Phytochemical analysis of *Achyranthes aspera* and its activity on sesame oil induced lipid peroxidation

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**Abstract**

The effect of *Achyranthes aspera* on lipid peroxidation were studied in rats fed with Sesame Oil. Increase in the levels of LPO in sesame oil treated groups returned towards normalcy in the plant extract treated groups revealing the antioxidant potential of the plant. Phytochemical studies revealed the presence of secondary metabolites. According to the results obtained *Achyranthes aspera* inhibited Ferrous Ascorbate stimulated LPO.

**Keywords**: *Achyranthes aspera*, Sesame oil, LPO, Ferrous, Ascorbate, Secondary metabolites.

**Introduction**

Lipid peroxidation is free radical mediated process, which has been implicated in variety of diseases. It is involved in the formation and propagation of lipid radicals, the uptake of oxygen re-arrangement of the unsaturated lipid that result in the variety of degraded products like alkanes, malondialdehyde, conjugated dienes and lipid hydroperoxides and eventually destruction of membrane lipids, variety of secondary metabolites, which have a large importance, as they are included in the trade of the plant itself. Besides producing drugs the plant secondary metabolites are also important for the production of colour, fragrance, antioxidants, flavours, dyes, insecticides and pheromones.

Natural products have served as a major source of drugs for centuries and about half of the pharmaceuticals in use today are derived from natural products. *Achyranthes aspera* belongs to the family of Amaranthaceae. It is
a perennial herb with 1 – 3 feet height, often woody below. Whole plant possesses medicinal property, which include astringent, diuretic, expectorant etc. The polyunsaturated fatty acids or PUFAs in vegetable seed oils are the bane of human health — they actually cause cancer, diabetes, obesity, aging, thrombosis, arthritis, and immunodeficiencies. The main problem is that polyunsaturated oils contain long-chain fatty acids, which are extremely fragile and unstable. The present study focuses on the phytochemical analysis of *Achyranthes aspera* and its activity on sesame oil induced lipid peroxidation.

**Materials and Methods**

**Plant Material**

Fresh plant, *Achyranthes aspera* were collected from the wild during the month of August – November 2005. Taxonomic authentication was done by the Department of Botany, Kongunadu Arts and Science College, Coimbatore, Tamilnadu, India. Aqueous extract was prepared by collecting fresh seeds of *Achyranthes aspera* and grinding one part of the seed with ten parts of water.

**Preparation of plant extracts**

The fresh plant parts (leaves, seed, stem and root) were collected, washed and then extracted with water, methanol and benzene by homogenizing it in the ratio of 1:10. The plant parts were shade dried and the dried plant material was powdered and extracted with water, methanol and benzene by immersing for 48 hours.

**Chemicals**

All the chemicals used in the present study were of analytical reagent grade.

**Selection of Oils**

Locally available and widely used brands of sesame oil were used for the study.

**Experimental animals**

Male albino rats weighing 150 – 200 g were procured from small Animal’s breeding centre of Kerala Agricultural University, Mannuthy, Thrissur. The animals were housed in spacious cages and fed with standard pellet diet supplied by AVM foods, Coimbatore, Tamilnadu, India. Food and water were provided ad libitum. A week’s time was allowed for the animals to get acclimatized to the laboratory conditions. The experiments were carried out as per the Institutional Ethics committee.

**Animal diet**

The diet given for the experimental animal constituted:

- Wheat flour - 15g
- Roasted Bengal Gram Flour - 58g
- Groundnut Flour - 10g
- Milk Powder - 5g
- Health Mix - 4g
- Salt - 4g
- Sesame oil - 4g

Everyday the feed for each animal was weighed separately, transferred into a separate container and mixed with sufficient water and steamed to a

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semisolid consistency. The feeding was continued for a period of 45 days.

**Experimental Design**

After one week adaptation period, the animals were divided into three groups with six animals in each group.

- Group I: Served as normal control rats
- Group II: Rats which received diet containing 6g/kg/BW of sesame oil for 45 days
- Group III: Rats fed with 1.33g/kg/BW of aqueous plant extract in addition to 6g/kg/BW of sesame oil for 45 days

At the end of the experimental period, the rats were deprived of food overnight and then sacrificed by cervical dislocation. Liver, heart and kidney tissues were dissected out, blotted off blood, rinsed in phosphate buffered saline (pH 7.4) and then 10% tissue homogenate were prepared.

**Biochemical Analysis**

The tissue homogenate were assayed for lipid peroxidation by the method of Buege and Aust, 1984\(^5\). The primary phytochemical studies of the aqueous, methanol and benzene extracts of *Achyranthes aspera* performed for alkaloids, flavonoids, tannins, saponins, phenols, glycosides, resins and steroids according to the published standard methods\(^6\).

