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Impact of Alkaloids Fraction from Jatropha Curcas on Monocytes

KEYWORDS: Alkaloids, Cycloxygenase (COX), Lipoxygenase (LOX), Jatropha curcas (EEJC)

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ABSTRACT *Jatropha curcas is a tropical and subtropical plant. It has a wide distribution habitat.The ethanolic extract of the Jatropha curcas was extracted and their phytochemical and antioxidant assays were performed. Using that ethanolic extract of Jatropha curcas (EEJC) the alkaloids were isolated and their invitro activity was investigated by culturing the monocytes and their viability was assessed. RNA was isolated from the alkaloid treated monocytic cells and were processed to RT-PCR for amplification, whereas Cycloxygenase (COX) and Lipoxygenase (LOX) assays were carried out by the same alkaloid treated cultured cells. The results were compared and it will conclude that the plant extract having the capacity to reduce the COX and LOX assay activity. Therefore, the Jatropha curcas having a potent bioactive principle compounds treated against the monocytes. In future these active principle compounds of Jatropha curcas can be assayed for curing different human aliments on pathogenic diseases.*

INTRODUCTION

The genus Jatropha has 175 known species of the plant belonging to the family Euphorbiaceae [Dehgan *et al*., 1992] and are widely cultivated and distributed in India. Many plants contain proteins that are capable of inactivating ribosome and accordingly are called ribo-some-inactivating proteins (RIP). At present, it is useful to look for new RIP to identify those with the highest antitumor activity. The alkaloids comprise the largest single class of secondary plant substances. They have a remarkable range of pharmacological activity. The matrix metalloproteinase's (MMPs) a family of extracellular zinc and calcium- dependent endopeptidase that are structurally and functionally related endoproteinases that degrade the extracellular matrix and other extracellular proteins [Stamenkovic *et al*., 2003] and they can be classified according to their substrate specificity in collagenases, gelatinases, stromelysins and matrilysin [Egeblad *et al*., 2002]. MMPs were thought to be contribute to tumour metastasis via their matrix degrading activity [Fu *et al*.,1990]. Zymography is widely used to study various aspects of matrix metalloproteinase (MMP) function [Harza *et al*., 2010] and visualization depends directly on proteolytic activity [Feitose *et al*., 1998.]. The lipo polysaccharide (LPS) treated monocytes can stimulate the activity of COX and mediating inflammatory reactions. LOX has proinflammatory actions and LO-1 expression is induced by IL-13 in human blood monocytes [Deleuran *et al*., 1995.]. It have protective role in inflammatory disorders due to formation of anti-inflammatory lipoxins [Linda *et al*., 2003].

The objective of this study was to define the anti inflammation a cascade of biochemical reaction taking place in body in response to an immunological reaction and leading to severe pain, swelling and cell death. To know about the current anti-inflammatory effects and increased role of molecular enzymes such as matrix metalloproteinase's raise options to design a new therapeutic drugs. The current study was aimed to isolate and purify the alkaloid content of ethanolic extract of *Jatropha curcas (*EEJC (Ethanolic Extract of Jatropha curcas) and their invitro cytotoxic effect, antioxidant and anti-inflammatory effect were assessed. Further the effect of purified alkaloid of EEJC on TNFα (Tumor Necrosis Factor) expression and their inhibitory effect in LPS treated HPC (Human Peripheral Cells) were studied.

MATERIALS AND METHODS

Plant Material Collection

The selected plant materials were collected from in and around surrounding areas of Trivandrum district, Kerala. Verify that the plant materials being collected was the species desired. The collected plant materials were washed under the running tap water and once in distilled water. Then the plant materials were shade dried and ground by mixer griender as power form. The sample was packed by filter paper and then filled in soxhlet apparatus [Linda *et al*., 2003].

Extraction of Alkaloid from Plant Sample

The extraction protocol is found to be in accordance with the Amador Perez et al., 2007.

Preparative Thin Layer Chromatography

Preparative TLC silica gel plates were used for isolation and purification of alkaloid compounds were found in accordance with the Sitifaridah et al, 2009.

Phytochemical Analysis for Alkaloids

To 0.5ml of the extract, 2ml of HCL was added and followed by the addition of 1ml of drangondrof's reagent. An orange red precipitate was produced indicates the presence of alkaloids.

Antioxidant Assay

The anti-oxidant assay was measured in accordance with Hazra et al., 2010.

