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Antioxidant and Anti-Inflammatory Activities of *Lactuca sativa*: An *In Vitro* Study

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Bhaskara Reddy Nallamilli[*1], Kalakonda S Nataraj[2], Rakesh Kumar Jat[3]

[1]*Research Scholar, Shri JJT University, Jhunjhunu, Rajasthan*
[2]*Professor, Shri Vishnu College of Pharmacy, Bhimavaram*
[3]*Prof. & Principal, Institute of Pharmacy, Shri JJT University, Jhunjhunu, Rajasthan.*

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ABSTRACT

The present study was designed to evaluate the in vitro antioxidant and anti-inflammatory activity of aqueous leaf extract of *Lactuca sativa* (ALLS). Antioxidant activity of the ALLS was determined by following well established methods for free radical scavenging such as superoxide, hydroxyl, hydrogen peroxide, nitric oxide and DPPH radical scavenging activities using ascorbic acid as standard. The anti-inflammatory activity was evaluated by using HRBC membrane stabilization assay and albumin denaturation assay using diclofenac as standard. Treatment with ALLS showed dose dependent activity against superoxide, hydroxyl, hydrogen peroxide, nitric oxide and DPPH radicals. The ALLS showed significantly stabilization of membrane and denaturation of protein at a concentration range of 100-500 µg/ml. The antioxidant and anti-inflammatory activity of *Lactuca sativa* might be due to the triterpenoids and phenols.

INTRODUCTION

The production of free radicals (reactive oxygen and nitrogen species) is a steady-state event in normal cells and is now recognized that uncontrolled production of these reactive species is the primary cause of numerous disease conditions. Oxidative stress is defined as an imbalance between cellular production of ROS and antioxidant defense mechanisms. ROS (e.g., superoxide radical, peroxynitrite, hydroxyl radical, and hydrogen peroxide) are key signaling molecules in the progression of inflammatory disorders.

Traditionally, herbal medicines with antioxidant properties have been used for various purposes and epidemiological data also points at prevalent acceptance of use of these agents. Nowadays, research in the field of natural products has increased considerably in finding naturally occurring antioxidants to replace synthetic antioxidants, which are being restricted due to their side effects such as carcinogenicity.

The *Lactuca sativa* (*L. sativa*) belongs to the family *Asteraceae* commonly known lettuce and used as an important leafy vegetable in healthier food consumed in salads. The leaves of *L. sativa* contain variety of phytochemicals as triterpenoids, saponins, phenols, carotene, vitamin C and vitamin D [1] and are reported to have multiple biological uses, antimicrobial activity, anticancer activity, anticonvulsant activity, analgesic activity, antiviral and cardioprotective activity [2-4] (Bennett MH., 1994; Nicolle C., 2004; Chu YF., 2002). Based on the literature, the study was designed to evaluate the effect of aqueous leaf extract of *Lactuca sativa* (ALLS) for antioxidant activity and anti-inflammatory activity *in vitro*.

MATERIALS:

Plant Material

The lettuce (*Lactuca sativa*) were obtained from local market. The leaves were manually separated and shade dried. The leaves were powdered in a grinder to get 40-mesh size powder. The moisture content of the dried powder was found to be 13.5%. The extract was prepared with methanol using Soxhlet apparatus. The extract was suspended in 2% gum acacia and used for experimental studies.

Methods:

Superoxide radical scavenging activity

The riboflavin-light- NBT system contained 58 mM phosphate buffer, pH 7.6, 20 µM riboflavin, 6 mM EDTA, and 50 µM NBT, final volume made up to 3 ml, added in that sequence. Reaction was started by illuminating 40 volts. Immediately after illumination, the absorbance was measured at 560 nm. The potency of ALLS measured in terms of NBT photo reduction capacity [5].

***Hydroxyl radical scavenging activity*:**

The Fenton reaction ($Fe3^{+}$-ascorbate-EDTA-H_2O_2 system) reaction mixture contained 2-deoxy-2-ribose (2.8 mM), phosphate buffer (0.1 mM, pH 7.4), ferric chloride (20 µM), EDTA (100 µM), hydrogen peroxide (500 µM), ascorbic acid (100 µM) and various concentrations of the test sample in a final volume of 1 ml. The mixture was incubated for 1 h at 37°C. After the incubation an aliquot of the reaction mixture (0.8 ml) was added to 2.8% TCA solution (1.5 ml), followed by TBA solution (1% in 50 mM sodium hydroxide, 1 ml) and sodium dodecyl sulphate (0.2 ml). The mixture was then heated (20 min at 90°C) to develop the color. After cooling, the absorbance was measured at 532 nm against an appropriate blank solution. The percentage of inhibition of ALLS was expressed deoxyribose degradation capacity [6].

