# Modulatory Influence of *Abutilon Indicum* Leaves on Hepatic Antioxidant Status and Lipid Peroxidation against Alcohol-Induced Liver Damage in Rats

**Dharmendra Singh and Radhey Shyam Gupta***

Reproductive Physiology Section, Centre for Advanced Studies**,**
Department of Zoology, University of Rajasthan, Jaipur – 302 004, India.

**Summary**

To evaluate the modulatory influence of ethanol extract of *Abutilon indicum* leaves on hepatic antioxidant status and lipid peroxidation against alcohol-induced liver damage in rats. The 30% alcohol (3.0 ml/150 g b wt/day, PO, for 21 days) treated rats have been more susceptible to peroxidative damage as measured by thiobarbituic acid reactive species. After the 30% alcohol induction to rats, the concentration of lipid peroxidation was significantly higher in liver and serum, alongwith concomitant significant decrease in the levels of enzymic and non enzymic antioxidants such as SOD (Superoxide dismutase), CAT (Catalase), GPx (glutathione peroxidase), GR (glutathione reductase), GST (glutathione-S-transferase), GSH (glutathione), vitamin C, vitamin E, Ceruloplasmin and $\beta$-carotene were significantly ($P \leq 0.001$) lower in liver and serum of 30% alcohol treated rats compared with normal controls. When these rats received *A. indicum* leaves extract, at the doses of 100 and 200 mg/kg b wt/day, PO, for 21 days, peroxidative damage was minimal in both liver and serum, dose-dependently alongwith effectively inducing the antioxidant potential in alcohol treated rats. In conclusion, the increased peroxidative damage in liver is likely to be associated with alcohol induction pathology, which could be reduced by *A. indicum* leaves extract by enhancing the antioxidant potential through free radical scavenging activity due to its some flavonoids and micronutrients.

**Key words** : *Abutilon indicum*, antioxidant defense system, lipid peroxidation, oxidative stress.

***Correspondence to:** Dr. R. S. Gupta,
TR – 03, Teacher's Hostel, University of Rajasthan, Jaipur – 302 004 (India)
**Email :** d_s1976@yahoo.co.in ; **Fax :** +91-141-2701137;
**Phone No.** +91-141- 2711228

## Introduction

Alcohol dependency is a major health and socio-economic problem throughout the world. Several animal studies have revealed that almost all ingested alcohol is metabolised in the liver and excessive alcohol use can lead to acute and chronic liver disease. In addition, a group of metabolic products called free radicals can damage liver cells and promote the impairment of vital functions such as energy production. The Natural defenses of body against free radicals are inhibited by alcohol consumption, leading to liver damage and progression of alcohol liver diseases[1].

Despite great progress made in the field in the past two decades, development of suitable medications for the treatment of alcohol dependency or alcohol-induced health injury remains a challenging goal for alcohol research. It is widely accepted that alcoholism is a complexes heterogeneous disorder. Traditionally, indigenous medicinal plants have been used for the treatment of alcohol dependency in India for centuries and also attracted the attention of western scientists. Because, mainly indigenous plant derived constituents may have stimulating or regenerating effect on hepatocytes and restored the activities of hepatic system.

*Silybum marianum* has been commonly standardized for 70% to 80% silymarin content. A hepatology clinic patient survey found that 31% were using over-the-counter "alternative agents" for the therapy for their liver diseases, the most common being milk thistle (Silymarin)[2]. In 2001, milk thistle ranked # 12 of the 20 top selling herbs in the mainstream U.S. market[3]. Therefore, familiarity with this herb, its constituents, current usage and potential drug interactions is increasingly important for health care provides as growing numbers of those with liver conditions elect to use this supplement[4].

*Abutilon indicum* (Malvaceae), commonly known as “Thuthi”, is distributed throughout the hotter parts of India[5]. It has been reputed in the siddha system of medicine as a remedy for jaundice, piles, ulcer and leprosy[6]. In some places, juice from the leaves of the plant is used in combination with the liquid extract of *Allium cepa* to treat jaundice, and hepatoprotective studies on experimental animals confirmed the above activity[7,8].However, no attention has been paid to its hepatic antioxidant status against alcohol induced hepatic injury.

Hence, in the present investigation, our aim being to study the modulatory influence of *Abutilon indicum* leaves extract on hepatic antioxidant status and lipid peroxidation against alcohol-induced liver damage in rats.

