of Clinical and Basic Biochemistry, Pasteur Institute of Côte d'Ivoire, 01 BP 490 Abidjan 01, Côte d'Ivoire.

Authors' contributions

This work was carried out in collaboration between all authors. Author YAA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors KAJ and BKD managed the analyses of the study. Author YHF managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJBCRR/2017/35071
Editor(s):
(1) Sahar Samir Mahmoud Abd El-Rahman, Department of Pathology, College of Veterinary Medicine, Cairo University, Egypt.
Reviewers:
(1) Anthony E. Ojieh, Delta State University, Nigeria.
(2) Cuauhtemoc Pérez González, Univertsidad Autónoma Metropolitana Unidad Xochimilco, Mexico.
Complete Peer review History: http://www.sciencedomain.org/review-history/20565

Original Research Article

Received 26th June 2017
Accepted 11th August 2017
Published 19th August 2017

ABSTRACT

Aims: Our work consisted to evaluate the anti-inflammatory activity of the bark of *Entandrophragma angolense* in the laboratory.
Methods: The experiments were carried out on the model of acute edema of the rat's paw induced by carrageenan and the determination of C-reactive protein. The aqueous and ethanolic extracts, at doses of 100 and 200 mg/kg body weight were administered orally to the rats. The extracts were administered an hour before induction of acute inflammation with 1% carrageenan. The results obtained were compared to those of Diclofenac Sodium and those of physiological control.
Results: After administration of the distilled water, the carrageenan induced edema which increased gradually with a maximum of 38.41% after five hours of observation. The administration of

**Corresponding author: E-mail: aurelieachie@yahoo.fr;*

Diclofenac Sodium at dose of 10 mg/kg b.wt as well as aqueous and ethanolic extracts of *E. angolense* significantly prevented the increase in the diameter of the rat's paw. From the 1st to the 4th hour, the aqueous extract at dose of 200 mg/kg b.wt respectively shows a percentage of inhibition similar to that of Diclofenac Sodium. Then, the administration of the extracts and Diclofenac Sodium decreased the concentration of CRP induced by the injection of carrageenan compared to the control.

Conclusion: *E. angolense* has a mechanism of anti-inflammatory action similar to that of non-steroidal anti-inflammatory drugs which could be attributed to its phytochemical constituents.

Keywords: Entandrophragma angolense; inflammation; CRP; anti-inflammatory.

1. INTRODUCTION

Inflammation is a complex reactional process of the vasculo-conjunctive system of the body in response to an aggression that may be physical, biological or infectious origin, in order to maintain its homeostasis and integrity [1]. It is usually a beneficial process, but it can be harmful because of the aggressiveness of the pathogen, its persistence, the site of inflammation due to abnormality of the regulations of inflammatory process or by quantitative or qualitative abnormality of the cells Involved in inflammation [2]. Inflammation, when it is visible, manifests classically itself by four clinical signs: redness, pain, swelling and an increase in heat. These clinical manifestations are treated by the anti-inflammatory drugs which are drugs permitting to suspend or slow down the inflammation process. A lot of non-steroidal anti-inflammatory drugs (NSAIDs) are prescribed because of their effectiveness. However, their therapeutic use in the long term is often associated with adverse effects such as gastro-intestinal ulcers and renal insufficiency [3]. Moreover, plants are sources, rich in drugs because they produce many bioactive molecules, most of which probably play the role of chemical defense against predators or infectious agents [4]. These plants could constitute an alternative to the anti-inflammatory therapeutics because of their better accessibility and their less toxicity in general. *Entandrophragma angolense,* subject of our work, is used in the treatment of malaria, peptic ulcers, rheumatic or arthritic pains… [5,6]. The stem bark, in decoction, is used for the treatment of fever or malaria in Cameroon and Ivory Coast. In Congo, it is used as analgesic and anti-inflammatory [7]. The aim of this study was to investigate the anti-inflammatory activity of the aqueous and ethanolic extracts of the bark of *E. angolense* on acute inflammatory edema of the rat paw and the serum concentration of CRP induced by carrageenan.