Hydrogen peroxide radical scavenging activity:

The hydrogen peroxide scavenging assay was carried out as per the procedure described. The principle of this method is that there is a decrease in absorbance of H_2O_2 upon oxidation of H_2O_2. A solution of 43 mM H_2O_2 was prepared in 0.1M phosphate buffer (pH 7.4). ALLS at concentration in 3.4 mL phosphate buffer was added to 0.6 mL of H_2O_2 solution (43 mM) and absorbance of the reaction mixture was recorded at 230 nm. A blank solution contained the sodium phosphate buffer without H_2O_2. [7]

DPPH radical scavenging activity:

The potential AA of extracts, fractions and pure compounds was determined on the basis of the scavenging activity of the stable 1,1 -diphenyl-2-picrylhydrazyl (DPPH) free radical. Aliquots of 1 mL of a methanolic solution containing each pure compound were added to 3

mL of 0.004% MeOH solution of DPPH. Absorbance at 517 nm, against a blank of methanol without DPPH, was determined after 30 min (UV, Perkin-Elmer-Lambda 11 spectrophotometer) and the percent inhibition activity was calculated. IC50 values denote the concentration of sample required to scavenge 50% DPPH free radicals. All tests were run in triplicate and averaged [8].

Nitric oxide radical scavenging activity:

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which were measured by the Griess reaction. The reaction mixture (3 mL) containing sodium nitroprusside (10 mM) in phosphate buffered saline (PBS) and ALLS in different concentrations were incubated at 25°C for 150 min. After incubation 1.5 mL of the Griess reagent (1% sulphanilamide, 0.1% naphthyl ethylenediamine dihydrochloride in 2% H_3PO_4) was added. The absorbance of the chromophore formed was measured at 546 nm. The percentage inhibition of nitric oxide generated was measured by comparing the absorbance values of control and test samples [9].

***HRBC membrane stabilization assay*:**

The HRBC membrane stabilization has been used as method to study the anti-inflammatory activity. Blood was collected from healthy volunteer and was mixed with equal volume of sterilized Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% sodium chloride in water). The mixer was centrifuged at 3000 rpm and packed cell was washed with isoaline (0.85%, pH 7.2) and a 10 % (v/v) suspension was made with isosaline. The assay mixture contained the drug, 1 mL of phosphate buffer (0.15M, pH 7.4), 2 ml of hyposaline (0.36%) and 0.5 mL of HRBC suspension. Diclofenac was used as reference drug. All the assay mixture were incubated at 37ºC for 30 min and centrifuged. The hemoglobin content in the supernatant solution was estimated using spectrophotometer at 560 nm [10].

Albumin denaturation inhibition assay:

Different concentrations of ALLS (1 mL) was mixed with 1 mL of 1 mM albumin solution in phosphate buffer and incubated at $27^0 \pm 1^0$C in BOD incubator for 15 min. Denaturation was induced by keeping the reaction mixture at 60° ± 10° C in water bath for 10 min. After cooling, the turbidity was measured at 660 nm (UV-Visible Spectrophotometer SL-159, Elico India Ltd.). The diclofenac sodium was used as standard drug. Percentage of inhibition of

denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and average was taken [11].

RESULTS AND DISCUSSION:

Free radicals are continuously generated and metabolized as the result of metabolic processes in the body and interact with the environmental stimuli. Under normal physiological conditions wide range of antioxidant defense mechanism protect the body against free radicals [12]. In normal conditions, the human body undergoes a number of the physiological and biochemical processes leads to the production of several radicals.

Oxidative stress is a consequence of imbalance between the production of reactive oxygen species (ROS) and antioxidants defense system of human body [13]. Many studies indicate that antioxidants systems have the ability to treat chronic metabolic disease by scavenging ROS due to oxidative stress systems [14]. Both enzymatic and nonenzymatic antioxidants need to protect organisms from inflammation caused by excessive generation of ROS like superoxide, hydroxyl, hydrogen peroxide and nitric oxide.

