Research Article

Evaluation of Anti-Inflammatory Effect of Ethyl Acetate and Methanol Extracts of *Loranthus europaeus* in Experimental Models of Acute Inflammation in Rats

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ABSTRACT

Loranthus europaeus was extracted by maceration with 70% ethanol then step wise fractionated with petroleum ether, chloroform, ethyl acetate and the remaining methanol fraction. Ethyl acetate and methanol fractions were analyzed by HPLC for its flavonoids contents & tested for its anti-inflammatory effect by induction paw edema in rats by using fresh egg albumin and measure the paw edema before 30 min. from induction and 30 min, 1 hr, 1.5 hr and 2 hr after induction & compared with piroxicam. Both fractions of *loranthus europaeus* show non-significant decrease in the mean of increase of paw edema after half hour when compared with negative control, but after one hour of induction ethyle acetate fraction shows significant decrease in the mean of increase in paw edema, while methanol fraction show non- significant decrease in the same parameter when compared with negative control . After one and half hour and two hour of starting measuring, both fraction both fraction show significant decrease in measure parameter when compared with negative control.

Keywords: Ethanol, Ethyl acetate, Flavonoid, Loranthus, Piroxicam.

INTRODUCTION

Loranthus is a genus of parasitic plants that grow on the branches of woody trees. It belongs to the family Loranthaceae (the showy mistletoe family).[1] In most earlier systematic treatments it contains all mistletoe species with bisexual flowers (though some species have reversed to unisexual flowers), while most modern systematists treat it as a monotypic genus with the only species *Loranthus europaeus* Jacq. The summer mistletoe or European yellow mistletoe. In contrast to the wellknown European or Christmas mistletoe (*Viscum album* L., Santalaceae or Viscaceae) this species is deciduous. The systematic situation of *Loranthus* is not entirely clear, and some showy mistletoe in Asia may be true parts of this genus.

Historically, mistletoe had been use in several cases like (swellings, tumours, epilepsy, hysteria, delirium, vertigo, antispasmodic, tonic and narcotic, diseases of spleen and liver, labor-pains, weakness of the heart' and edema, eczema, ulcers of the feet, burns, and granulating wounds.[2] Recent scientific research has confirmed the folklore with evidence that mistletoe extracts (a) induce apoptosis, (b) stimulate immunocompetent cells which slow the growth of cancer cells, and (c) protect the DNA of mononuclear cells.[3] Loranthus species are known to produce variety of bioactive compounds for examples kaempferol 3-*O*-α-D-rhamnoside, kaempferol 3, 7-di-*O*-β-D-glucoside, quercetin 3-*O*-α-D-rhamnoside and quercetin 3-*O*-β-D-glucoside from the leaves of L. kaoi and from L. europaeus.[4]

Inflammation is the response of living tissue to mechanical injuries, burns, microbial infections, and other noxious stimuli that involve changes in blood flow, increased vascular permeability, activation and migration of leucocytes and the synthesis of local inflammatory mediators.[5]

All the steroidal and non-steroidal anti-inflammatory drugs (NSAIDs), despite their great number, cause undesired and serious side effects. Therefore, development of new and more powerful drugs is still needed. Medicinal plants have long been used worldwide in folk medicine as an alternative treatment of inflammatory processes of diverse origins.[6]

Egg albumin-induced inflammation in the rat paw represents a classical model of edema formation and hyperalgesia, which has been extensively used in the development of no steroidal anti-inflammatory drugs and selective COX1-2 inhibitors .Several lines of evidence indicate that the COX-2-mediated increase in prostaglandin (PG) E2 production in the central nervous system (CNS) contributes to the severity of the inflammatory and pain responses in this model. COX-2 is rapidly induced in the spinal cord and other regions of the CNS following fresh albumin injection in the paw.[7]

MATERIALS AND METHODS

Plant extraction and phytochemical investigation

100 grams of loranthus dry seed were triturated by mortar, then weighted again, then macerated with 500 ml of 70% ethanol, after maceration over night, the extract was filtered & the marc was macerated again overnight.

The filtrates were combined together & concentrated under vacuum then mixed with 100 ml of distilled water & fractionated using 70 ml x 3 times of petroleum ether,

chloroform, ethyl acetate. All the organic fractions were dried over anhydrous sodium sulfate, filtered & evaporated to dryness. The remaining methanol fraction was mixed with equal volume of methanol and evaporated to dryness under vacuum.[8]

Phytochemical investigations

Preliminary investigations for the chemical constituents of the ethyl acetate & methanol extracts were done using ammonia vapor, ethanolic KOH, Meyer's & Dragendorff's reagents. Utilizing HPLC (Waters, USA), ethyl acetate & methanol fractions were analyzed for their flavonoids contents using C_{18} (25 Cm) column, methanol : water 70:30 as a mobile phase with a flow rate 0.5ml /min & detection at 280nm , using quercetin, kaempherol, & rutin standards.