2. MATERIALS AND METHODS

2.1 Plant Material

The stem bark of *E. angolense* was collected in the area of Abidjan (southern Ivory Coast) in March 2014. It was identified at the National Floristic Centre of University Felix Houphouët-Boigny where a herbarium specimen of the plant was deposited. The bark was cut out then dried in the shade, at the room temperature for two weeks. Then, it was pulverized using an electric crusher in order to obtain a powder.

2.2 Experimental Animals

Female and male Rats of Wistar strain of body weight ranging between 110-170 g were used for this study. The animals were housed in cages and acclimatized for two weeks in the animal house of the Higher Teacher Training School. They had been maintained under standard conditions (room temperature 25℃ ± 3℃, humidity 35 to 60%, light and dark period 12/12 hours). All animals had regular supply of clean drinking water and food.

2.3 Preparation of Extracts by Decoction or Maceration

One hundred grams (100 g) of plant powder was boiled for 20 minutes in 2 liters of distilled water. The cooled juice was filtered thrice off through a cotton plug and once with a Whatman No.3 filter paper. The obtained filtrate was concentrated under reduced pressure at temperature below 50℃ through a Büchi rotary evaporator and gave the dry aqueous extract of *E. angolense*. As for the maceration, one hundred grams (100 g) of plant bark powder and 1 liters of 70% ethanol were introduced into an Erlenmeyer and subjected to magnetic stirring for 24 hours at room temperature. The mixture was then filtered thrice on cotton and one on Whatman No.3 filter

paper and was dried at temperature below 50℃ through a Büchi rotary evaporator. The powder obtained is called ethanolic extract [8].

2.4 Inhibition Test of Rat Paw Edema to Carrageenan

Rats were fasted for 16 hours before the experiment with free access to water. At the time of experiment, they were weighed and divided into six groups of six animals each. For each rat, the diameter at zero time of the right hind paw was determined using the caliper rule. Then, the distilled water (10 ml/kg) was orally administrated to rats of group 1, those of group 2, Diclofenac Soduim solution at the dose of 10 mg/kg b.wt, groups 3 and 4 respectively 100 and 200 mg/kg b.wt of the aqueous extract of *E. angolense* and groups 5 and 6 received respectively ethanolic extract at dose of 100 and 200 mg/kg b.wt. One hour after drug treatment, 0.1 mL of 1% (w/v) carrageenan solution was injected subcutaneously into the subplantar aponeurosis of right hind paw of each rat. The evolution of right hind paw edema was measured at 1 h, 2 h, 3 h, 4 h, 5 h, 6 h and 24 h [9]. The anti-inflammatory activity expressed as inhibition percentage (% Inh) of the edema was calculated for each group of rats treated compared with control group.

$$\%Inh = \frac{Pc-Pt}{Pc} \times 100$$

Pc = Percentage of increase of the paw of control group;
Pt = Percentage of increase of the paw of treated group.

The percentage of increase (% Inc) of the rat paw was given by the formula:

$$\%Inc = \frac{Dt-Do}{Do} \times 100$$

Do = Paw diameter in mm, before carrageenan administration;
Dt = Paw diameter in mm, at time t, after carrageenan administration.

2.5 Determination of CRP

Before the determination of CRP, the rats were put in 16 hours water diet. They were weighed and divided into five groups of five animals each. The various compounds were administrated orally in the following way:

- Normal group (group 1) and control group (group 2), distilled water (10 ml/kg);
- Group 3, Diclofenac Sodium solution (10 mg/kg b.wt);
- Group 4, aqueous extract of *E angolense* (200 mg/kg b.wt);
- Group 5, ethanolic extract of *E angolense* (200 mg/kg b.wt).

One hour after administration, the rats of groups 2, 3, 4 and 5 received 0.1 mL of 1% (w/v) carrageenan solution subcutaneously into the subplantar aponeurosis of right hind paw. Five hours after injection, blood was collected in rats by incision of the tail. The determination of CRP was made from serum of the animals by the technique of immunoturbidimetry on Cobas C_{311} (Hitachi). The principle is based on the photometric measurement of the disorder brought by the antigen-antibody reaction in the final point method at 340 nm [10].

