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Evaluation of hepatoprotective activity of *Andrographis paniculata* extract on paracetamol induced liver damage in albino rats

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Abstract
Traditionally, a number of medicinal plants are used to treat various hepatic disorders, but only few of them have been pharmacologically evaluated for their safety and efficacy. The plant *Andrographis paniculata* (AP) (Kalmegha) was traditionally used in Indian system of medicine (Ayurveda) for the treatment of various liver and other disorders. In the present study, an attempt was made to substantiate the ethnopharmacological use of *A. paniculata* in hepatoprotection against the paracetamol-induced hepatotoxicity. In this study, to induce hepatotoxicity, paracetamol was given at the dose rate of 2 g/kg body weight orally to albino rats. Serum levels of alanine transaminase, aspartate aminotransferase, and alkaline phosphatase were used as indicator to evaluate the liver injury. In addition, creatinine, BUN, and blood parameters were also assayed using the standard procedure. Extract of *A. paniculata* (APE) @ 400 mg/kg bw orally for 7 consecutive days exhibited significant protection of liver against paracetamol induced hepato-toxicity as depicted by normalization of biochemical and haematological parameters after administration of *A. paniculata*.

Keywords: *Andrographis paniculata*, paracetamol, APE, SGOT, SGPT, BUN

Introduction
Herbal medicine is still the primary health care source for approximately 75-80 percent of the world's population mainly in developing countries (Kamboj, 2000) [12]. Herbal drugs are essential components of traditional medicine and are used in ayurvedic, homoeopathic, naturopathic and other systems of medicine. Herbal medicines are generally thought to have least side effects and are inexpensive.

Andrographis paniculata is known as ‘kalmegh’ meaning "dark cloud" also known as Bhui-neem meaning "neem of the ground", Andrographis and Kirayat. It is widely cultivated in Southern Asia, and is an herbaceous plant, commonly known as “King of Bitters”, belonging to the family of Acanthaceae. *Andrographis paniculata* is an annual herb found in Sri Lanka, Pakistan, Java, Malaysia, Indonesia and throughout India, specifically in Maharashtra, Karnataka, Uttar Pradesh, Tamil Nadu, Andhra Pradesh and Madhya Pradesh.

A. paniculata possesses a broad category of pharmacological activity and some of them are enormously beneficial, such as anti-inflammatory (Kapil *et al*., 1993) [13], antiviral (Mishra *et al*., 1992) [15], antimalarial (Rahman *et al*., 1999) [18], hepatoprotective (Handa and Sharma, 1990) [10], cardiovascular (Zhang and Tan, 1997) [28], anticancer (Matsuda *et al*., 1994) [14]. Immuno-stimulatory, antipyretic, astringent, gastric and liver tonic, diuretic, carminative, and anthelmintic properties are also present in *Andrographis paniculata*. It is extensively used for medicinal purposes in India, Thailand, China and Mauritius (Niranjan *et al*., 2010) [17].

Andrographolide is the main active compound of *A. paniculata*. Andrographolide shows noticeable hepatoprotective effect in preventing carbon tetrachloride and galactosamine induced liver damage (Handa and Sharma, 1990) [10]. Andrographolide produce hepato-protection by reducing lipid peroxidation product malondialdehyde (MDA), as well as by maintaining high level of reduced glutathione, glutamic pyruvate transaminase and alkaline phosphatase as observed in various studies (Kapil *et al*., 1993) [13].

Acetaminophen is a non-steroidal anti-inflammatory drug (NSAID) that inhibits cyclooxygenase (COX) and prostaglandin synthesis in the brain and central nervous system (CNS) respectively and also cause hepatic toxicity in higher doses (Budnitz *et al.,* 2011) [4]. Acetaminophen has been standardized as a model for laboratory study of potential hepatoprotective agents.

Materials and Methods

The purpose of this study was to evaluate the hepatoprotective properties of *A. paniculata* against paracetamol-induced liver damage in albino rats. The experiment was carried out on healthy adult inbred albino rats of both sexes weighing 150–200 g. The rats were housed in clean polycarbonate cages in Lab Animal House at College of Veterinary Science and Animal Husbandry in Jabalpur. The rats were fed standard pellet diet with free access to water. The animals were kept under observation for two weeks before the start of the experimentation. During this time, animals were subjected to clinical examinations to rule out the possibility of any disease condition. All the necessary management procedures were implemented to keep the animals free from stress. The experimental protocol and use of animals in the experiment was approved by Institutional Animal Ethics Committee (IAEC) No: 116/IAEC/Vety/2018. Guidelines of CPCSEA were implemented for the care and management of animals during experimentation.

Drugs and Chemicals

Paracetamol was purchased from Micro Labs Ltd., Bengaluru, Karnataka. Ethanolic extract of *A. paniculata* (Kalmegh) was received as a gift from Amsar Pvt. Ltd., Indore. Other essential chemicals used in this study were purchased from Hi Media Laboratory Pvt. Ltd., Mumbai.