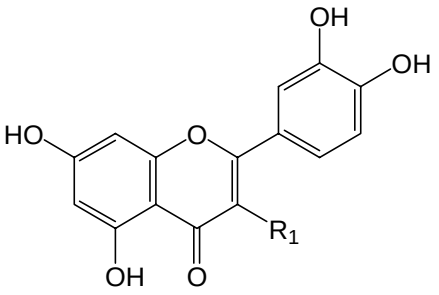
## Materials and methods

### Plant material and extraction

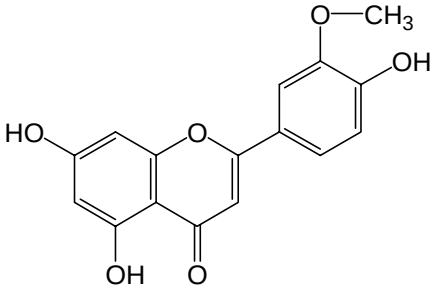
The leaves of *Abutilon indicum* were collected from University campus, Jaipur during the month of August, 2006 and identified from the Department of Botany, University of Rajasthan, Jaipur and placed in the herbarium for future reference (Voucher No. RUBL-19910). The leaves were shade dried and pulverized. The powder was treated with petroleum ether for defatting as well as to remove chlorophyll. The powder was packed into a soxhlet apparatus and subjected to hot continuous percolation using ethanol (95% $^{v}/_{v}$) as solvent. The extract was concentrated under vacuum and dried in a vacuum desicator (yield, 6.5% $^{w}/_{w}$) and then suspended in olive oil just before oral administration. Silymarin was obtained from German Remedies Ltd. Mumbai, for experimentation as a standard reference.

**Isolation of active compounds**

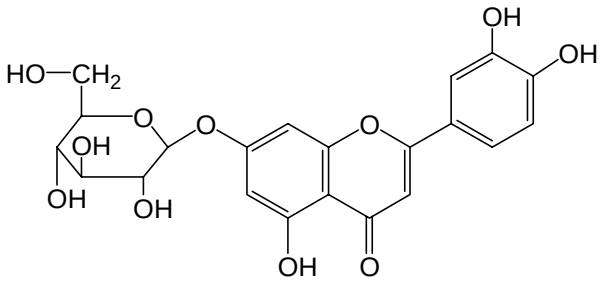
The dark brown ethanolic extract of *Abutilon indicum* leaves was washed with petroleum ether to remove the fatty portion and chlorophyll. The fat free extract was than extracted with chloroform and then subjected to column chromatography. For this purpose , a column (1.2 m × 5 cm) filled with Si-gel (800g) was used. The column was eluted with petroleum ether and chloroform (9:1) to afford some flavonoids (i) Luteolin, (ii) Chrysoeriol, (iii) Luteolin-7-O-beta glucopyranoside, (iv) Chrysoeriol-7-O-beta glucopyranoside, (v) Quercetin-3-O-beta glucopyranoside. The structures of above active compounds were confirmed by $^{1}H$ NMR and mass spectrometric data.



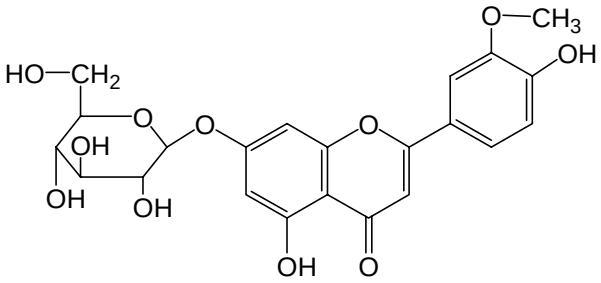

(i) Luteolin



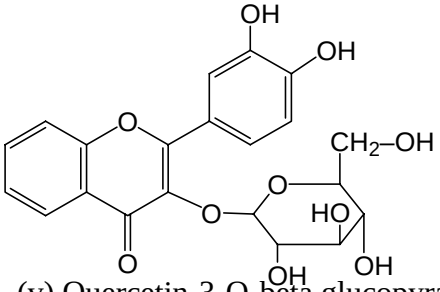

(ii) Chrysoeriol




(iii) Luteolin-7-O-beta glucopyranoside




(iv) Chrysoeriol-7-O-beta glucopyranoside




(v) Quercetin-3-O-beta glucopyranoside

**Experimental animals**

Adult, male wistar albino rats (140-150 g each), maintained under standard animal housing conditions (10h light : 14h dark cycle), were used as a experimental animals, performed on six rats each. The rats were allowed standard laboratory feed and water *ad libitum*.

**Chronic toxicity of *A. indicum* leaves extract**

The leaves extract was administered to the test groups in graded doses ranging upto 3g/kg body wt. and the rats were observed for signs of toxicity and mortality for 21 days afterward. The extract was found to be practically nontoxic when administrated orally to rats and its $LD_{50}$ values was found to be higher than 3g/kg body wt. The minimum dose levels viz. 100 and 200 mg/kg body wt. were used for the experimentation[8].