2.6 Statistical Analysis

The results were expressed as mean followed by the standards errors of mean (mean ± SEM). The graphic representation of data was carried out from the software Graph Pad PRISM 5.0 (Microsoft U.S.A). Statistical analysis of results was performed using the analysis of variances (ANOVA). The differences between the means were determined according to Dunnett's comparison test. $P < 0.05$ is considered as significant.

3. RESULTS

3.1 Effect of Aqueous and Ethanolic Extracts of *E. angolense* on the Edema Induced by Carrageenan

Edema was created in the right hind paw of rat. The variation of edema was appreciated after administration of the aqueous and ethanolic extracts of *E. angolense* at dose of 100 and 200 mg/kg b.wt orally. The results obtained were compared with those of Diclofenac Sodium®, a nonsteroidal anti-inflammatory and control (distilled water). After administration of distilled water, carrageenan induced edema which increased gradually with a maximum of 38.41% after five hours of observation (Table 1). Administration of Diclofenac Sodium at dose of 10 mg/kg b.wt significantly prevents the increase in the diameter of rat paw. It varies from 7.38% to 15.82% after administration of carrageenan (Fig. 1). The results were significantly different from

those of control (distilled water) (P <0.05). Administration of the aqueous extract of *E. angolense* at dose of 100 and 200 mg/kg b.wt significantly (P <0.05) prevents acute edema of the rat paw during 24 hours. The effect of ethanolic extract of *E. angolense* was less pronounced compared to that of the aqueous extract in the prevention of acute edema of the rat paw. From the first hour to the fourth hour, the aqueous extract at dose of 200 mg/kg b.wt respectively shows inhibition percentages similar to those of Diclofenac Sodium® at dose of 10 mg/kg b.wt (Table 2).

3.2 Effect of Aqueous and Ethanolic Extracts of *E. angolense* on the Serum Concentration of CRP after Induction of Edema by Carrageenan

The CRP value of DW group + carrageenan group is significantly high (p <0.05) compared to the treated groups. On the other hand, the administration of extracts and Diclofenac Sodium decreased the concentration of CRP induced by the injection of carrageenan compared to the control (Fig. 2).

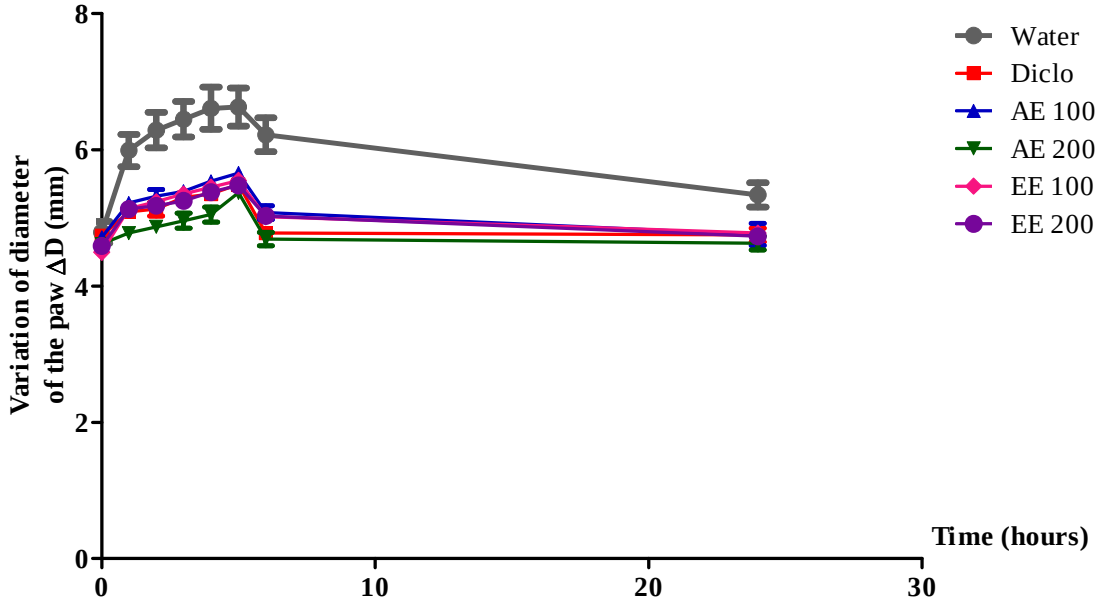

Fig. 1. Variation of the rat paw diameter of the treated groups compared with control according to time