Experimental design

Adult male Wistar albino rats weighing 200–215 g rats obtain from the animal house of department of pharmacology and toxicology-college of pharmacy, as animal model in the induction of acute inflammation. We make 4 groups each group with 5 rats.

First group: Control group: receive only solvent (dimethylsulfoxid) I.P. in a dose 2ml/kg before30 minute of induction of acute inflammation.

Second group: Receive 50 mg/kg bodyweight ethyl acetate fraction I.P. before 30 minute of induction of acute inflammation.

Third group: Test group: receive 50 mg/kg bodyweight methanol fraction I.P before 30 minute of induction of acute inflammation.

Fourth group: Standard group receive 5 mg/kg bodyweight piroxicam I.P before 30 minute of induction of acute inflammation.

Induction of Acute inflammation

The acute inflammation technique is done by using fresh egg albumin –induced edema in rats model according to the technique establish by winter.[9]

Rats were fasted overnight, and deprived of water during the experiment to ensure uniform hydration and to minimize variability in edematous response.[10]

All the drug were administered intraperitonealy and 30 minute post-treatment ,inflammation was induced by injection 0.1 ml of fresh egg albumin (phlogisic agent) into the sub planter surface of the right hind paw[11]. The increase in paw edema, because of inflammation was measure by using vernier caliper before and after 0.5 hour, 1 hour, 1.5 hour and 2 hour after induction of inflammation. The differences in paw thinness before and after induction of inflammation was calculated and presented as mean increase in paw thickness (mm). The ability of anti-inflammatory drug to suppress paw inflammation was expressed as percent of inhibition of paw edema [12].

All result express mean increase significance was measured by using students T-test in which when P-value <0.05 were considered significant. The percentage of inhibition calculated as in previous models.

Percent of increase = (a-b/b)*100

In which, A mean the measure of paw at any time.

B means the measure of paw at zero time.

In addition, the inhibition percent was calculated as follow:

Inhibition percent = 100(1- {a-x/b-y})

Where;

a = mean paw volume of treated animals after egg albumin injection

x = mean paw volume of treated animals before egg albumin injection

b = mean paw volume of control animals after egg albumin injection

y = mean paw volume of control animals before egg albumin injection

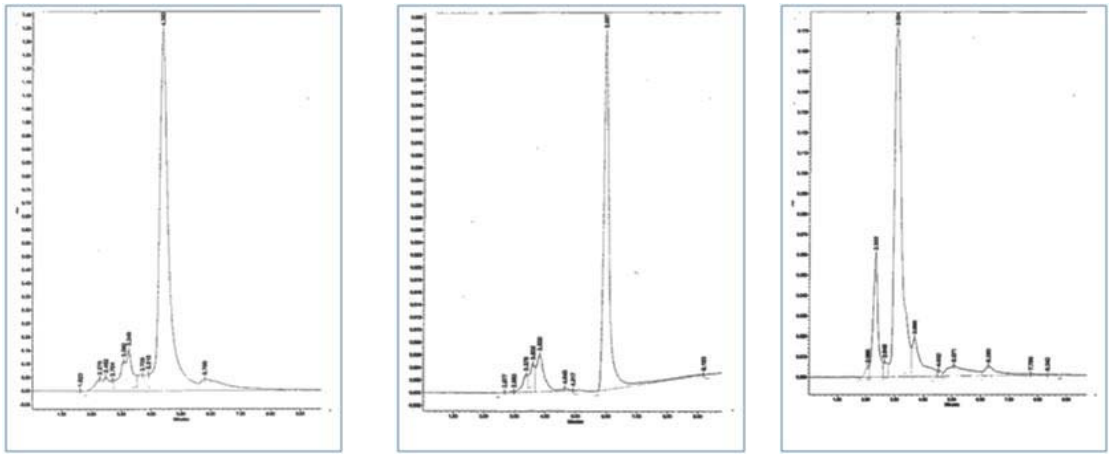
Statistical Methods

Data are expressed as mean±SD; unless otherwise indicated, statistical analyses were performed using unpaired *t*-test. If the overall F value was found statistically significant ($P<0.05$), further comparisons among groups were made according to post *hoc* Tukey's test. All statistical analyses were performed using SPSS GraphPad InStat 3 (GraphPad Software Inc., La Jolla, CA, USA) software.

