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EVALUATION OF THE ANTI-ARTHRITIC ACTIVITY OF *BAUHINIA ACUMINATA* (LINN) LEAVES EXTRACTS IN EXPERIMENTAL MODELS

JAMADAR MJ[*1, 2], KHULBE P[2] AND MOHITE SK[3]

1: Appasaheb Birnale College of Pharmacy, Sangli-416416, India

2: School of Pharmacy, Suresh Gyan Vihar University, Mahal, Jagatpura, Jaipur, Rajasthan 302017, India

3: Rajarambapu College of Pharmacy, Kasegaon, Taluka - Walwa, Dist-Sangli, Maharashtra, India

[*]**Corresponding Author: Mohsin J. Jamadar: E Mail: mohsinjamadar999@gmail.com**

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ABSTRACT

Medicinal plants are playing an imperative role in the therapy for treating various chronic ailments including arthritis. Using *In*-vivo arthritis models, the current study was started to assess the anti-arthritic effects of ethanol extract and aqueous extracts of *Bauhinia acuminata* Linn. *In-vivo* anti-arthritic activity of ethanolic and aqueous extract at a dose of 200 mg/kg and 400 mg/kg respectively was assessed using formaldehyde-induced arthritis on 0th day, 7th day, 14th day, 21st day and 28th day.*Bauhinia acuminata* Linn produced significant ([**]p<0.01) dose dependent inhibition of paw volume edema and markedly improved hematology profile. In formaldehyde-induced arthritis models, the ethanolic extract of leaves of *Bauhinia acuminata* Linn at 400 mg /kg significantly ([**]p<0.01) reduced paw volume edema by 70.24%on 28thday. Whereas ethanolic extract of leaves of *Bauhinia acuminata* Linn 200 mg/kg, b.w., p.o.,) appears to show significant activity ([*]p<0.05) on day 28th with 61.157% inhibition. The aqueous extracts of leaves of *Bauhinia acuminata Linn* at 400 mg/kg appears to show significant activity ([*]p<0.05) on day 28th reduced paw volume edema by 58.677%. Whereas aqueous extract of leaves of *Bauhinia acuminata* Linn 200 mg/kg, b.w., p.o.,) appears to show significant activity ([*]p<0.05) on day 28th with 54.54% inhibition.It could be concluded that *Bauhinia acuminata* Linn holds anti-arthritic potential, supporting its traditional use in treatment of rheumatoid arthritis.

Keywords: *Bauhinia acuminata* Linn, Anti-Arthritic Activity, Medicinal plants

INTRODUCTION:

One of the most prevalent chronic autoimmune disorders is rheumatoid arthritis (RA). Synovial joints initially experience localised swelling and stiffness before the condition develops into a chronic multisystem illness. RA commonly manifests between the ages of 30 and 50 and involves symmetrical polyarthritis of both major and small joints **[1]**. Due to lack of movement, morning symptoms are more acute. The pathological characteristics of RA include an increase in the cellularity of synovial tissue as well as joint degeneration brought on by inflammatory responses **[2]**. Non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and disease-modifying anti-rheumatoid drugs (DMARDs) are frequently used to treat inflammatory illnesses including arthritis **[3]**. NSAIDs are traditionally preferred in RA because of their analgesic and anti-inflammatory properties, which work by inhibiting cyclooxygenases 1 and 2, or "COXs," which produce prostaglandins. However, these medications have side effects, including gastrointestinal, renal, cardiac, and hepatic disorders for COX-1 and myocardial infarction and strokes for COX-2 **[4]**. Finding a new strategy for treating RA through alternative medicine is critical given all these negative effects. Natural products are more accessible and affordable than manufactured treatments since they have less toxicity and adverse effects. Because it has been shown that they are effective and useful in the treatment of RA, many medicinal plants are now being investigated for the creation of innovative medicines **[5, 6]**. The risk-benefit ratio is higher for traditional medicinal plants, which are widely used to treat inflammatory diseases. Ayurveda recommended utilising a single herb or a combination of herbs to treat RA **[7]**.