Media Preparation for Monocyte Culture :(RPMI -1640 Media)

550mg of medium was suspended in 25ml of tissue culture grade water, 1.47g of sodium bicarbonate was added and 55ml of the medium was prepared .The pH was adjusted to 0.2-0.3.Then the medium was sterilized immediately by filtering through a sterile membrane filter with a porosity of 0.22 micron and stored at 2.8°c in dark till use.

Viability Assay

Viability assay was calculated in accordance with the Sampson et al., 2011.

Lipopolysaccharide Isolation (LPS)

The LPS isolation was found to be accordance with the Darveau et al, 1983.

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Zymography Protocol
Zymography protocol was found in accordance with Linda et al, 2003.

Reverse Transcriptase - Polymerase Chain Reaction (RT-PCR):
For Control Reaction:

To the 575µl of the reaction Mix, 50µl of Enzyme Mix was added followed by the addition of the Control Reverse Primer (100ng/µl) 12µl (Beutler et al.,1989) and Control Forward Primer (100ng/µl) 6µl (Daniel et al.,1995). After that control RNA of 5µg was added (Add 50µl Diethyl bicarbonate (DEPC) water and incubate at 65°c for 15min to suspend the RNA) and finally made to 1ml with DEPC water.

For Test Reaction:
5µl of RNA template (~1µg) was mixed with 400ng of reverse primer (gene specific) and 20µl of reaction mix was made with DEPC water. The above mix was incubated at 65°c for 10 min and immediately chills on ice. Test RT PCR reactions was carried out by adding the components in the following order: 25µl of reaction mix was added ,followed by the addition of 20µl RNA-primer mix, 300ng of forward primer (gene specific) and finally 2µl of enzyme mix was added. The final volume was made to 50µl with DEPC water. Place the above reaction mix in a thermocycler. Finally, agarose gel electrophoresis was proceed with the help of the PCR product and viewed under UV transilluminator.

Assay of Cyclooxygenase (Cox) Inhibitory Assay
The assay procedure was found in accordance with Dannhardt et al, 2001.

Assay of 5-Lox
The assay procedure was found in accordance with Li et al, 2006.

RESULTS AND DISCUSSION
1g of crude extract has given a yield of approximately 10% of alkaloids. Chromatography on silica is a conventional preparative method for the separation of alkaloids. The purity of alkaloids after column chromatography was determined by TLC analysis. UV exposure of glass plates developed in ethylacetate: formaldehyde solvents and with the locating reagent giving single band fluorescence when observed under 320nm (**Plate 1**). The chromatograms viewed under UV light demonstrated a much better separation for the elution mode [Loren *et al.,* 2010.]. Better elution was obtained in the current study by visualization under UV light. From the keen observation of the colors developed it was revealed that the *Jatropha curcas* contain more alkaloids (**Plate 1**). A significant reduction up to 50% was observed in samples when compared with the control. The reduction in the absorbance can be attributed to antioxidant effect of plant extract (**Table 1**).

Table 2 reveal that *Jatropha* extracts shows significant reducing activity. The increasing absorbance can be considered as effectiveness of plant extract in reducing the generated free radicals. The hydroxyl radical scavenging effect of EEJC was determined by quantifying the amount of Thio barbituric acid reactive substances (TBARS) formed by reaction with hydroxyl radicals and deoxy ribose. From the obtained results, it clearly states that the plant extracts produced significant increase in percentage inhibition (**Table 1**). From the results it was observed that the plant extract have the capacity to reduce the viability of the monocytic cells. The concentrations above 220µg have more capacity to reduce the viability of the cells. If increasing the concentration of the plant extract it reduces the total count of the cells up to 53.96% refer **Table 1**.

Plate 1 shows the active bands corresponding to the enzymes are seen as white bands with a blue background and the bands were measured to determine the digestion of the substrates refer **Table 2**. The RNA that was isolated from the given monocyte culture (**Plate 2**) after the addition of the plant extracts were checked with the gel electrophoresis to identify the quality of it. Later, it was ready to proceed RT-PCR. After amplification the primer TNFα, they are the central proliferating cytokine produced by macrophages and dentritic cells. Deregulations of TNFα are associated with autoimmune disease such as rheumatoid arthritis where TNFα was successfully inhibited by purified EEJC.COX-2 inhibition leads to the production of prostaglandins which produces inflammatory signals leading to production of cytokines IL and TNFα. *Jatropha curcas* extract was added to LPS activated HPLS and spectrophotometrically determined the COX and LOX inhibition.

