Department of Biochemistry

Anti inflammatory properties of some detected plant extraction on Lipo polysaccharide induced inflammation in white blood cells

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In

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SUMMARY

Inflammation is a normal protective response to tissue injury. It's role to remove injurious stimuli as well as initiate the healing process for the tissue. It involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair. Harmful stimuli including pathogens, irritants or damaged cells initiate response of vascular tissue as inflammation. However, if inflammation is not treated it leads to onset of diseases like cancer, vasomotor rhinorrhoea, rheumatoid arthritis and atherosclerosis.

Oxidative stress is presently thought to make a significant contribution to pathophysiology of human diseases such as inflammation, viral Infections, autoimmune pathologies and ulcer. The impairment in the oxidant/antioxidant equilibrium results in oxidative stress in numerous pathological conditions leading to cellular damage. Reactive oxygen and nitrogen species, assume an essential part in inflammation then carcinogenesis. The most common ROS are superoxide anion, hydroxyl radicals and hydrogen peroxide. An excess of oxidative stress can prompt the oxidation of lipids and proteins, which is related with changes in their structure and functions.

It is believed that current drugs available such as opoids and NSAIDS are not useful in all cases of inflammatory disorders, because of their side effects and potency. As a result, a search for other alternatives seems necessary and beneficial. The study of plants that have been used traditionally for curing inflammation is still fruitful and logical research strategy in the source of new anti-inflammatory drugs with fewer side effects. Nature has been a source of medicinal agents since the beginning of time. There is an increasing interest in finding natural anti-oxidants from plant materials to replace synthetic ones.

The medicinal values of these plants lie in their phytochemical components such as alkaloids, tannins, flavonoid and phenolic compounds and sulphated polysaccharide contents. Phytochemicals which are widely distributed in plants are capable of terminating a free radical-mediated oxidative reaction and would have beneficial activities in protecting the human body from many diseases.

The present investigation focuses on the *in vitro* anti-oxidant and anti-inflammatory activities of some promising natural plant extracts from several sources for treatment of inflammation promoted by LPS-stimulated WBCs culture. LPS induced inflammation in WBCs through interaction of LPS with LPS-binding protein, followed by subsequent interaction with membrane-localized CD14, membrane-bound toll-like receptor (TLR) 4 and MD-2 using short term cultures of WBCs which have been demonstrated as a valuable method to assess monocyte derived cytokine production.

The examined plant extracts are *Tribulus terrestris, Calluna vulgaris, Ferula hermonis, Erica multiflora, Sonchus oleraceus L* and *Taraxacum officinale* in parallel with anti-inflammatory synthetic drug piroxicam as reference.

Each examined plant extract was prepared in total ethanolic extract then lyophilized and prepared with concentration 1mg lyophilized plant extract per 1ml distilled H_2O and 10 drops ethanol. Phytochemical analysis were performed to investigated total phenolic, alkaloids, flavonoids and sulphated polysaccharide contents of each examined plant ethanolic extract.

Part 1; *In vitro* anti-oxidant assays were assessed using ferric reducing power, DPPH, nitric oxide, hydroxyl radical and superoxide anion scavenging activity.

Part 2; an average of (16- 20) healthy human volunteer blood (who had not taken any anti-inflammatory drugs for at least 2 weeks prior to the experiment) were transferred to heparinized centrifuge tubes. Each separated collected blood was kept at room temperature overnight then used for assessment of anti-inflammatory effect of the examined plant extract on human RBCs. Then their cytotoxicity and EAICs also measured on WBCs culture.

Part 3 and 4; WBCs were stimulated by LPS for 24 h then treated with each plant extract effective dose for 72 h in parallel with LPS stimulated WBCs alone, normal untreated WBCS and piroxicam treated cells. After centrifugation at 1650 rpm for 10 min, each supernatant was collected and used for the TNF-α level, NO level and lipid peroxidation assays. Furthermore, pellets of untreated and treated cells were collected for estimating the antioxidant indices (GSH, GPx, SOD) and the other half

was used for molecular assays (determination of COX -2, GAPDH and iNOS expression level).