MATERIALS AND METHODS

Plant material and extraction:

Fresh *Bauhinia acuminata* (Linn.) plants were collected in Kolhapur, Maharashtra, and authenticated by the Botanical Survey of India in Pune. Using the Soxhlet apparatus, the coarse powder plant material was extracted with ethanolic and aqueous to obtain semisolid mass, the solvent was removed under reduced pressure. To determine the phytoconstituents in the extract, standard methods were used for preliminary phytochemical screening. The extract was discovered to contain alkaloid, flavonoids, glycosides, steroids, and tannins.

Animals:-

Male Albino rats weighing between 150-220 gm were procured from the Animal House, Appasaheb Birnale College of Pharmacy, Sangli for the present study. The animals were

randomly assigned to treatment groups and housed in cages with paddy husk as bedding. The animals were kept at a temperature of 24±2ºC and a humidity of 30-70%. A light: day cycle of 12:12 was used.

All animals had unrestricted access to water and were fed standard commercial pellet. The (IAEC) reviewed all of the investigational procedures and protocols used in this study, and they were all as per the IAEC's guidelines. Animal handling was carried out in accordance with Good Laboratory Practice (GLP). The Institutional Animal Ethics Committee granted ethical approval, and the experiment was Perform in accordance with the Indian National Science Academy's guidelines for the use and care of experimental animals. R. No: (IAEC/ABCP/19/19-20)

Chemicals and reagents:-

All the chemicals like Formaldehyde, Anaesthetic ether used in this study were of Standard analytical grade. Diclofenac sodium was purchased from Novartis Pharma.

Experimental groups:-

Formaldehyde induced arthritis:-

Group 1: Negative control (1% v/v Tween 80, p.o. for 28 days).

Group 2: Positive Control (Formaldehyde 0.1 ml 2% v/v by sub-plantar region).

Group 3: Standard (Diclofenac 10 mg/kg p.o. for 28 days)+ Formaldehyde 0.1 ml 2% v/v.

Group 4: AQ (200 mg/kg p.o. for 28 days) + Formaldehyde 0.1 ml 2% v/v.

Group 5: AQ (400 mg/kg p.o. for 28 days) + Formaldehyde 0.1 ml 2% v/v.

Group 6: ETH (200 mg/kg p.o. for 28 days) + Formaldehyde 0.1 ml 2% v/v.

Group 7: ETH (400 mg/kg p.o. for 28 days) + Formaldehyde 0.1 ml 2% v/v.

Animals were divided into six groups (n = 6).

Group I received the vehicle (2 ml/kg, 1% v/v Tween 80) and served as the Negative (normal) control.

Group II received Formaldehyde, served as a Positive control.

Group III received the Standard Diclofenac sodium (10 mg/kg body weight),

Groups IV received AQ 200mg/kg, b.w. of *Bauhinia acuminata* (Linn.)

Group V received AQ 400mg/kg, b.w. of *Bauhinia acuminata* (Linn.)

Group VI received ETH 200mg/kg, b.w. of *Bauhinia acuminata* (Linn.)

Group VII received ETH 400mg/kg, b.w. of *Bauhinia acuminata* (Linn.)

Thirty minutes after oral administration of vehicle/drugs, arthritis was induced by sub-plantar administration of 0.1 ml formaldehyde (2% v/v) into the left hind paw of all the animals except normal control. This was designated as day 1.

Vehicle/drug treatment was continued for the duration of 28 more days. Formaldehyde (0.1 ml 2% v/v) was again injected into the same paw on the third day **[8, 9]**. Paw volume was measured at 0 days, 7thday, 14th day, 21stday and 28thday by using Plethysmometer (UGO BASILE, Italy).

% inhibition of paw edema concerning untreated groups was calculated using the following formula:

$$\mathbf{i= [1-(\Delta V_{treated}/\Delta V_{untreated})] \times 100}$$

Where,

i= % inhibition of paw edema

$\Delta V_{treated}$= mean change in paw volume of treated rat

$\Delta V_{untreated}$= mean change in paw volume of untreated rat

Method for collection of a blood sample: On the 28thday, the blood (2 ml) collected by retro-orbital cavity under the influence of ether anaesthesia. The collected blood was used to perform various hematological studies to estimate WBC, RBC & Platelets and serum biochemical parameter C-reactive protein (CRP).