RESULTS AND DISCUSSION

Preliminary phytochemical investigations revealed the presence of flavonoids in both ethyl acetate & methanol fractions, & the presence of traces of alkaloids in the ethyl acetate fraction & the absence of them in the methanol fraction. HPLC chromatograms of standard quercetin, kaempherol, & rutin are shown in Figure (1). HPLC of the ethyl acetate fraction shown in Figure (2) revealed the presence of rutin, quercetin & kaempherol in extract, while HPLC of methanol fraction shown in Figure (3) revealed the presence of rutin.

In table (1) and figure (4). methanol and ethyl acetate fractions of *loranthus europaeus* show non-significant decrease in the mean of increase of paw edema after half hour when compared with negative control, but after one hour of induction ethyl acetate fraction shows significant decrease in the mean of increase in paw edema, while methanol fraction show non- significant decrease in the same parameter when compared with negative control. After one and half hour and two hour of starting measuring, both fraction both fraction show significant decrease in measure parameter when compared with negative control.



Standard quercetin Standard kaempherol Standard rutin

Figure 1: HPLC for quercetin, kaempherol and rutin

Table 1: Represent the mean increase in paw edema in rats induced acute inflammation

Mean increase	0.5 hr	1 hr	1.5 hr	2 hr
Negative control	3.20±0.9	3.51±0.54	3.71±0.67	3.93±05
Positive control (piroxicam)	2.55±0.3[a]	1.98±0.2*[a]	1.12±0.4*[a]	0.22±0.2*[a]
Methanol fraction	2.98±0.6[a]	3.19±0.3[b]	3.06±0.4*[b]	2.88±0.2*[b]
Ethyl acetate fraction	2.48±0.3[a]	2.24±0.4*[c]	1.92±0.39*[a]	1.74±0.5*[a]

- The data were express as mean ± SEM; number of animal =5; p<0.05with respect to control group; values with non-identical superscripts (a, b,c) are considered significant different (p<0.05).

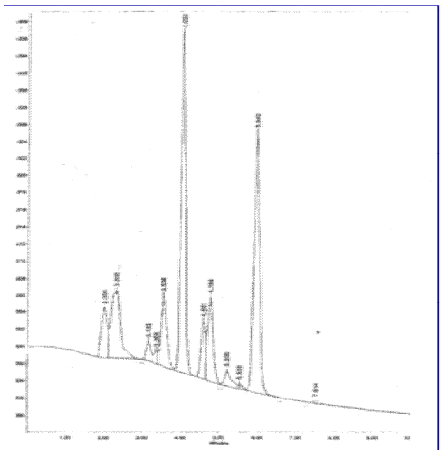


Figure 2: HPLC of ethyl acetate fraction of Loranthus europaeus.

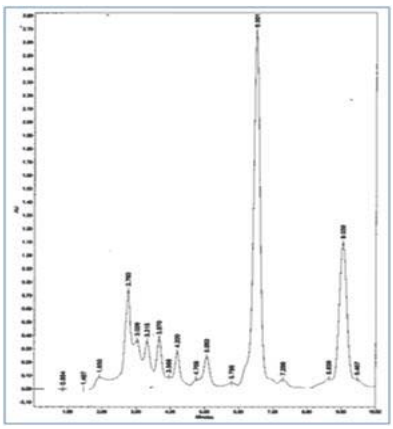


Figure 3: HPLC of methanol fraction of Loranthus europaeus.

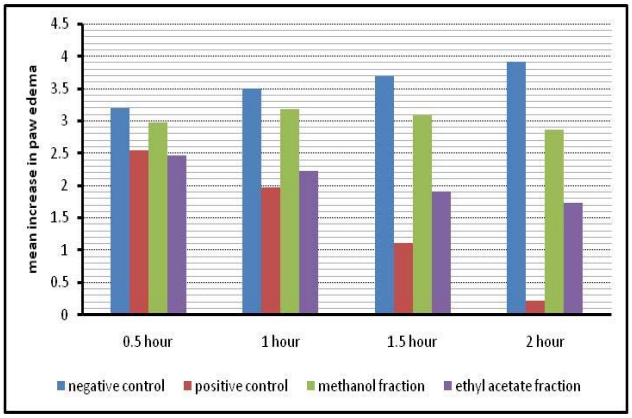


Figure 4: Represents the effects of methanol and ethyl acetate fraction on mean increase in paw edema in rat's induced acute inflammation.

In table (2), figure (5) both methanol and ethyl acetate fraction of *loranthus europaeus* at a dose 50 mg/kg showed decreasing in the percent of increase at all time that measured when compared with negative control meanwhile these fractions when compared with piroxicam showed lower effect of inhibition (higher values of the percent of increase) at all times that measured.

