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Research Article

ANTIOXIDANT AND HEPATOPROTECTIVE ACTIVITY OF METHANOLIC EXTRACT OF *HYGROPHILA SCHULLI* LEAVES

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ABSTRACT
The objective of this study was to investigate the antioxidant effects of methanolic extract of leaves of *Hygrophila schulli* against paracetamol induced liver damage. The material was dried in shade; they were powdered and extracted with methanol. The antioxidant effects of the methanol extract was assessed in paracetamol induced hepatotoxic rats. Alteration in the levels of antioxidant markers of hepatic damage like LPO, SOD, CAT, GPx and GST were tested in both paracetamol treated and untreated groups. Treatment of methanolic extract of *Hygrophila schulli* leaves (500 mg/kg) has brought back the altered levels of antioxidant markers to the near normal levels in the dose dependent manner. Our finding suggested that *Hygrophila schulli* methanol leaf extract possessed Antioxidant activity.
KEY WORDS: Antioxidants, Lipid peroxidation, *Hygrophila schulli*, Paracetamol

INTRODUCTION

The world health organization has defined traditional medicine as comprising therapeutic practice that has been in existence for hundreds of years [1]. The traditional preparations comprise medicinal plants, minerals and organic matter. Herbal drug constitute only those traditional medicines which primarily use medicinal plant preparations for therapy [2]. Liver is the one of the largest organ in human body and the chief site for intense metabolism and excretion. So it has a surprising role in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction [3]. Liver disease mainly caused by chemical agents, excess consumption of alcohol, infection and autoimmune disorder. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damage in liver [4].

Hygrophila schulli (Acanthaceae) is described in ayurvedic literature as Ikshura, Ikshugandha and Kokilasha "having eyes like the Kokila or Indian Cuckoo". The plant is widely distributed throughout India, Srilanka, Burma, Malaysia and Nepal. The whole plant, roots, seeds, and ashes of the plant are extensively used in traditional system of medicine for the treatment of Pnemeham (Diabetes), athisaram (Dysentery) etc., [5, 6]. The plant is known to possess antitumor [7, 8], hypoglycemic [9], antibacterial [10, 11] and hepatoprotective [12] activities.

MATERIALS AND METHODS

Plant material

The fresh plant leaves of *Hygrophila schulli* were collected from Narasipuram, Coimbatore, Tamil Nadu, and India. The plant material was taxonomically identified by the Botanical Survey of India (BSI), Coimbatore, Tamil Nadu, and India.

Preparation of extract

The plant leaves was collected and dried under shade and powdered leaves (200 gm) were extracted with methanol in a Soxhlet extractor for 36 hr. The extract was concentrated and last trace of solvent was removed by Rotary Vacuum evaporator and used for further investigation.

Animals

Male Wistar rats (150 – 200 g) and were procured from Small Animal Breeding Station, College of Veterinary Animal Science, Mannuthy, and Kerala. They were housed in microloan boxes with standard laboratory diet and water ad libitum.

Experimental design

Rats were divided into five groups, each group consisting of six animals.

Group I : Control received the vehicle viz.,

Group II: Received paracetamol (750 mg/kg P.O.) 13 at every 72h for 21 days

Group III : Received methanol extract of Hygrophila schulli 250 mg/kg P.O. for 21 days and simultaneously administered paracetamol 750 mg/kg every 72 h.

Group IV : Received methanol extract of Hygrophila schulli 500 mg/kg P.O. for 21 days and simultaneously administered paracetamol 750 mg/kg every
72 h.

Group V: Received Silymarin 50 mg/kg (P.O.) for 21 days and simultaneously administered Paracetamol 750 mg/kg every 72 hrs.

ESTIMATION OF LIVER MARKAR ENZYMES

After 21 days animals are sacrificed and the liver was dissected out washed in ice cold saline, and a homogenate was prepared in 0.05M sodium phosphate buffer pH 7.0. The homogenate was centrifuged at 3000rpm for 10 minutes and the supernatant was used for the assay of marker enzymes. Lipid peroxidation (LPO) [14], Superoxide dismutase (SOD) [15], Catalase (CAT) [16], Glutathione preoxidase (GPx) [17] and Glutathione S-transferase (GST) [18].