Table 1: Effect of aqueous & ethanolic extract of leaves of *Bauhinia acuminata* Linn on paw volume of Formaldehyde induced arthritis rats

Groups	Paw volume edema (Mean ± SEM) in rats				
	0th day	7th day	14th day	21th day	28st day
Negative (Normal)Control	0.40±0.17	0.41±0.012	0.39±0.51	0.37±0.03	0.32±0.014
Positive Control (Formaldehyde induced) 0.1 ml 2% v/v	0.79±0.31	0.90±0.075	1.16±0.14	1.26±0.13	1.21±0.20
Diclofenac sodium (10 mg/kg p.o)	0.61± 0.10 *** (↓22.78)	0.39±0.07 *** (↓56.66)	0.36±0.014 *** (↓68.965)	0.27±0.06*** (↓78.57)	0.18±0.05*** (↓85.123)
AQ (200 mg/kg)	0.76±0.13* (↓3.797)	0.71±0.08* (↓21.11)	0.68±0.03* (↓41.38)	0.60±0.11* (↓52.38)	0.55±0.05* (↓54.54)
AQ (400 mg/kg)	0.66±0.32* (↓16.45)	0.63±0.01* (↓30.00)	0.59±0.099* (↓49.14)	0.55±0.091* (↓56.35)	0.50±0.03* (↓58.677)
ETH (200 mg/kg)	0.62±0.104* (↓21.51)	0.58±0.09* (↓35.55)	0.56±0.105* (↓52.173)	0.50±0.05* (↓60.32)	0.47±0.057* (↓61.157)
ETH (400 mg/kg)	0.60±0.16* (↓24.05)	0.47±0.06** (↓47.77)	0.50±0.19** (↓56.89)	0.48±0.07** (↓61.90)	0.36±0.014** (↓70.24)

Figures in bracket indicate percentage inhibition of edema

Values are represented as mean±S.E.M. (n=6), Data was analysed -using one–way ANOVA followed by Dunnett's-multiple comparison test by using GraphpadPrism 9 for Windows. Disease Control vs. Treated Groups: (* p<0.05, ** p<0.01, ***p<0.001)