Several researchers reported that natural herbs or plant based medicine could suppress the production of oxidative stress by increasing the antioxidants systems [15]. The activity might be due to the presence of phenolics compounds, carotenoids, vitamins, and terpenoids. These compounds have potency to scavenging the free radical in order to reduce the development of oxidative stress in many chronic diseases [16].

Superoxide is the first reduction product of molecular oxygen, a highly toxic radical, and the most abundantly produced in all aerobic cells by several enzymatic and non-enzymatic pathways, attacks a number of biological molecules and leads to unfavorable alterations of biomolecules including DNA [17]. It also forms an important source of other deleterious radicals such as hydroxyl and hydroperoxides, which initiate free radical chain reactions [18]. The ALLS were capable of scavenging of superoxide radical in a concentration dependent manner (Fig 1). The present study reveals that ALLS is a potent scavenger of deleterious free radicals formed during metabolic reactions as well as endogenous free radicals such as O^- and OH^-, at very low concentrations (Fig 2).

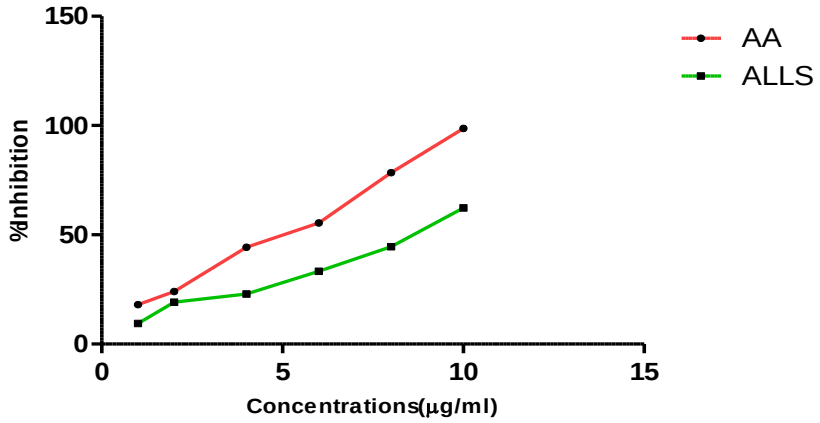

Figure 1: Effect of ALLS on superoxide radical scavenging activity

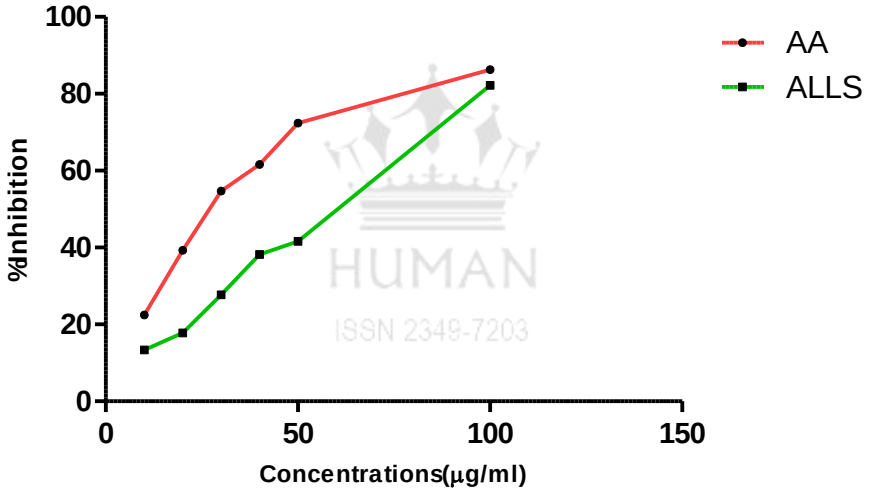

Figure 2: Effect of ALLS on hydroxyl radical scavenging activity

The ALLS were shown similar activity like AA in scavenging the hydrogen peroxide radicals in a dose dependent manner (Fig 3). Biological systems can produce hydrogen peroxide by several oxidizing enzymes such as superoxide dismutase [19]. It can cross-membranes and may slowly oxidize a number of compounds. Nitric oxide free radicals generated from the reactive oxygen free radicals and are also implicated in inflammation, cancer, diabetes and other pathological conditions [20]. The ALLS shown to scavenging the nitric oxide free radical in a dose dependent manner (Fig 4).