Expressed results in mean ± S.E.M (n = 6). p <0.05 statistically significant compared to the control
DICLO: Diclofenac; AE: Aqueous Extract at dose of 100 and 200 mg/kg b.wt;
EE: Ethanolic Extract at dose of 100 and 200 mg/kg b.wt

Table 1. Percentage of increase in edema of rats treated with diclofenac and extracts of *E. angolense* bark

Compounds	Dose	% of increase						
		T_{1h}	T_{2h}	T_{3h}	T_{4h}	T_{5h}	T_{6h}	T_{24h}
DW	10 ml/kg	25.05	31.31	34.65	37.99	**38.41**	29.85	11.48
DICLO	10	7.38	8.22	11.81	12.86	15.82	0.84	0.21
AE	100	10.59	12.71	14.19	17.37	19.91	7.62	0.84
AE	200	3.46	5.41	7.35	9.30	16.23	1.51	0.21
	mg/kg							
EE	100	14.22	16.44	18.88	21.11	23.33	11.55	6.22
EE	200	11.76	12.85	14.37	17.21	19.38	9.58	3.05

DW: Distilled Water (control); DICLO: Diclofenac; AE: Aqueous extract at dose of 100 and 200 mg/kg b.wt;
EE: Ethanolic extract at dose of 100 and 200 mg/kg b.wt

Table 2. Inhibition percentage of edema of rats treated with diclofenac and extracts of *E. angolense* bark

Compounds	Dose mg/kg	Inhibition percentage						
		T_{1h}	T_{2h}	T_{3h}	T_{4h}	T_{5h}	T_{6h}	T_{24h}
Control		-	-	-	-	-	-	-
DICLO	10	**70.53**	**73.74**	**65.91**	**66.14**	58.81	97.18	98.17
AE	100	57.72	59.40	59.04	54.27	48.16	74.47	92.68
AE	200	**86.18**	**82.72**	**78.78**	**75.51**	57.74	94.94	98.17
EE	100	43.23	47.49	45.51	44.43	39.26	61.30	45.81
EE	200	53.05	58.95	58.52	54.69	49.54	67.90	73.43

Expressed results in mean ± S.E.M (n = 6). P <0.05 statistically significant compared to the control
DICLO: Diclofenac; AE: Aqueous extract at dose of 100 and 200 mg/kg b.wt;
EE: Ethanolic extract at dose of 100 and 200 mg/kg b.wt

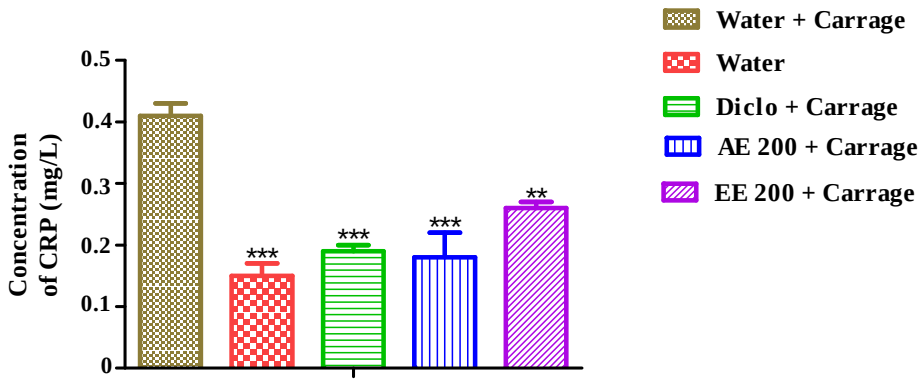

Fig. 2. Effect of *E. angolense* extracts on serum CRP concentration 5 hours after injection of the carrageenan

*Values in Mean ± S.E.M (n = 5); ** P < 0.01 and *** p < 0.001statistically significant compared to the Distilled water + carrageenan group.*
Distilled water + Carrageenan; Diclofenac + Carrageenan; Aqueous extract at 200 mg/kg b.wt + Carrageenan; Ethanolic extract at 200 mg/kg b.wt + Carrageenan