LPS administration induced inflammation confirmed by up-regulation of pro-inflammatory cytokines coupled with elevation of pro-oxidant molecules and depletion of anti-oxidant defense enzymes system.

Treatment with each ethanolic plant extract enhance the level of GSH, GPx specific activity, SOD specific activity and GAPDH expression level after being depleted in LPS stimulated WBCs, In contrast, treatment with each plant extract decreased the level of NO, TBARS, TNF-α, COX-2 expression level and iNOS expression level after being elevated in LPS stimulated WBCs.

Each ethanolic examined plant extract was found to contain polyphenols, alkaloid, flavonoids and sulphated polysaccharide which responsible for anti-oxidant and anti-inflammatory properties with variable amounts. The ability of phenolic compounds to serve as antioxidants has been recognized by donating a hydrogen atom. Also flavonoids may inhibit lipid oxidation by scavenging radicals or by other mechanisms such as singlet oxygen quenching, metal chelation, and lipoxygenase inhibition.

Up on evaluation of anti-oxidant properties of each ethanolic plant extract, *Tribulus terrestris* was found to be the most potent in scavenging DPPH, nitric oxide, hydroxyl radical and superoxide anion. *Tribulus terrestris* extract also have the highest anti-inflammatory activity with the lowest EAICs. While *Erica multiflora* extract was found to be effective in ferric reducing power assay.

The control of cellular TNF-α synthesis is very important for regulating the inflammatory responses which was performed by ELISA method. *Sonchus oleraceus L* extract corresponds to be the safest and most potent in ameliorating TNF-α level without significanc with *Tribulus terrestris* extract.

Normal activities of GSH-dependent enzymes such as GPx, GRx, GST, CAT, SOD, and GPx are the first line of cellular defense against oxidative injury and also have a vital for maintaining the anti-oxidant status. Thus, the loss of GSH/GSSG redox balance contributes to tissue hyperplasia and inflammation. The decreased

levels of GSH resulted in decreased activities of GPx, GRx, and GST. So; Increasing GSH content can prevent cellular damage. Increased level of ROS leads to lipid peroxidation then pathogenesis of several diseases.

Treatment with *Ferula hermonis* extract ameliorated the depletion of GSH level, GPx specific activity, SOD specific activity and GAPDH expression level. Also, *Ferula hermonis* extract significantly suppressed the increase of the level of TBARS, NO and the expression level of both COX-2 and iNOS.

CONCLUSION

1. The ethanolic extract of *Tribulus terrestris* was found to be the most potent in scavenging DPPH, nitric oxide, hydroxyl radical and superoxide anion with the highest anti-inflammatory activity and the lowest EAICs. Through which *Tribulus terrestris* extract is considered the most potent anti-oxidant plant extract due to the presence of saponins and trace metals like Cr, Cu, Fe, Mg, Zn, Ca, K and Na.

2. Treatment with *Ferula hermonis* ethanolic extract ameliorated the depletion of GSH level, GPx specific activity, SOD specific activity and GAPDH expression level, it also significantly suppressed the increase in the level of TBARS, NO and the expression level of both COX-2 and iNOS. Through which *Ferula hermonis* extract is considered the most potent anti-inflammatory plant extract due to the presence of high amount of sesquiterpenes like ferutinin and teferin. More importantly, Ferula demonstrated the highest potential in suppressing the expression of COX-2 and iNOS genes and upregulating GAPDH expression.

Recommendation

It was recommended to use *Tribulus terrestris* as a natural anti-oxidant plant extract and *Ferula hermonis* as a natural anti-inflammatory plant extract at their EAICs which can be introduced in a pharmaceutical form to test their efficacy (as single or combined) using the animal model for inflammatory diseases.

Limitation

Avoid high concentration of *Tribulus terrestris* and *Ferula hermonis* that was recorded to be toxic at dose higher than (41.3 and 66.1) µg/ml respectively.