STATASTICAL ANALYSIS

The results were expressed as mean ± SD of six animals from each group. A column means followed by different superscript are significant at 5% DMRT [19].

RESULT

Analysis of LPO levels by thiobarbituric acid reaction showed a significant ($P<0.05$) increase in the paracetamol treated rats. Treatment with *Hygrophila schulli* (250 and 500 mg/kg) significantly ($P<0.05$) prevented the increase in LPO level which was brought to near normal. The effect of *Hygrophila schulli* was comparable with that of standard

drug silymarin (Fig: 1). Paracetamol treatment caused a significant (P<0.05) decrease in the activities of SOD, catalase, GPx and GST in liver tissue when compared with control group (Fig: 2-5). The treatment of *Hygrophila schulli* at the doses of 250 and 500 mg/kg resulted in a significant increase in the activities of SOD, catalase, GPx and GST when compared to paracetamol treated rats. The liver of silymarin treated animals also showed a significant increase in antioxidant enzymes levels compared to paracetamol treated rats.

DISCUSSION

Paracetamol, a widely used antipyretic-analgesic drug, produces acute hepatic damage on accidental over dosage. It is established that, a fraction of paracetamol is converted via the cytochrome P450 pathway to a highly toxic metabolite, N–acetyl–p–benzoquinamine (NAPQI) [20], which is normally conjugated with glutathione and excreted in urine. Overdose of paracetamol depletes glutathione stores, leading to accumulation of NAPQI, mitochondrial dysfunction [21], and development of acute hepatic necrosis. Several P450 enzymes are known to play an important role in N-acetyl-p-aminophenol (APAP) bioactivation to NAPQI. P450 2E1 (CYP2E1) have been suggested to be primary enzymes for paracetamol bioactivation in liver microsomes [22]. Studies demonstrated that paracetamol induced hepatotoxicity can be modulated by substances that influence P450 activity [23].

The increase in LPO levels in liver induced by paracetamol suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defence mechanism to prevent formation of excessive free radicals. As mentioned above, one of the phenomena often observed in combination with oxidative stress is lipid peroxidation. Reactive oxygen species (hydrogen peroxide, superoxide anions, and hydroxyl radicals) are required for its initiation as NAPQI is expected to be incapable of initiating a radical hydrogen abstraction from lipid molecules. However, reduction of NAPQI, which could occur in the presence of flavoproteins, followed by reoxidation by oxygen could give rise to superoxide anions with a consequent formation of reactive reduced oxygen species. Even protein bound NAPQI was suggested to be liable to one electron reduction. LPO has been regarded to be an important initiation event in the toxicity mechanism of paracetamol [24]. Treatment of the rats with *Hygrophila schulli* significantly reduced the elevated levels of LPO on dose dependent manner.

Decrease in enzyme activity of SOD is a sensitive index in hepatocellular damage [25]. SOD has been reported as one of the most important enzymes in the enzymatic antioxidant defence system. It scavenges the superoxide anion to form hydrogen peroxide and thus diminishing the toxic effect caused by this radical. *Hygrophila schulli* causes a significant increase in hepatic SOD activity and thus reduces reactive free radical induced oxidative damage to liver.

Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues, and the highest activity is found in the red cells and liver. CAT decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals [26]. Therefore reduction in the activity of CAT may result in a number of deleterious effects due to the assimilation of superoxide radical and hydrogen peroxide. A higher dose (500 mg/kg) increases the level of CAT as produced by silymarin, the standard hepatoprotective drug.

Glutathione S-Transferase (GST) is one of the most abundant tripeptide, non-enzymatic biological antioxidants present in the liver. It removes free radical species such as hydrogen peroxide, superoxide radicals and maintains membrane protein thiols. Decreased level of GST is associated with an enhanced lipid peroxidation in paracetamol treated rats. Administration of *Hygrophila schulli* significantly increased the level of GPx and GST in a dose dependent manner.