CONCLUSION
In the present study results indicate that the plant extract possess anti-inflammatory and antioxidant activity. This may be due to the strong occurrence of polyphenolic compound such as alkaloids. The alkaloids tend to be rather toxic, although the toxicity appears to be well below the therapeutic levels. The alkaloids appear to offer the considerable promise for further investigation as anti-inflammatory components and some appears to be remarkably active. These extract reduced the activity of COX and LOX purification of each bioactive compound can be need and purified compound can also increase their activity. This report proposing its potential application as a lead compounds for designing potent anti-inflammatory activity and they can be used as a lead compounds for designing potent anti-inflammatory activity and they can be used for treatment of various diseases (cancer, neurological disorder, aging, and inflammation).

Table – 1: Estimation of Nitric Oxide Scavenging Activity, Reducing Power Activity, Hydroxyl Radical Scavenging Activity and Viability Assays

Concentration Of Plant Extract (µG)	Nitric Oxide Scavenging Activity (Inhibition %)	Reducing Power Activity (OD @ 700NM)	Hydroxyl Radical Scavenging Activity (Inhibition %)	Viability Assay (%)
Control	100	0.033	100	78.2
40	55.12	0.114	11.01	71.4
120	51.28	0.284	19.17	68.7
220	32.05	0.408	27.16	36

Table – 2: Estimation of COX and LOX assays

Concentration Of Sample(µg)	COX ASSAY		LOX ASSAY	
	Crude Extract (OD @ 632nm)	Pure Extract (OD @ 632nm)	Crude Extract (OD @ 234nm)	Pure Extract (OD @ 234nm)
CONTROL	0.573	0.563	0.221	0.153
CONTROL+LPS	0.532	0.717	0.191	0.036
40	0.424	0.400	0.040	0.014
220	0.714	0.584	0.148	0.075

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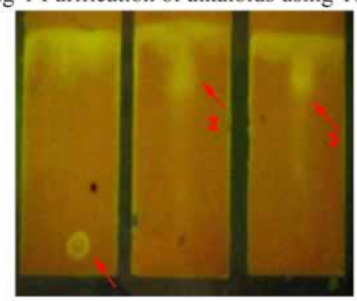
PLATE -1 & 2

Plate-1

Fig-1 Purification of alkaloids using TLC



1 -Spotting of the crude plant extract
2,3 - Solvent front for the separation of the pure alkaloids

Fig-2 Dragendroff's test

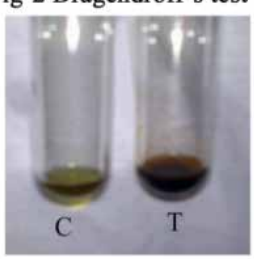
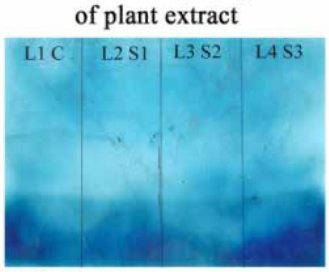


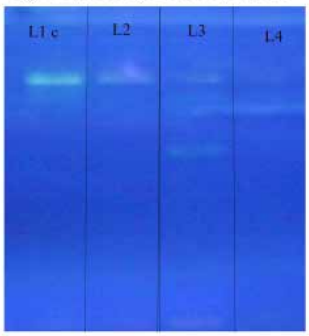
Fig-3 Gelatin Zumography- Different of plant extract



L1 C - Control
L2 - S1 (40 Microgram of plant extract)
L3 - S2 (120 Microgram of plant extract)
L4 - S3 (220 Microgram of plant extract)

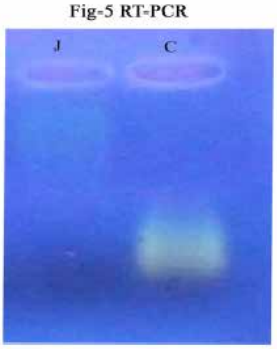
Plate- 2

Fig-4 Agarose gel electrophorosis



L1 - Control (RNA isolate)
L2 - 40 Microgram of plant extract
L3 - 120 Microgram of plant extract
L4 - 220 Microgram of plant extract

Fig-5 RT-PCR



J - Test (Plant extract added amplified RNA)
C - Control (Amplified Monocytes RNA)