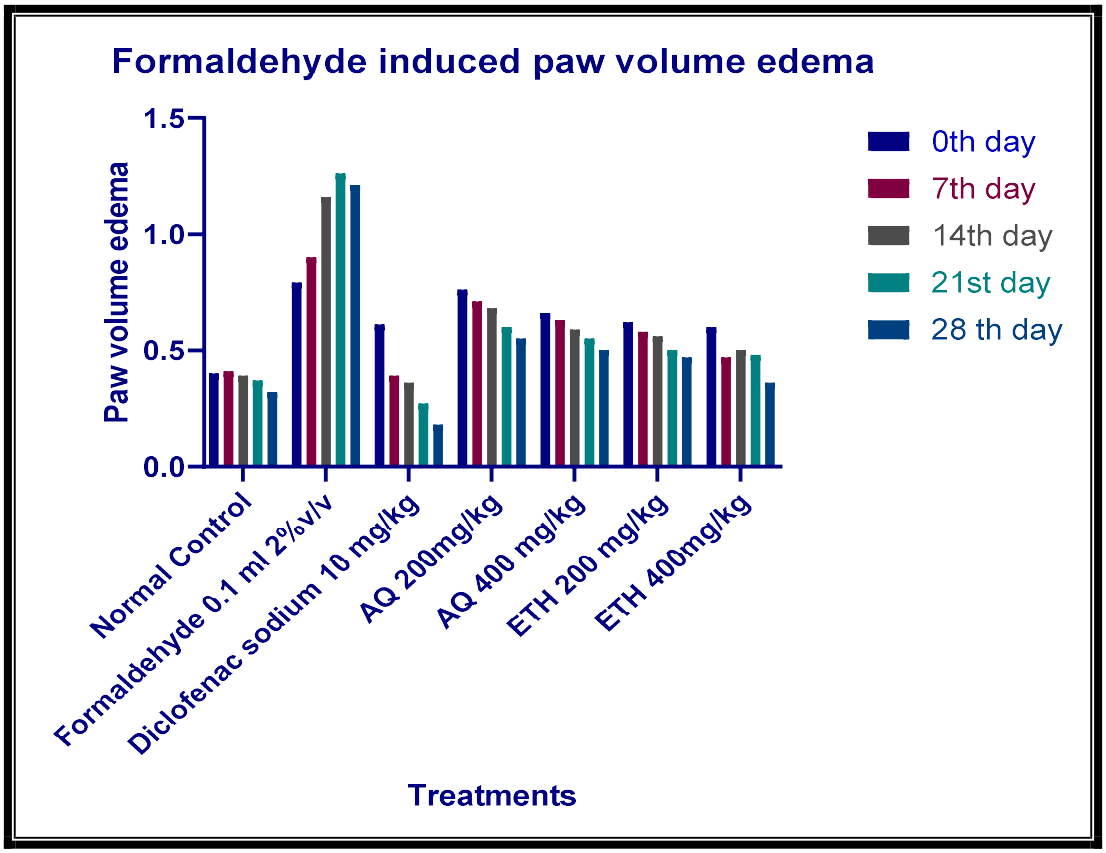

Figure 1: Effect of AQ & ETH extract of leaves of *Bauhunia acuminata* Formaldehyde induced arthritis rat

Table 2: Effect of aqueous & ethanolic extract of leaves of *Bauhinia acuminata Linn* on Formaldehyde induced changes in hematological profile

Hematological Profile				
Treatment (Dose mg/kg)	**WBC×10^3/mm^3**	**RBC×10^6/mm^3**	**Platelet (lakhs/Cu mm)**	**CRP (mg/l)**
Non arthritic control	**11.43 ± 0.12**	**9.32 ± 0.62**	**3.36 ± 1.19**	**1.333 ±0.33**
Arthritic control	**19.31 ±021**	**5.14 ±0.041**	**10.58 ±1.01**	**14.33 ±1.44**
Diclofenac sodium (10 mg/kg p.o)	**11.06 ±0.36*****	**6.57 ±0.016*****	**5.20 ±1.60*****	**4.33 ±0.69*****
AQ 200	**19.07 ± 1.32***	**4.41 ± 0.01***	**10.25 ±0.83***	**12.46 ± 1.27***
AQ 400	**17.18 ± 1.61***	**5.17 ± 1.23***	**9.17 ± 0.08***	**10.33 ±1.53***
ETH 200	**15.05 ± 1.23***	**4.13 ± 0.07***	**6.36 ±0.14***	**8.20 ± 0.57***
ETH 400	**14.18 ±0.34****	**7.13 ± 1.23****	**5.63 ± 0.70****	**7.41 ±0.23****

Values are represented as mean±S.E.M. (n=6), Data was analysed -using one–way ANOVA followed by Dunnett's-multiple comparison test by using Graphpad Prism 9 for Windows. Disease Control vs. Treated Groups: (* p<0.05, ** p<0.01, ***p<0.001)