Free radical mediated process has been implicated in pathogenesis of most of the diseases. The protective effect of *Hygrophila schulli* on paracetamol induced hepatotoxicity in rats appears to be related to inhibition of lipid peroxidation and enhancement of antioxidant enzyme levels in addition to free radicals scavenging action. Preliminary phytochemical studies reveal the presence of flavonoids in methanolic extract of *Hygrophila schulli.* [27-28] the observed antioxidant effects of *Hygrophila schulli* may be due to the presence of flavonoids.

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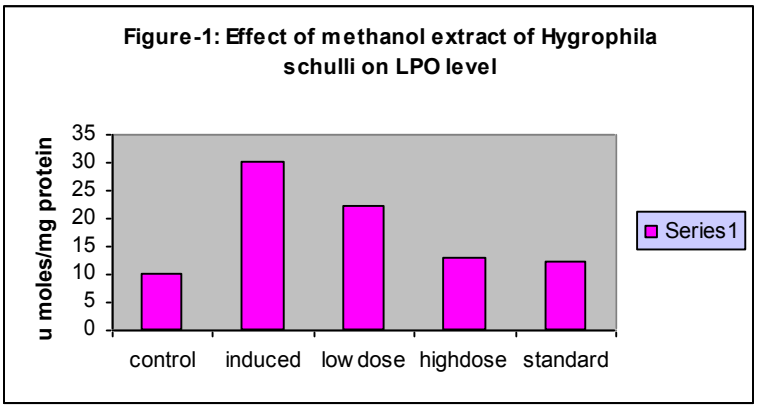
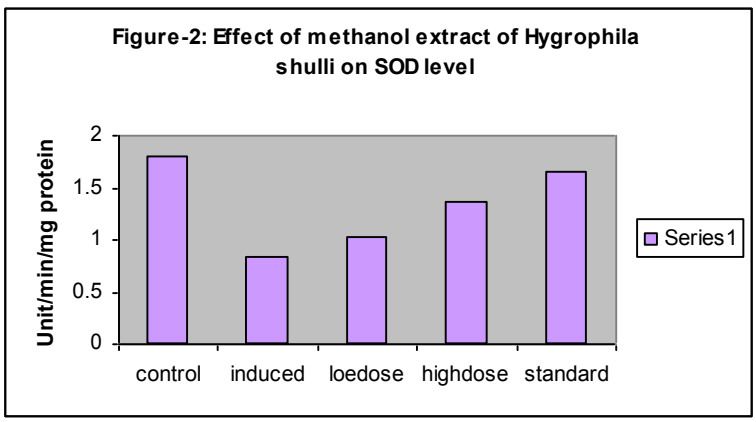
Table:1 Effect of *Hygrophila schulli* on antioxidant activities in paracetamol induced hepatotoxicity rats.

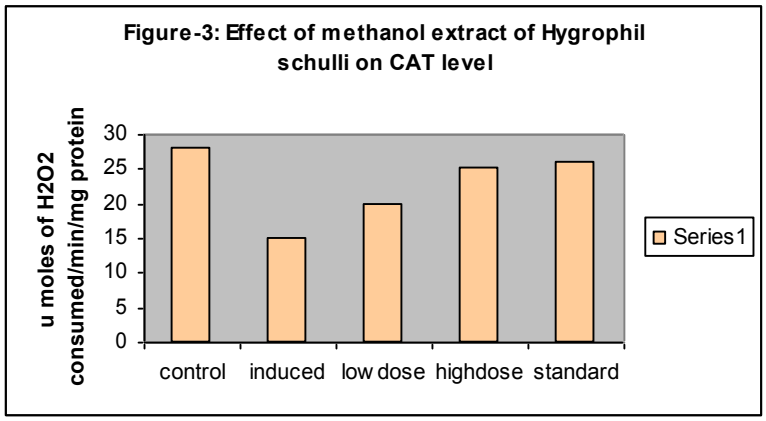
Group	Treatment	LPO	SOD	CAT	GPx	GST
I	Normal control	10.09±0.71[a]	1.79±0.11[a]	28.71±0.96[a]	91.32±0.55[a]	110.61±0.93[a]
II	Paracetamol Induced 750 mg/kg	30.72±0.98[b]	0.83±0.07[b]	15.76±0.23[b]	61.37±0.99[b]	60.81±1.59[b]
III	Paracetamol +*Hygrophila schulli* 250 mg/kg (Low dose)	22.00±0.74[c]	1.01±0.02[c]	20.01±0.65[c]	70.13±1.51[c]	80.53±0.68[c]
IV	Paracetamol +*Hygrophila schulli* 500 mg/kg(high dose)	13.13±0.55[d]	1.35±0.02[d]	25.01±0.63[d]	85.85±1.00[d]	98.86±0.40[d]
V	Paracetamol+ Standard Silymarin 50mg/kg	12.27±0.46[e]	1.65±1.10[e]	26.04±0.59[e]	88.53±0.77[e]	102.72±2.11[e]