REFERENCE

1. Amador Perez Mc. Munoz Ocotero,V. Garcia Castaneda,JM., Gonzalez Esqunia, AR. (2007). Alkaloids in Solanum torvum SW (Solanaceae). International Journal of Experimental Botany. 76. 39-45. | 2. Dannhardt Gerd, and Werner Kiefer. (2001). Cycloxygenase Inhibitors-current status and future prospects. Eur J Med chem. 36.109-126. | 3. Darveau P. Richard and Robert E.W.Hancock.(1983). Procedure for isolation of bacteria LPS from both smooth and rough Pseudomonas aeruginosa and Salmonella typhimurium strains. Journal of bacteriology. 831-838. | 4. Dehgan Bijan and Emily J Lolt. (1992). Systemic Botany. American society of plant Taxonomists.17 (3). 363-366. | 5. Deleuran, B.Iversen, L.Deleuran, M.Yssel, H.Kragballe, K.Stengaard Pedersen, K.Thestrup-Pedersen, K. (1995). Interleukin 13 suppresses cytokine production and stimulates the production of 15-HETE in PBMC. A comparison between IL-4 and IL-13. Cytokine. Arthritis Research and Therapy. 7. 319-324. | 6. Egeblad Mikala, and Zena Werb. (2002). New functions for the matrix metalloproteinase's in cancer progression. Nature Review. 2. 161-174. | 7. Feitose, Gremski ,L. Veiga,W. Elias,S.S., Graner,M.C.M., Mangali ,E. O.C., and Bretani, R.R. (1998). Detection and chacterization of metalloproteinase's with geltinlyic, Fibronctionlyic and fibringenolyic activities in brown spider (Loxosceles intermedia) venom. Toxicon. 36.1039-1051. | 8. Fu, J.Y., Masferrer,J.L., Seibert,K. Raz,A.Needleman ,P.(1990). The induction and suppression of prostaglandin H2 synthase (cyclooxygenase in human monocytes). J.Biol. Chem. 265.16737-16740. | 9. Harza Bibhabasu Hazra, Rhitajit Sarkar, Santanu Biswas, and Nirpendranath Mandal.(2010). comparative study of the antioxidant and reactive oxygen species scavenging properties in the extracts of the parts of Terminalia chebula,Jerminalia helerica and Emlica officinalis. BMC complement allera me. Doi: 10.1186/1972-6882-10-20. | 10. Itoh,Y.Binner, S., and Nagase, H. (1995). Steps involved in the activation of pro-matrix metallopeptidase 2 (progelatinase A) and tissue inhibitor metallopeptidases (TIMP)-2 by 4-aminophenyl-mercuric acetate. Biochem. J. 308.645-651. | 11. Li W Rachel,David,G.Leach,N. Petu ,G.Waterman, Stephen P. Myers. (2006). Inhibition of COX s and 5-LOX and activation of PAARS by Australian dematis species. Journal of Ethanopharmacology. 1-2. 138-143. | 12. Linda Troeberg, and Hideaki Nagase. (2003). Current protocols in protein science by John Wiley and Sonc,Inc. Dol:10.1002/0471140864. | 13. Loren St. John, Daniel G.Olge,Joe Scianna, Susan Winslow, Derek Tilley, Chris Hoag. (2010). Plant material collection guide.Natural Resource Conservation Service. 2-15. | 14. Matysik.G, and Soczewinski.E.(1988).Gradient thin-layer chromatograrhy of plant extracts. Medical academy.6.20-81. | 15. Sampson,J. Josiah, Isaac O. Donkor, Tien ,L. Huang, Samuel E. (2011). Adungah.Novel piperazine induce apoptosis in U937 cells. Int J Biochem Mol Biol. 2 (1).78-88. | 16. Sitifaridah, BT.,and Rahman, AB. (2009). Analysis of agarwood oil composition via preparative TLC. Chemical and natural resources. 9-16. | 17. Stamenkovic,MD.,and Werb, Z. (2003). How matrix metalloproteinase regulate cell behaviour. Annu Rev Cell EV Boil.17. 463-516. | 18. Sternlicht ,MD.,and Werb, Z. (2001). How matrix metalloproteinase's regulate cell behavior. Annu Rev Cell Biol. 17. 463-516. | 19. Wittwer, J.,and Hersberger, M. (2007). The two faces of the 15-lipoxygenase in atherosclerosis. Prostaglandins Leukot Essent Fatty Acids. 77. 67-77. |