**Experimental protocol**

After acclimatization the animals were divided into following groups:

| | | |
|---|---|---|
| Group I | : | Vehicle treated rats served as control (0.5 ml olive oil/rat/day) for 21 days. |
| Groups II | : | Animals were orally treated with 30% alcohol (3.0 ml/twice a day) for 21 days. |
| Group III | : | Animals received *A. indicum* extract (100 mg/kg b wt/day, dissolved in olive oil, PO) for 21 days and 30% alcohol as groups II. |
| Group VI | : | Animals received *A. indicum* extract (200 mg/kg b wt/day, dissolved in olive oil, PO) for 21 days and 30% alcohol as group II. |
| Group V | : | Animals received silymarin (100 mg/kg b wt/day, dissolved in olive oil, PO) for 21 days and 30% alcohol as group II. |

At the end of experimental period, all treated rats were kept on starvation condition for 24 hrs. afterthat all rats were anesthesized, blood samples were collected by cardiac puncture and serum was analysed for various antioxidant markers such as GSH[9], Vit.C[10], Vit. E[11], Ceruloplasmin[12] and $\beta$-carotene[13]. Livers were frogen (-20°C) for biochemical analysis of SOD[14], CAT[15], GPx[16], GR[17], and GST[18], respectively. Simultaneously, lipid peroxidation[19] was monitored in both serum and liver as a indicator of unsaturated fatty acid formation in the hepatic cells.

**Ethical aspects**

This study was approved by the ethical committee of the University Department of Zoology, Jaipur, India. Indian National Science Academy, New Delhi (INSA, 2000) guidelines were followed for maintenance and use of the experimental animals.

**Statistical analysis**

All values are expressed as mean ± SEM. Data were analysed by Student '*t*' test.

## Results

The results of biochemical parameters revealed that the administration of 30% alcohol to rats caused significant ($P \leq 0.001$) alterations as evidenced by antioxidant defense system through liver and serum contents (Table 1 and fig. 1, fig. 2, fig. 3).

Table 1 depicts that treatment with 30% alcohol elevated hepatic lipid peroxidation (fig. 3) with a concurrent decline in hepatic antioxidant enzymes level such as SOD, CAT, GPx, GR, and GST in group II. These altered hepatic antioxidant defense system markers were significantly brought about towards normalization by *A. indicum* leaves extract dose dependently, in group III and IV. The significant protection against 30% alcohol induced hepatic antioxidant aberrations were achieved with silymarin in group V.

The activities of GSH, Vit. C, Vit. E, Ceruloplasmin and $\beta$-carotene in serum were decreased in 30% alcohol treated rats alongwith the significant ($P \leq 0.001$) elevation of serum lipid peroxidation in group II (fig. 1, fig. 2, fig. 3). Simultaneous treatment with *A. indicum* leaves extract afforded a significant protection dose dependently, against 30% alcohol induced alterations in the serum antioxidant levels and lipid peroxidation contents in the group III and IV. The remarkable protection were monitored with the silymarin treated group V against 30% alcohol treated group II (fig. 1, fig. 2, fig. 3).

**Table 1 : Modulatry activities of antioxidant enzymes in the liver of control and experimental rats.**

| Groups | SOD (μ mole/mg protein) | CAT (μ mol $H_2O_2$ consumed/min /mg protein) | GPx (n mole NADPH consumed min/mg protein) | GR (n mole NADPH consumed/min/mg protein) | GST (μ mole CDNB-GSH conjugate formed/min/mg protein) |
|---|---|---|---|---|---|
| **Normal (Vehicle treated) (Gp. I)** | **10.33±0.65** | **65.48±3.61** | **13.01±05.0** | **17.47±0.98** | **6.56±0.24** |
| **30% alcohol (3.0 ml/day, PO.) (Gp. II)** | **6.10±0.23[a]** | **36.19±1.19[a]** | **7.87±0.45[a]** | **9.28±0.62[a]** | **3.99±0.17[a]** |
| **30% alcohol + *A. indicum* extract (100 mg/kg b wt/day, PO.) (Gp. III)** | **7.24±0.12[b]** | **44.16±0.75[b]** | **9.76±0.37[b]** | **11.40±0.49[c]** | **4.65±0.15[c]** |
| **30% alcohol + *A. indicum* extract (200 mg/kg b wt/day, PO.) (Gp. IV)** | **8.97±0.29[a]** | **56.14±1.15[a]** | **10.82±0.42[a]** | **14.77±0.54[a]** | **5.34±0.22[a]** |
| **30% alcohol + silymarin (100 mg/kg b wt/day PO.) (Gp. V)** | **9.27±0.39[a]** | **61.33±1.17[a]** | **12.08±0.38[a]** | **16.17±0.56[a]** | **6.08±0.28[a]** |