**Statistical Analysis**

The results were presented as mean ± SD. One way Analysis Of Variance (ANOVA) was performed to analyze statistical significance of the data \(^7\).

**Results And Discussion**

The phytochemical studies with aqueous methanol and benzene extracts of various parts of the plant *Achyranthes aspera* showed to possess secondary metabolites which is clearly given in table 1. Medicinal plants and their active principals have received great attention as potential antiperoxidative agent. Plant products are known to exert their protective effects by scavenging free radicals and modulating carcinogen detoxification and antioxidant defense system\(^8\). The qualitative study of *Achyranthes aspera* reveals that the presence of number of secondary metabolites which has therapeutic values and that is considered to be good constituents which leads to further detailed isolation, purification studies to elucidate the value based secondary metabolite component which is indulged in *Achyranthes aspera*.

All the plant parts of *Achyranthes aspera* are said to possess alkaloids in fresh and dried conditions of aqueous, benzene and methanol extracts. Flavonoids are found to be seen only in fresh and dried leaves under aqueous, benzene and methanol extracts. Seed and stem parts are found to contain saponins and glycosides. Tannins are to be found only in stem of the plant. Resins are seen in leaf, seed and stem parts of the plant. The plant does not possess phenols and steroids.
The basal, ascorbate and ferrous sulphate induced LPO in the liver, heart and kidney homogenate of control and experimental rats are depicted in table 2. LPO generated by feeding with sesame oil is eliminated by the conversion of reduced GSH to oxidised GSH in the presence of enzyme GPx, thus curbing the propagation of LPO. In our study declined in the activities in LPO in Achyranthes aspera treated rats revealed that LPO and oxidative stress elicited by sesame oil have nullified due to the effect of Achyranthes aspera. Our result goes in accordance with the results of Thirunavukarasu et al.,

The LPO level was significantly increased in liver, heart and kidney of hyperlipidemic rats, which may be due to the release of chemical mediators during phagocytosis. After the herbal extract (Achyranthes aspera) administration the LPO level was reduced to near normal which may be due to the presence of secondary metabolites in the medicinal plants which was selected for this study.

Table 1: Phytochemical studies on Achyranthes aspera

| Constituent | Leaf | Seed | Stem | Root |
|-------------|------|------|------|------|
|             | Fresh | Dried | Fresh | Dried | Fresh | Dried | Fresh | Dried | Fresh | Dried |
| W           | B     | M     | W     | B     | M     | W     | B     | M     | W     | B     |
| Alkaloids   | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| Flavonoids  | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| Tannins     | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     |
| Saponins    | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     |
| Phenols     | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     |
| Glycosides  | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     |
| Resin       | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| Steroids    | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     |

W – Water; B – Benzene, M - Methanol
Table 2: Protective effect of *Achyranthes aspera* on tissue LPO

| Particulars in n mol of MDA / min. / mg. protein | Group I Control | Group II Sesame Oil | Group III Sesame Oil + A.a |
|-----------------------------------------------|----------------|---------------------|---------------------------|
| Liver                                         |                |                     |                           |
| Basal                                         | 1.79 ± 0.15    | 2.62 ± 0.22€        | 1.93 ± 0.16 x            |
| Ascorbate                                     | 2.63 ± 0.21    | 3.72 ± 0.25€        | 2.79 ± 0.19 x            |
| Ferrous sulphate                              | 1.53 ± 0.14    | 2.79 ± 0.24€        | 1.66 ± 0.13 x            |
| Heart                                         |                |                     |                           |
| Basal                                         | 3.21 ± 0.29    | 4.01 ± 0.31€        | 3.41 ± 0.32 x            |
| Ascorbate                                     | 3.76 ± 0.31    | 4.89 ± 0.40€        | 3.88 ± 0.30 x            |
| Ferrous sulphate                              | 3.42 ± 0.28    | 4.38 ± 0.34€        | 3.57 ± 0.31 x            |
| Kidney                                        |                |                     |                           |
| Basal                                         | 1.89 ± 0.71    | 2.81 ± 0.22€        | 2.01 ± 0.19 x            |
| Ascorbate                                     | 1.93 ± 0.18    | 2.98 ± 0.26€        | 2.08 ± 0.20 x            |
| Ferrous sulphate                              | 2.42 ± 0.21    | 3.64 ± 0.29€        | 2.62 ± 0.25 x            |

Values are expressed as mean±SD (n=6)

*Significant at p< 0.05 compared to control / € Significant at p< 0.05 compared to Group II

Unit: n mol of MDA / min. / mg. Protein

*A.a - Achyranthes aspera*

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