

Hepatoprotective Activity of Herbal Extracts in Carbon Tetrachloride Intoxicated Albino Rats by Measuring Anti-oxidant Enzymes

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Abstract: The plants *Tinospora cordifolia*, *Aloe Vera* and *Mangifera indica* are used in the ayurvedic system as antidiabetic, antibacterial, antifungal, antitumor, immunostimulant and also to treat liver disorders. The aim of the study was to evaluate the hepatoprotective activity of these three plants extracts against carbon tetra chloride induced intoxicity in wistar albino rats. Stock solution (1mg/ml) of these extracts was prepared and administered to experimental rats for 6 days. On the seventh day single dose of CCl_4 (0.25 ml/100 gm) was given and hepatoprotective activity of extracts was evaluated by estimation of the antioxidant enzymes- superoxide dismutase, catalase, peroxidase and also lipid peroxidation level. The toxicity induced by CCl_4 was controlled significantly by restoration of improved level of superoxide dismutase, catalase, peroxidase and decreased lipid peroxidation when compared to the toxic control group. Increased levels of these enzymes showed good antioxidant potential and thus hepatoprotective effect. *Aloe Vera* extract showed the best antioxidant potential followed by *Tinospora cordifolia* and *Mangifera indica*. *Aloe vera* extract could be recommended for improving the antioxidative status of patients undergoing antiviral therapy.

Key words: Liver, carbon tetrachloride, superoxide dismutase, catalase, peroxidase, lipid peroxidation.

Introduction and Experimental

The liver is a vital organ present in the animals and it has wide range of functions (1). Liver plays important role in detoxification of drugs through metabolizing enzymes. Human beings are exposed to foreign compounds through environmental exposure, consumption of food or during intake of medicines and produce a variety of toxic manifestations due to damage to hepatocytes (2). In spite of tremendous strides in modern medicine, there are hardly any drugs that stimulate liver function, offer protection to the hepatocytes from damage. Therefore, many folk remedies from plant origin are evaluated for their possible antioxidant and hepatoprotective effect against chemical induced liver damage in experimental animals. CCl_4 induced hepatotoxicity model is frequently used for the investigation of hepatoprotective effects of drugs and plant extracts. The present study was designed to

investigate hepatoprotective effects of *Tinospora cordifolia*, *Aloe vera* and *Mangifera indica* (3-5) against CCl_4 induced hepatotoxicity in albino rats.

Collection of Plant Material

Fresh and mature plants of *Tinospora cordifolia* and *Aloe vera* were collected from nursery in Kurukshetra where as fruits of *Mangifera indica* were collected from general market in Kurukshetra.

Preparation of Extracts

Tinospora cordifolia:

Stem and bark (200 gm) of *Tinospora cordifolia* were dried in shade and powdered. Defatting of the dried powder (100 g) was done with 200 ml petroleum ether in a soxhlet apparatus (60-80°) for 12 hours. The marc was air-dried and was further extracted with methanol (100 ml) in a Soxhlet apparatus for 12 h at 60°. The extract was filtered and concentrated to dryness on hot water bath. 5 gm of dried powder was obtained after

drying on water bath (6,7). Aqueous solution of dried powder was prepared for feeding the animals. Two doses of different concentration were prepared. For 250 mg/kg dose, 2500 mg of powder was dissolved in 100 ml water and for 500 mg/kg dose, 5000 mg of powder was dissolved in 100 ml water.

Aloe vera:

Gel was exudated from *Aloe vera* leaf. Gel (200 gm) was dissolved in 200 ml glycerol solution. The resulting solution was filtered and evaporated on water bath till it reduced to one fifth. The dried solution was sterilized in an autoclave.

Mangifera indica:

Pulp of *Mangifera indica* was separated using a simple knife. 200 gm of pulp was dissolved in 200 ml of acetone. The resulting solution was evaporated on water bath till it dried to powder form. Nearly 4 gm of dried powder was obtained and was used as mango peel extract.

Animals

Wistar albino male rats (100-150 gm) were taken and were maintained under control condition of light and temperature in Animal House, Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra. Food pellets (DRDE, Gwalior) and tap water were provided *ad libitum*. For experiments, animals were kept fasting overnight but were allowed free access to water.