Levels of significance : Data are mean ± SEM (n = 6)

a = $P \leq 0.001$ G.p II compared with control (Gp. I)

b = $P \leq 0.01$; c = $P \leq 0.05$ Gp. III compared with Gp. II

a = $P \leq 0.001$ Gp. IV compared with Gp. II

a = $P \leq 0.001$ Gp. V compared with Gp. II

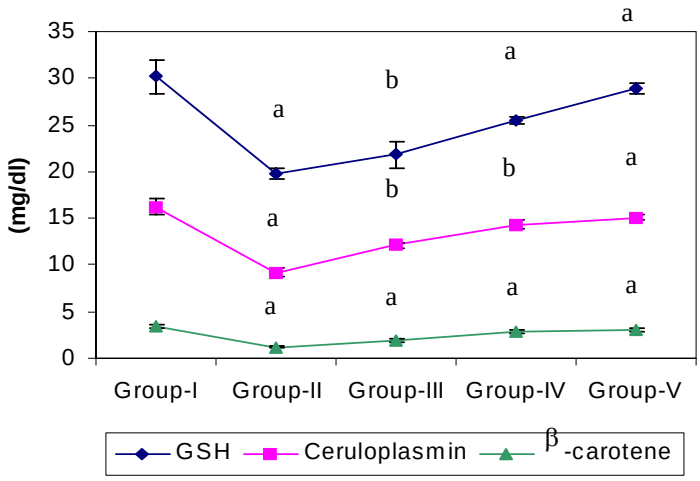



**Fig. 1 : Modulatory activity of non-enzymatic antioxidants in the serum of control and experiment rats. Data points with different letter notations (a,b) are significantly different at a = P < 0.001; b = P < 0.01.**

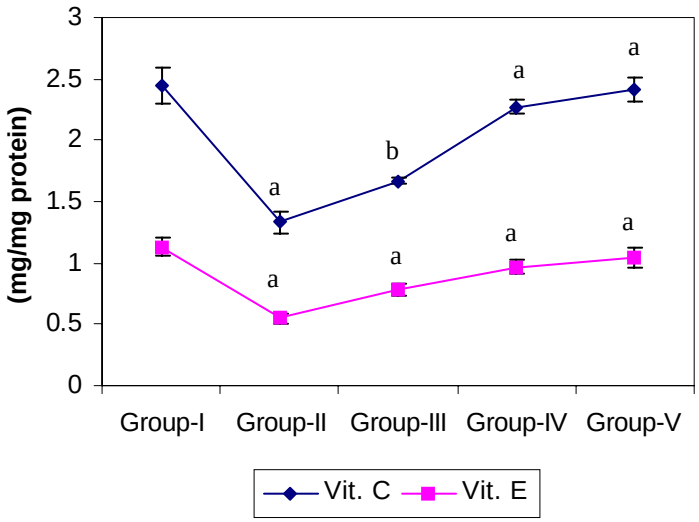



**Fig. 2 : Modulatory activity of non-enzymatic antioxidants in the serum of control and experiment rats. Data points with different letter notations (a,b) are significantly different at a = P ≤ 0.001; b = P ≤ 0.01.**

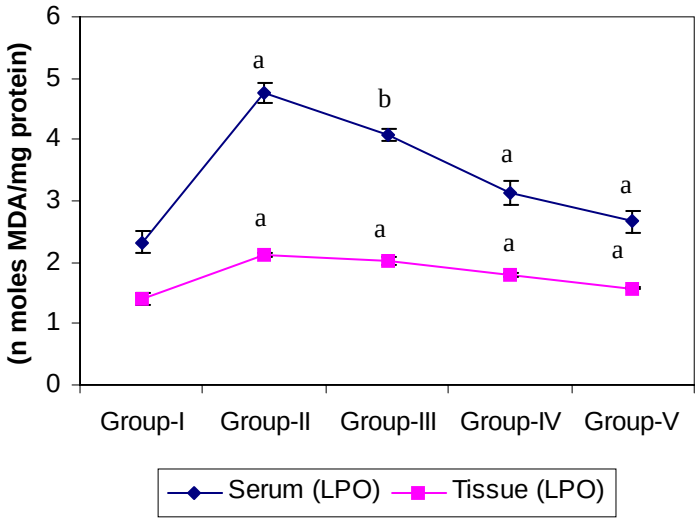



**Fig. 3 : Modulatory activity of lipid peroxidation in the serum and liver of control and experiment rats. Data points with different letter notations (a,b) are significantly different at a = P ≤ 0.001; b = P ≤ 0.01.**

## Discussion

Oxidative stress has been implicated in the pathology of many diseases and conditions including diabetes, cardiovascular diseases, inflammatory conditions, cancer and aging[20]. Antioxidants may offer resistance against the oxidative stress by scavenging the free radicals, inhibiting the lipid peroxidation and by many other mechanisms and thus prevent diseases[21].