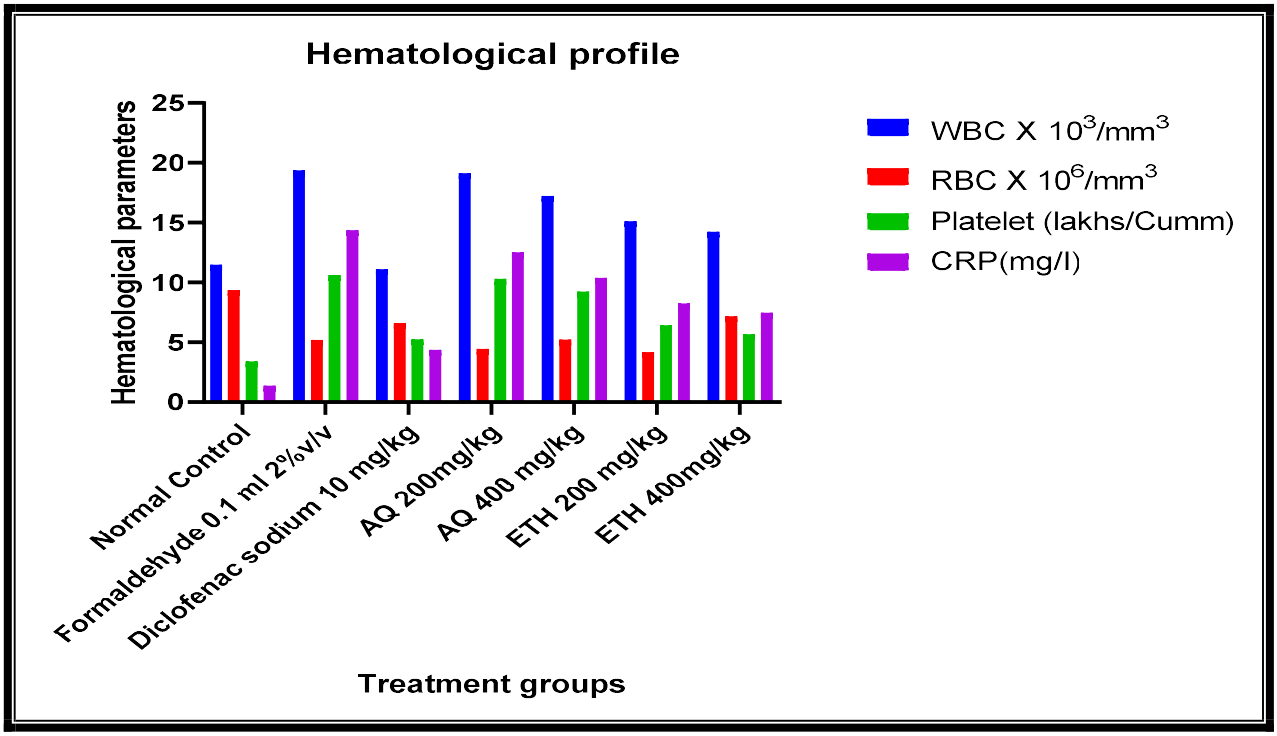

Figure 2: Effect of AQ & ETH extract of leaves of *Bauhunia accuminata* (Linn.) on Hematological profile in Formaldehyde induced arthritis

Statistical analysis

Values are represented as Mean±S.E.M. (n=6), Data was analysed -using one–way ANOVA followed by Dunnett's-multiple comparison test by using Graphpad Prism 9 for Windows. Disease Control vs. Treated Groups: (* p<0.05, ** p<0.01, ***p<0.001).

RESULT

Effect of aqueous & ethanolic extract of leaves of *Bauhinia acuminata* (Linn.) in Formaldehyde induced paw volume edema. Induction of Formaldehyde caused significant increase in paw volume was seen in all animals throughout the observation period. Maximum paw volume was observed on day 28th in arthritic group. Group-3 rats (i.e. Diclofenac sodium (10mg/kg, b.w., p.o.) appears to exhibit significant inhibition on day 7th (***p<0.001) and day 14th with 56.666% and 68.965% inhibition respectively. It appears to show highly significant (***p<0.001) on day 21th to 28st with 78.57% and 85.123% inhibition. Group-4 rats (i.e. AQ exts. of leaves of *Bauhinia acuminata* (Linn.) 200 mg/kg, b.w., p.o.,) appears to show significant activity (*p<0.05) on day 7th and day 14th with 21.11% and 41.38% inhibition. It appears to show significant activity (*p<0.05) on day 21th and day 28st with 52.38% and 54.54% inhibition. Group-5 rats (i.e. AQ exts. of leaves of

Bauhinia acuminata (Linn.) 400 mg/kg, b.w., p.o.,) appears to show significant activity ($^{*}p<0.05$) on day 7th and day 14th with 30.00% and 49.14% inhibition. It appears to show significant activity ($^{*}p<0.05$) on day 21th and day 28st with 56.35% and 58.677% inhibition. Group-6 rats (i.e. ETH exts. of leaves of *Bauhinia acuminata* (Linn.) 200 mg/kg, b.w., p.o.,) appears to show significant activity ($^{*}p<0.05$) on day 7th and day 14th with 35.55% and 52.173% inhibition. It appears to show significant activity ($^{*}p<0.05$) on day 21th and day 28st with 60.32% and 61.157% inhibition. Group-7 rats (i.e. ETH exts. of leaves of *Bauhinia acuminata* (Linn.) 400 mg/kg, b.w., p.o.,) appears to show significant activity ($^{**}p<0.01$) on day 7th and day 14th with 47.77% and 56.89% inhibition. It appears to show significant activity ($^{**}p<0.01$) on day 21th and day 28st with 61.90% and 70.24% inhibition.