In table (3) and figure (6), the ability of methanol fraction to suppress the acute inflammation is higher than what find in ethyl acetate fraction at all times that measured but both fractions did not suppress inflammation with same efficacy than what find in piroxicam at all the time that measured.

Table 2: Represents the percent of increase in paw edema in rats induced acute inflammation

Percent of increase	0.5 hr	1 hr	1.5 hr	2 hr
Negative control	98.50	106.02	87.04	93.37
Positive control (piroxicam)	79.19	61.80	35.09	2.79
Methanol fraction	96.61	85.13	81.07	75.98
Ethyl acetate fraction	95.22	88.65	55.52	53.13

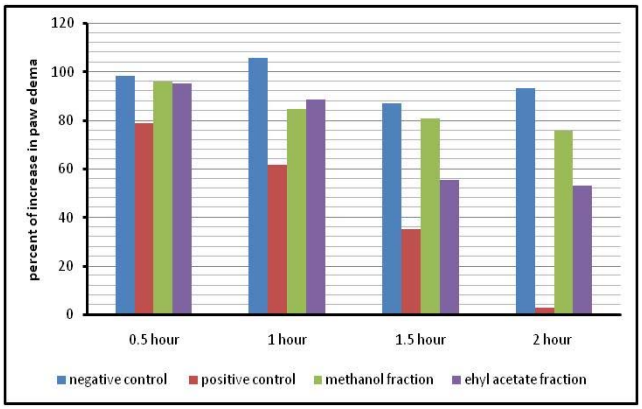


Figure 5: Represents the effects of methanol and ethyl acetate fraction on percent of increase in paw edema in rat's induced acute inflammation

Table 3: Represents the percent of inhibition in paw edema in rats induced acute inflammation

Percent of inhibition	0.5 hr	1 hr	1.5 hr	2 hr
Positive control (peroxicam)	22.02	43.47	60.9	97.1
Methanol fraction	9.18	9.38	35.65	42.59
Ethyl acetate fraction	1.48	4.58	6.92	7.42

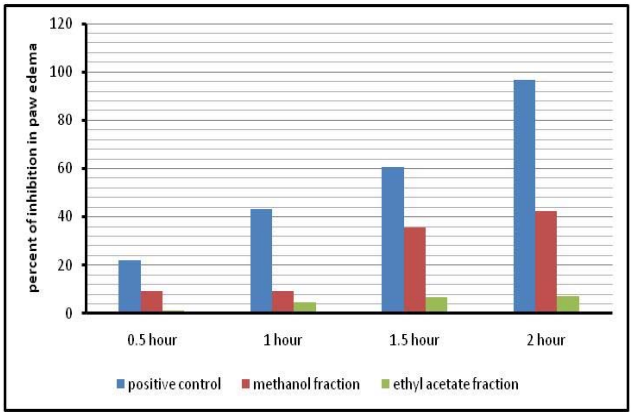


Figure 6: Represents the effects of methanol and ethyl acetate fraction on percent of inhibition in paw edema in rat's induced acute inflammation

The immune system is a highly complex, intricately regulated group of cells whose integrated function is essential for health. Cells of the immune system may interact in cell-cell manner and may also respond to intercellular messages including hormones, cytokines, and autacoids elaborated by various cells, the immune system can be modified by diet, pharmacological agents, environmental pollutants, and naturally occurring food chemicals such as vitamins and flavonoids.[13] Egg albumin-induced paw edema in rats an *in vivo* model of inflammation, which has long accepted as a useful tool to study and evaluate drugs with anti-inflammatory activity in acute inflammation.[14]

According to HPLC results, both fractions contain flavonoids, which identified as (kaempherol, quercetin and rutin). Flavonoids are reported to have a different pharmacological activity, especially as antioxidant, anti-proliferative, anti-inflammatory, antiviral, hepatoprotective, anti-thrombotic, anti-allergic and anti-carcinogenic activities.[15]

CONCLUSION

The physicochemical property of flavonoids plays a crucial in the ability of suppression of acute inflammation. These properties make the methanolic fraction have higher amount of these flavonoid that what present in ethyl acetate fraction. This could be obviously notice on the ability of acute inflammation suppression at which methanol fraction suppresses the acute inflammation relatively stronger than ethyl acetate fraction. The method of extraction and fractionation provided a sufficient degree of separation between glycoside (which present mostly in methanol fraction due to highly water solubility) and a glycon part (which present mostly in ethyl acetate fraction due to lower water solubility) and according to results of this experiment it could be realized that the glycoside have higher efficacy in suppression of acute inflammation than a glycon part. In spite of that, these two fractions cannot suppress the inflammation as stronger as piroxicam

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