Formation of ROS (reactive oxygen species), oxidative stress and hepatocellular injury have been implicated to alcoholic liver disease. It has been documented that Kupffer cells are the major sources of ROS during chronic alcohol consumption, and these are primed and activated for enhanced formation of pro-inflammatory factors[22]. Additionally, alcohol induced liver injury has been associated with increased amount of lipid peroxidation[23].

Lipid peroxidation is viewed as a complicated biochemical reaction involving free radicals, oxygen, metal ions and a host of other factors in the biological system. Since lipids constitute nearly 60% of the components in biomembrans, only major perturbation is bound to affect structure and function of the cell. In recent years, lipids and their derivatives have been recognized as important molecules in signal transduction and lipid peroxidation is the focus of intense research in relation to its possible involvement is health and disease[24].

In our present investigation, the measurement of lipid peroxidation in the liver is a convenient method to monitor oxidative cell damage. Inhibition of elevated LPO has been observed in *A. indicum* extract, dose-dependently and silymarin treated groups due to its antioxidant and free radical scavenging activities.

The antioxidant defense enzymes have been suggestive of playing an important role in maintaining physiological levels of oxygen and hydrogen peroxide and eliminating peroxides generated from inadvertent exposure to xenobiotics and drugs. Any natural compound with antioxidant properties may help in maintaining health when continuously taken as components of dietary food, spices or drugs[25]. The increase in the levels of antioxidant profiles i.e. SOD, CAT, GR and GPx by *A. indicum* extract dose-dependantly and silymarin may be attributed to have biological significance in eliminating reactive free radicals that may affect the normal functioning of cells.

In the present study, wherein CDNB was used as a non-specific substrate for assaying hepatic GST, (as CDNB is active for different degrees with all the isoenzymes of GST)[18], the hepatic GST activity was found to be enhanced by the *A. indicum* extract significantly in a dose dependent manner and by silymarin. The elevated level of GST by the extract may have facilitated the conjugation reaction of xenobiotic metabolism and may have increased the availability of non-critical nucleophiles for inactivation of electrophiles and therefore might be playing a major role in hepatic antioxidant system.

Alcoholic liver disease is characterized by steatosis, inflammation, necrosis and ultimately fibrosis and cirrhosis [26].

Our study further revealed that chronic exposure to alcohol significantly, decreased the activities of non-enzymic antioxidants namely GSH, Vit-C, Vit.-E, $\beta$-carotene and ceruloplasmin in serum might be responsible for hepatocellular injury. The supplementation of *A. indicum* extract, dose-dependently and silymarin were enhanced non-enzymic antioxidants, significantly in 30% alcohol treated rats. GSH, Vit. C and Vit. E exist in their interconvertible forms and participate in the detoxification of the toxic reactive oxygen species.

Regeneration to their reduced forms is brought about by reduced glutathione. The circulating GSH reflects increased utilization to trap the free radicals[27]. Vit. C is considered to be the most important antioxidant in extracellular fluids[28] and also acts to protect membranes against peroxidation by enhancing the activity of α-tocopherol, the chief lipid soluble and chain breaking antioxidant. *β*-carotene is a potent free radical quencher, singlet oxygen scavenger, and lipid peroxidation [29].

The decreased serum lipid peroxidation as MDA formation in the present study is in correlation with the induction of antioxidant enzymes above basal level by *A. indicum* extract dose-dependently and silymarin.

## Conclusion

It may be concluded that *A. indicum* leaves extract prevents alcohol induced oxidative stress and hepatic injury by the combined synergistic effects of its flavonoids viz. (i) Luteolin, (ii) Chrysoeriol, (iii) Luteolin-7-O-beta glucopyranoside, (iv) Chrysoeriol-7-O-beta glucopyranoside, (v) Quercetin-3-O-beta glucopyranoside and micronutrients, which have been shown to possess various biological properties related to antioxidant mechanisms.

Further, the comparative evaluation of such flavonoids are in progress in our laboratory for the responsible of more antioxidant potential with molecular mechanism and will be reported elsewhere.

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