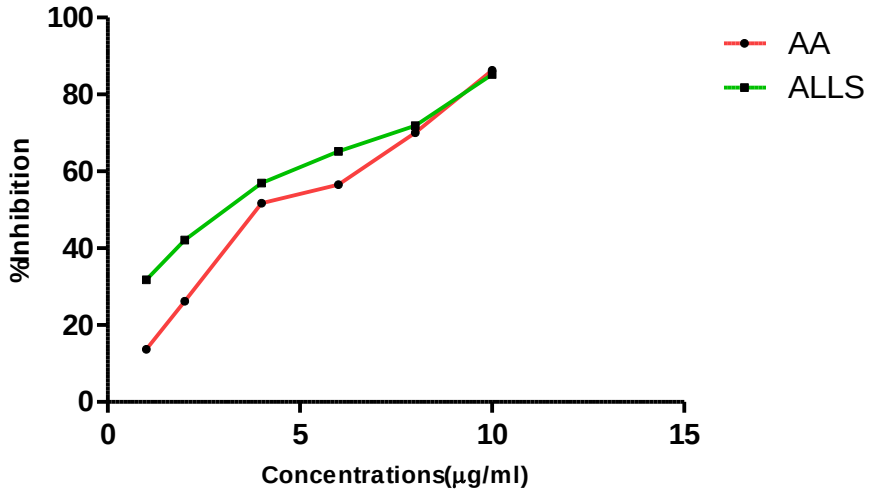

Figure 3: Effect of ALLS on hydrogen peroxide radical scavenging activity

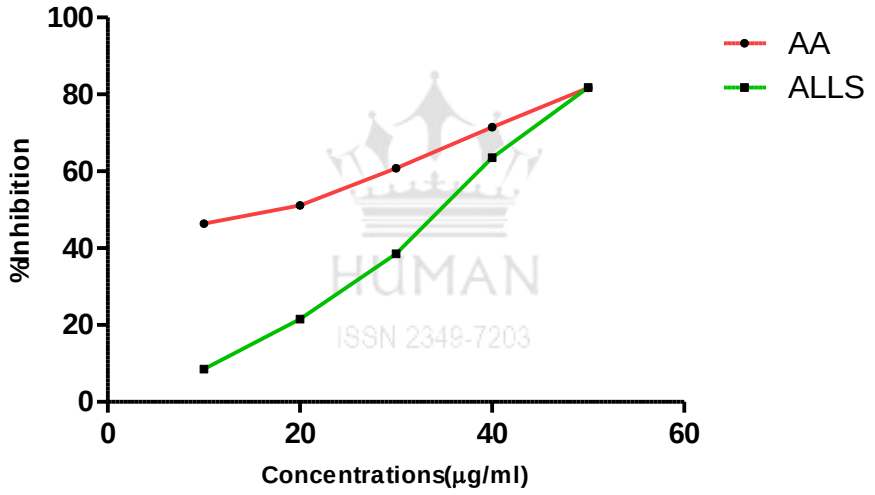

Figure 4: Effect of ALLS on nitric oxide radical scavenging activity

The DPPH antioxidant assay is based on the ability antioxidant to decolorize DPPH, a stable free radical. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured by the changes in absorbance [21]. The ALLS showed a significant dose-dependent inhibition of DPPH radical scavenging activity (Fig 5).