4. DISCUSSION

The carrageenan-induced rat paw edema test [11] and the CRP determination method were used to evaluate anti-inflammatory activity. Carrageenan (a phlogistic agent) induced edema in the rat paw considered as a characteristic sign of inflammation [12]. This technique has been used because of its simplicity of execution, its rapid induction of symptoms characteristic of inflammation (development of edema at the hour which follows the injection, with a maximum effect at the end of 5 hours) and also because of its reproducibility. In the evaluation of anti-inflammatory activity, one of the non-steroidal anti-inflammatory drugs is used for comparison of experimental and clinical parameters. Diclofenac was chosen for comparison in this study because it is most commonly used clinically [13]. Phytochemical analysis carried out on bark of *E. angolense* revealed the presence of alkaloids, polyphenols, sterols, terpenes, tannins, flavonoids, leucoanthocyanins, quinones, saponins and cardiotonic glycosides [14]. During this kinetic study, our extracts gave an inhibition of the edema compared to the control. Already at first hour the aqueous extract of *E. angolense* bark at dose 200 mg/kg b.wt gave the same effect as diclofenac sodium at dose of 10 mg/kg b.wt with respectively 86.18% and 70.53% inhibition of edema. This gives the plant a mechanism of anti-inflammatory action similar to that of nonsteroidal anti-inflammatory drugs and could be explained by the presence in the plant of tannins, polyphenols and flavonoids. However, studies have showed that the anti-inflammatory effect of flavonoids would be due to an inhibition of prostaglandin synthesis and polyphenols could act on the enzymatic activities of arachidonic acid metabolism [15]. Tanins are chemical

substances known for their ability to bind to proteins with a tendency to impermeability of the external layers and the protection of the underlying layers [16]. The extracts also possess sterols and terpenes, compounds responsible for various activities in plants and have beneficial effects in humans and animals. Thus, the effect of sterols could be due to an inhibition of pro-inflammatory cytokines and cyclooxygenase [17,18]. Terpenes are natural compounds which contribute to aroma of plants. Its anti-inflammatory properties would be due to the inhibition of inflammatory cell migration and a decrease in the release of pro-inflammatory cytokines [19]. C-reactive protein is a protein which reacts with pneumococcus polysaccharide C in the serum of patients with an acute inflammation [20]. Its physiological roles are the activation of classical complement pathway, mobilization and activation of leukocytes, stimulation of phagocytosis and cytokine secretion by monocytes [21]. Our results showed that administration of extracts and the reference molecule (Diclofenac Sodium) decreased the concentration of CRP induced by carrageenan injection compared to control (distilled water + carrageenan). The results confirm those obtained in the edema test. The various studies conducted on the protective effects of polyphenols in these pathological contexts have shown that these diminish the markers of inflammation.

5. CONCLUSION

The anti-inflammatory effect of the aqueous and ethanolic extracts of *E. angolense* was evaluated in this work. The results obtained show that the extracts have an anti-inflammatory activity. However, this activity is different according to the degree of solubility of secondary compounds in *E. angolense* extracts. During the carrageenin-induced rat paw edema test, the aqueous bark extract at dose of 200 mg/kg b.wt showed an anti-inflammatory activity more significant than the ethanolic extract. Also, the extracts decreased the CRP level induced by the injection of carrageenan.

Thus, these results constitute a scientific basis justifying the traditional use of *Entandrophragma angolense* in the management of pathologies with inflammatory component (malaria, ulcer, rheumatism ...).