Inducing Liver Damage:

Acute liver damage was induced by administering a single dose of 0.25 ml/100gm of body weight of CCl₄ (2:5 v/v in paraffin oil) intraperitoneal (IP) and no mortality from the single dose of CCl₄ were observed.

Wistar albino rats were divided into nine groups (A, B, C, D, E, F, G, H and I) of three (3) rats each. The aqueous extract of powdered *T. cordifolia*, *Aloe vera* and *M. indica* carbon tetrachloride, 5% gum acacia, olive oil were given with the help of feeding cannula. The group A served as control and rats were fed with aq. 5% gum acacia for 7 days. Group B served as toxic control and rats were given aq. 5% gum acacia for 6 days, 0.25 ml/100 gm of carbon tetrachloride and olive oil (1:1) on 7th day, groups C served as standard and were given 1 ml of 0.5% silymarin (25 mg/kg) for 6 days and 0.25 ml/100gm of carbon tetrachloride and olive oil (1:1) on 7th day. Rest of groups (i.e. group D, E, F, G, H, and I) served as test groups. Group D and E received 250 mg/kg and 500 mg/kg of *Tinospora cordifolia* extract respectively and group F and G received 250 mg/kg and 500 mg/kg extract of *Aloe vera* respectively, groups H and I received 250 mg/kg and 500 mg/kg of *M. indica* extract respectively. 0.25

ml/100 gm of CCl₄ was administered on 7th day after 6 days administration of the extracts. After 24 hrs of carbon tetrachloride administration, rats were sacrificed by anesthetizing for the analysis of blood anti-oxidant enzymes.

Preparation of Liver Homogenate

The animals were sacrificed, livers were rapidly excised and perfused with cold normal saline and processed. Liver homogenates (10%) were prepared in 0.25 M sucrose in phosphate buffer (0.2 M, pH 7.4). Homogenates were centrifuged at 800 x g for 10 minutes at 4°C. The supernatant obtained was collected and used for the estimation of antioxidant enzymes i.e. superoxide dismutase (SOD), catalase, peroxidase and lipid peroxidation levels.

Assessment of hepatoprotective activity

Estimation of the antioxidant enzyme SOD was performed by the method of Kono (8), catalases by method of Luck (9), peroxidase by the method of Alexander (10) while lipid peroxidation was estimated by the method of Okhawa et. al.(11).

Result and Discussion

The hepatoprotective activities of extracts of *Tinospora cordifolia*, *Aloe vera* and *Mangifera indica* were determined on the basis of antioxidant potential using two different doses (250 mg/kg and 500 mg/kg) of each extract and results were compared with respect to control, standard drug silymarin and CCl₄ treated animals. The effects of *Tinospora cordifolia*, *Aloe vera* and *Mangifera indica* extracts on antioxidant enzymes and lipid peroxidation are reported in Table-1.

Pretreatment of rats with 250 mg/kg of *Tinospora cordifolia*, *Aloe vera* and *Mangifera indica* extracts improved SOD, catalase, peroxidase and decreased lipid peroxidation level in comparison to CCl₄ treated group. The values obtained were 8.89±0.69 U/mg protein, 98.61±3.87 U/mg protein, 145.76±3.89 U/mg protein, 18.21±2.23 nm MDA/g protein for SOD, catalase, peroxidase and lipid peroxidation respectively on treatment with extracts of *Tinospora cordifolia*. These values were 19.48%, 30.50%, 30.14% higher than CCl₄ treated group for SOD, catalase and peroxidase respectively but 26.45% lower in case of lipid peroxidation. On pretreatment with *Aloe vera* extract the values obtained were 10.23±0.67 U/mg protein, 108.61±3.34 U/mg protein, 153.76±4.76 U/mg protein, 21.21±2.34 nm MDA/g protein for SOD, catalase, peroxidase and lipid peroxidation respectively. These values were 37.5%, 43.74%, 37.28% higher than CCl₄ treated group for SOD, catalase and peroxidase respectively but 14.70% lower in case of lipid peroxidation. On