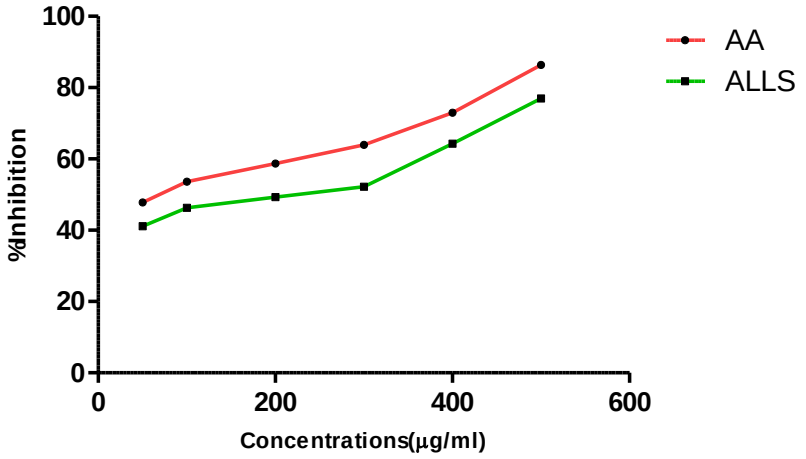

Figure 5: Effect of ALLS on DPPH radical scavenging activity

Several lysosomal enzymes are involved in the process of inflammation and causing damage to the surrounding tissues. The erythrocyte plasma membrane resemblances to the lysosomal membrane and it act as stabilizing effect of drugs on erythrocyte membrane [22]. The lysosomal membrane stabilization leads to the inhibition of release of the inflammatory mediators and consequent inhibition of the process of oxidative stress and inflammation [23]. The ALLS showed better membrane stabilization effect in a dose dependent manner (fig 6).

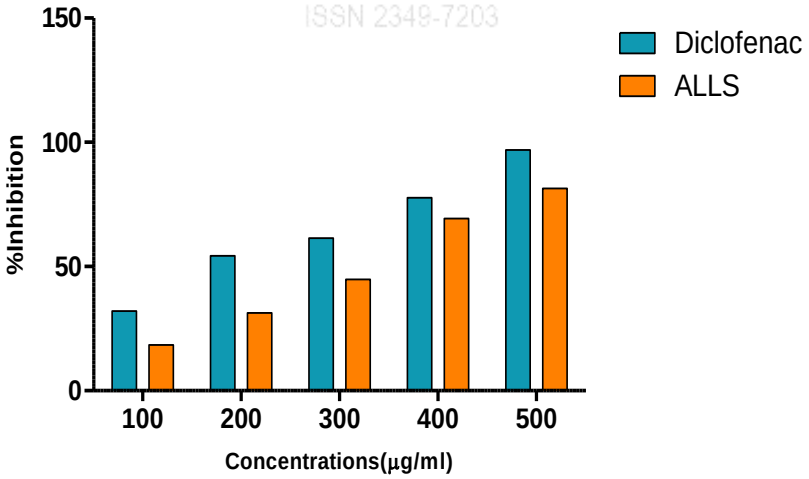

Figure 6: Effect of ALLS on HRBC membrane stabilization assay

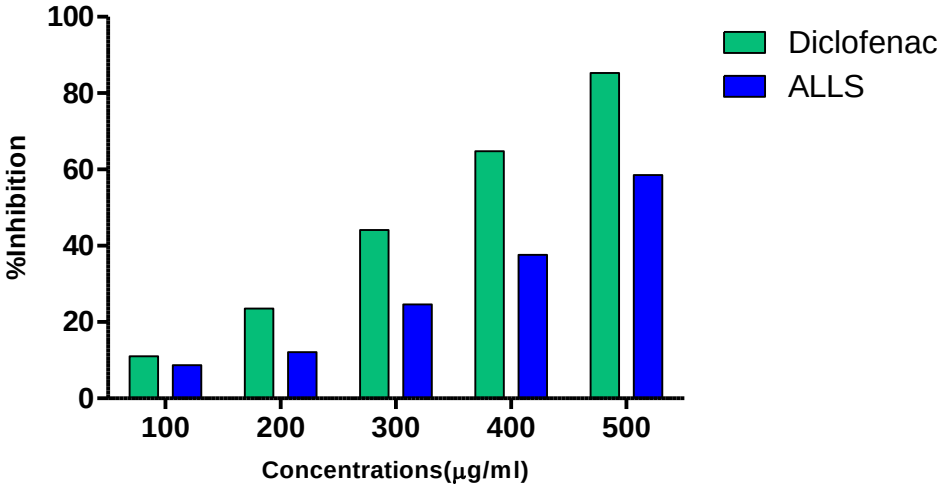

Figure 7: Effect of ALLS on albumin denaturation assay

The denaturation of proteins is one of the causes of inflammation. The reason might be due to the production of autoantigens involved in denaturation of protein and membrane lysis action. The ALLS had better activity against albumin denaturation among all the selected plant extracts (fig 7).

CONCLUSION:

The present study concluded that the ALLS showed antioxidant and anti-inflammatory activity. All the activities might be due to the presence of active constituents like triterpenoids, saponins and phenols in the leaf of *Lactuca sativa*.

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