ETHICAL APPROVAL

The experimental procedures and protocols used in this study were approved by the Ethical Committee of Health Sciences, University Felix Houphouët-Boigny. These guidelines were in accordance with the European Council Legislation 87/607/EEC for the protection of experimental animals. All efforts were made to minimize pain of animals and reduce the number of animals used.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sy GY, Wélé A, Ndiaye M, Diatta W, Barbosa FS, Dièye AM, Touré MT, Bassène E, Faye B. Evaluation of the anti-inflammatory activity of *Annona reticulata* (Annonaceae) leaves on acute edema of the rat leg induced by carrageenan. Pharm Med Trad Afric. 2008;15:23-25.
2. Rousselet MC, Vignaud JM, Hofman P, Chatelet FP. Inflammation and inflammatory pathology. Paris Maloine. 2005;320-331.
3. Corrado B, Marco T, Colucci R, et al. Role of coxibs in the strategies for gastrointestinal protection in patients requiring chronic non-steroidal anti-infllammatory therapy. Pharm Res. 2009;59:90-100.
4. Cox PA, Balick MJ. The ethnobotanical approach to drug discovery. Sc Am. 1994;270(6):82-7.
5. Bickii J, Tchouya GR, Tchouankeu JC, Tsamo E. Antimalarial activity in crude extracts of some Cameroonian medicinal plants. Afr J Tradit Compl Altern Med. 2007;4(1):107-111.
6. Bray DH, Warhurst DC, Connolly JD, O'Neill MJ, Phillipson JD. Plants as sources of antimalarial drugs. Part 7. Activity of some species of Meliaceae and their constituent limonoids. Phytother Res. 1990;4:29-35.
7. Bouquet. Fetish and traditional medicine of Congo (Brazzaville); O.R.S.T.O.M., Paris. 1969;157.
8. Bidié AP, Koffi E, N'guessan JD, Djaman AJ, Guédé-Guina F. Influence of *Mitragyna ciliata* (MYTA) on the microsomalactivity of ATPaseNa+/K+ dependent extract on a rabbit (heart). Afr J Trad CAM 5. 2008;294-301.

9. Anupama AS, Kishor NR, Rahul DK, Kanchan SM. Evaluation of anti-inflammatory and analgesic activities of *Tamarindus indica* seeds. Int J Pharm Sci Drug Res. 2012;4(3):213-217.
10. Marrack JR, Richards CB. Light-scattering studies of the formation of aggregates in mixtures of antigen and antibody. Immunology. 1971;20(6):1019-1040.
11. Winter CA, Risley EA, Nuss GW. Carrageenan-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med. 1962;111: 544-547.
12. Morris CJ. Carrageenan-induced paw edema in the rat and mouse. Inflammation Protocols. Totowa, NJ, Humana Press Inc. 2003;115-121.
13. Ravi SS, Mushtaq A, Zahoor AW, Vikas S, Vijay VM, Prerna U. Antiinflammatory effects of Dashmula, an ayurvedic preparation, versus Diclofenac in animal models. J Chem Pharm Res. 2011;3:882-888.
14. Aurélie AY, Yapi HF, Ayébé EK, Yapo AF, Nguessan JD, Djaman AJ. Acute toxicity and phytochemical screening of stem bark extracts of *Entandrophragma angolense* (Welw.) C.DC. (Meliaceae). Asian J Biom Pharm Sc. 2014;4(38):67-70.
15. Havsteen BH. The biochemistry and medical significance of flavonoids. Pharmacol Ther. 2002;96:67-202.
16. Bruneton J. Pharmacognosie, phytochimie et plantes médicinales. TEC & DOC et Médicales Internationales, éd., 3e, Paris, France. 1999;1120.
17. Bouic PJ, Lamprecht JH. Plant sterols and sterolins: A review of their immune-modulating properties. Altern Med Rev. 1999;4:170-7.
18. Awad AB, Burr AT, Fink CS. Effect of resveratrol and β-sitosterol in combinaison on reactive oxygen species and prostaglandin release by PC-3 cells. Prostaglandins Leukot Essent Fatty Acids. 2005;72(3):219-226.
19. Patocka J. Biologically active pentacyclic triterpenes and their current medicine signification. J Appl Biomed. 2003;1:7-12.
20. Ablij H. C-reactive protein: History and revival. Eur J Intern Med. 2002;13:412-422.
21. Hajek V, Pasquet F, Karkowski L, Lachenal F, Gerôme P, Pavic M. Etiologic and prognostic profile of extreme values (≥500 mg / L) of C-reactive protein: Retrospective study of 168 values in 113 patients. The Internal Med Revue. 2011;32:663-668.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/20565