pretreatment with dose of 250 mg/kg of *M. indica* extract, the values obtained were 6.64 ± 0.60 , 87.61 ± 3.65 , 114.76 ± 4.76 U/mg of protein and 14.21 ± 2.34 nm MDA/g of protein for SOD, catalase and peroxidase respectively. The values observed were 10.75%, 15.94%, 2.46% higher than CCl_4 treated group for SOD, catalase and peroxidase respectively but 42.60% lower levels in case of lipid peroxidation. The values obtained with silymarin were 14.11 ± 0.23 , 148.22 ± 0.55 , 198.2 ± 10.0 U/mg of protein for SOD, catalase and peroxidase and 14.56 ± 1.54 nm MDA/g of protein for lipid peroxidation. The result shows the beneficial effects of silymarin.

Pretreatment with 500 mg/kg of *T. cordifolia* extract further improved the antioxidant status as evident from the table 1. The values were 21.90%, 50.14%, 39.98% higher than CCl_4 treated group for SOD, catalase and peroxidase respectively but 44.42% lower in case of lipid peroxidation. The values observed were 48.79%, 62.05%, 64.98% higher than CCl_4 treated group for SOD, catalase and peroxidase respectively but 36.75%

lower levels in case of lipid peroxidation on treatment with 500 mg/kg *Aloe vera* extract. Levels of antioxidant enzymes measured at 500 mg/kg dose of *Aloe vera* extract were comparable with standard silymarin. Peroxidase level with silymarin corresponds to 198.2 ± 10.0 U/mg of protein whereas values were 184.78 ± 5.64 U/mg of protein with *Aloe vera* treated extract as shown in table 1.

500 mg/kg dose of *M. indica* extract further improved the level of superoxide dismutase, catalase, peroxidase and decreased lipid peroxidation level. The values observed were 4.97%, 30.29%, 4.26% higher than CCl_4 treated group for SOD, catalase and peroxidase respectively but 37.19% lower levels in case of lipid peroxidation. The effects were less on treatment with *M. indica* as compared to that observed with extracts of *T. cordifolia* and *Aloe vera*.

It can be concluded that *Aloe vera* has best antioxidant effects followed by *T. cordifolia* and *M. indica*. *Aloe vera* extract could be recommended for improving the antioxidative status of patients undergoing antiviral therapy.

Table 1: Effects of *Tinospora cordifolia*, *Aloe vera* and *Mangifera indica* extracts on antioxidant enzymes and lipid peroxidation. Values are mean $\pm$ SEM. N= 3 in each group

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Treatment	SOD (U/ mg of protein)	Catalase (U/ mg of protein)	Peroxidase (U/ mg of protein)	Lipid peroxidation (nm MDA/g of protein)
Normal control	12.75 ± 0.55	178 ± 28.8	265 ± 11.32	6.12 ± 0.87
CCl_4 treated control	7.44 ± 0.14	75.56 ± 0.67	112 ± 8.88	24.76 ± 2.56
CCl_4 + Silymarin	14.11 ± 0.23	148.22 ± 0.55	198.2 ± 10.0	14.56 ± 1.54
CCl_4 + extract of <i>T. cordifolia</i> (250 mg/kg)	8.89 ± 0.69	98.61 ± 3.87	145.76 ± 3.89	18.21 ± 2.23
CCl_4 + extract of <i>T. cordifolia</i> (500 mg/kg)	9.07 ± 0.47	113.45 ± 7.56	156.78 ± 4.73	13.76 ± 2.48
CCl_4 + extract of <i>A. vera</i> (250 mg/kg)	10.23 ± 0.67	108.61 ± 3.34	153.76 ± 4.76	21.21 ± 2.34
CCl_4 + extract of <i>A. vera</i> (500 mg/kg)	11.07 ± 0.44	122.45 ± 7.98	184.78 ± 5.64	15.66 ± 2.48
CCl_4 + extract of <i>M. indica</i> (250 mg/kg)	6.64 ± 0.60	87.61 ± 3.65	114.76 ± 4.76	14.21 ± 2.34
CCl_4 + extract of <i>M. indica</i> (500 mg/kg)	7.07 ± 1.73	98.45 ± 7.95	116.78 ± 4.64	15.55 ± 2.89

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