Rheumatoid arthritis is associated with hematological abnormalities during the course of the disease. The changes in hematological parameters in formaldehyde induced arthritic rats when compared to other groups are shown in **Table 1, Table 2**. There was a significant reduction in RBC count in arthritic group when compared to normal ($^{**}p<0.01$). A significant increase in WBC count and platelet count was also observed in Formaldehyde group compared to normal group ($^{*}p<0.05$). Treatment with the standard drug Diclofenac sodium (10 mg/kg p.o) and phytocompounds were followed by favourable outcome in the altered hematological parameters. Diclofenac sodium (10 mg/kg p.o) treatment significantly improved the RBC count with signification reduction in WBC and Platelet counts ($^{*}p<0.05$). Compounds treated at both the doses were found to be equally effective and significant in reverting the altered hematological parameters to normal ($^{*}p<0.05$). The CRP levels of arthritic group were significantly elevated compared to normal. Treatment with Diclofenac as well as extract treated (aqueous & ethanolic) groups at both doses could significantly reduce ($^{*}p<0.05$) the CRP levels indicating a decline in the disease severity.

DISCUSSION

Rheumatoid arthritis (RA) is a chronic, systemic, immune-mediated inflammatory disease associated with decreased life expectancy and quality of life **[10]**. Currently no treatment can effectively afford the complete amelioration of the symptoms while only offering temporary relief. Further the available treatments of DMARD's (Disease Modifying Anti Rheumatoid Drugs) therapy are often associated with severe side effects **[11]**.

Animal models of rheumatoid arthritis are used extensively in research on the pathogenesis of inflammatory arthritis as well as in the pharmaceutical industry for the testing of potential anti-arthritic agents. The use of RA animal models has substantially advanced our understanding of the mechanisms and mediators involved in the development of inflammation, cartilage loss, and bone resorption. These developments have had a significant impact on therapeutic intervention for this debilitating disease **[12]**. Even though numerous studies have advanced our understanding of a variety of potential explanations, including reactivity to cartilage proteoglycans, heat shock proteins, and interactions with intestinal flora, the pathogenesis and causes of adjuvant disease that develops after injection of arthrogenic preparations remain unclear **[13, 14]**. Formaldehyde induced arthritis is one of most commonly used acute model for assessing anti-arthritic potential of plant extract. The development of edema in the paw of the rat after injection of formaldehyde (0.1ml, 2% v/v) is due to the release of histamine, serotonin and the prostaglandin like substances at the site of injection **[15]**. Inhibition of paw edema in formaldehyde induced arthritis may be due to the anti-inflammatory potential of leaves of *Bauhinia acuminata* (Linn.). Hind paw swelling is a characteristic feature of formaldehyde induced arthritis which is monitored from day 0 (onset of disease) to 21 or greater depending on duration desired. Treatments are initiated on day 0 (prophylactic model dosing) or day 8 (therapeutic model) **[16, 17]**. Several lines of studies have reported the efficacy of paw swelling as an index to measure arthritis severity in adjuvant induced arthritis model.

Hematological abnormalities may show up in rheumatoid arthritis (RA) patients at the time of diagnosis or later on in the course of their illness. Neutropenia, thrombocytopenia, large granular lymphocytosis (LGL), and malignancies are infrequent, although anaemia, thrombocytosis, and eosinophilia are frequently seen **[18, 19]**. A decrease in the level of RBC, and increase in the level of WBC, Platelet were noted in the present study in arthritic rats which were reverted towards normal in treatment groups. These results were similar to those observed for several other studies **[20-22]**. Estimation of CRP level is one of the most reliable and objective measure and a useful prognostic factor for disease progression to joint damage associated with RA. CRP is a biomarker for inflammation associated with rheumatoid arthritis (RA) that corresponds with disease activity, synovial histological alterations, and radiographic

progression, reacting swiftly to changes in disease activity **[23]**.

CONCLUSION

Bauhinia acuminata (Linn.) leaves ethanolic extract at 400 mg/kg, have anti-arthritic potential as obvious from a for mentioned results. Further studies on the mechanistic analysis of anti-arthritic activity as well as isolation of pure compounds from leaves of *Bauhinia acuminata* (Linn.) should be carried out.

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