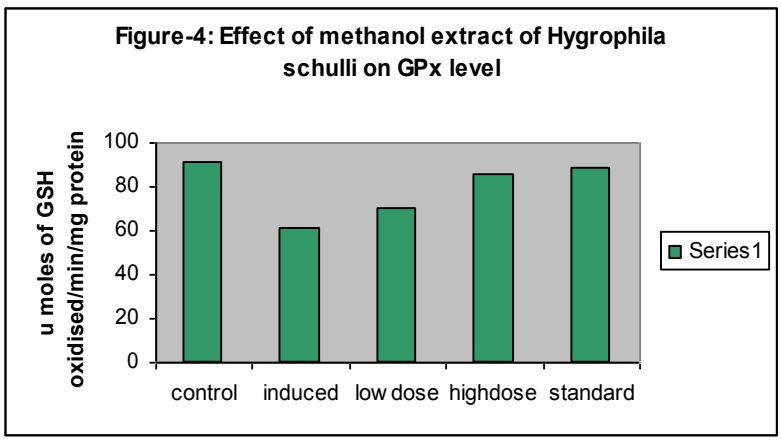
Values are expressed as mean ± S.D (n=6)

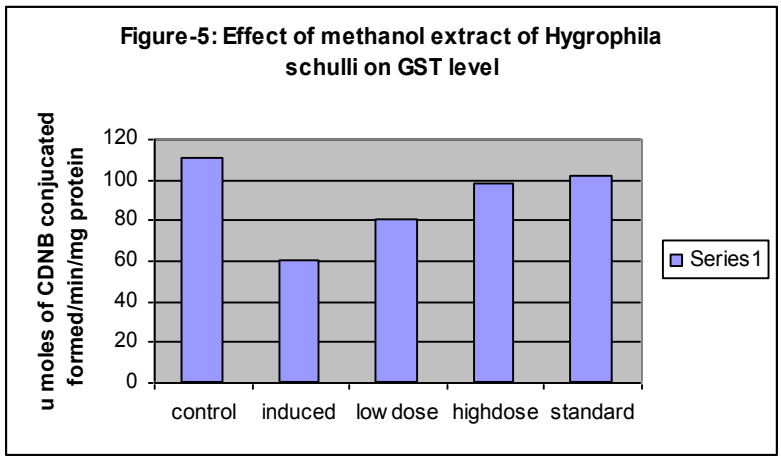
A Column means followed by different superscript are significant at 5% (P<0.05) DMRT. Units: LPO= µ moles of MDA/min/mg protein; SOD= Units/min/mg protein; CAT= µ mole of H_2O_2 consumed/min/mg protein; GPx= µ moles of GSH oxidized/min/mg protein; GST= µ moles of choloro-dinitro benzene(CDNB) conjucated formed/min/mg protein.











Control= Normal
Induced= Paracetamol 750mg/kg
Lowdose= Paracetamol +*Hygrophila schulli* 250 mg/kg
Highdose= Paracetamol +*Hygrophila schulli* 500 mg/kg
Standard= Paracetamol+Silimarin 50mg/kg