Animals Treatment

In this study, 12 rats were randomly divided into three groups, having four animals in each group. Rats of each group were housed in separate cages. The first group served as normal control and was only given normal saline orally for 10 consecutive days. To the second group, on the eighth day of the experiment, a single dose of paracetamol was given @ 2 g/kg bw to 24-hour fasted rats with no interruption in the water supply. In the third group of rats, *A. paniculata* extract (APE) was given @400mg/kg bw, orally for 7 consecutive days, and on the 8th day, a single dose of paracetamol was given @ 2 g/kg bw, orally.

Blood collection

On the 10th day of studies, all rats were sacrificed by decapitation method. Following decapitation, blood samples were collected in EDTA-coated and non-EDTA-coated test tube for evaluation of haematological and biochemical parameters, respectively. After allowing the blood to clot for 45 minutes at room temperature, the serum was separated by centrifugation at 4000 rpm for 20 minutes. In collected serum, serum aminotransferases such as SGOT (IU/L) and SGPT (IU/L), as well as alkaline phosphatase (ALP) (IU/L), albumin (g/dl), globulin (g/dl), total protein (g/dl), creatinine (mg/dl), and BUN (mg/dl) were measured using a semi-automatic biochemical analyser.

Results and Discussion

In this study, large oral dose of paracetamol caused significant hepatocellular changes, as evidenced by increased levels of SGPT (116.45^{a}±3.26 IU/L) and SGOT (139.43^{a}±3.56 IU/L) in comparison to normal control. Setty *et al*. (2007) [20] also noticed markedly increases in SGPT value on paracetamol administration at the dose rate of 2g/kg bw in rats. In normal doses, paracetamol is primarily metabolized via conjugation with sulphate and glucuronic acid, and N-acetyl-p-benzoquinone imine (NAPQI) is formed, which is subsequently metabolized by reduced glutathione. In the event of an overdose, the production of NAPQI exceeds the capacity of reduced glutathione detoxification (GSH). This causes liver toxicity by oxidative damage (Aubert *et al*., 2012) [2].

In this study, administration of APE caused significant reduction in the level of SGOT and SGPT with their respective value of 80.54^{cd}±1.82 and 88.44^{d}±3.33 IU/L. Babu, (2007) [3] exhibited marked hepatoprotective activity of aqueous and alcoholic extract of *A. paniculata*, and observed significant reduction in SGPT value in paracetamol induced liver toxicity. Shrivastava and Gilhotra (2017) [21] also evaluated the hepatoprotective activity in APE in CCl_4 treated rats induced liver damage and found significant reduction in SGPT value.

Nagalekshmi *et al*. (2011) [16] analysed the hepatoprotective potential of ethanolic extract of AP by oral route. The APE administration significantly prevented the elevation of SGOT in treated animals. Rajalakshmi *et al*. (2012) [19] also evaluated the hepato-protective activity of *A. paniculata* extract in paracetamol induced liver damage in albino rats and observed significant reduction in SGOT value in AP extract treated rats. Vetriselvan and Subasini (2012) [26] observed higher value of AST (162.50±5.80IU/L) in ethanol intoxicated rats and after the administration of *A. paniculata*, the same was reduced to 103.820±6.585 IU/L and 77.445±8.433 IU/L at the dose rate of 200 and 400 mg/kg bw orally, respectively.

The liver is the primary source of most serum proteins; the parenchymal cells of the liver are responsible for the production of albumin, fibrinogen, and other coagulation factors, as well as the majority of globulins. A low serum albumin level indicates poor liver function, so a drop in albumin level is generally indicative of liver disease (Thapa and Walia, 2007) [24].

On administration of paracetamol, statistically significant reduction was observed in the level of albumin (2.55^{c}±0.03 g/dl) and globulin (4.21^{c}±0.04 g/dl). However, in *A. paniculata* treated group, increase in the serum protein level was observed. Goldwasser and Feldman, (1997) [9] stated that liver necrosis could be the cause for reduction in albumin level by paracetamol administration.

Devaraj *et al*. (2010) [6] observed significant increases in total protein (TP) level on *A. paniculata* methanolic extracts treatment against paracetamol-induced hepatotoxicity. The increase TP level denotes significant hepatoprotective activity of the APE. Rajalakshmi *et al*. (2012) [19] reported that administering paracetamol (2 g/kg bw, p.o.) causes liver damage and decreases the level of total protein and administration of *A. paniculata* returned its value towards the normal level. Paracetamol induced renal injury may be due to the metabolic activation of paracetamol to reactive metabolite NAPQI (Hart *et al*., 1994) [11].

This study also observed the increase in serum creatinine level (0.84a±0.03 mg/dl) and BUN (14.10^{a} ±0.23 mg/dl) levels in paracetamol treated group in comparison to control group. In *A. paniculata* treated group, serum creatinine (0.73^{b}±0.03 mg/dl) and BUN levels (11.77^{b}±0.18mg/dl) were declined significantly (Table 01). Subramaniam *et al*. (2015) [23] in their study in rats also observed significant decrease in serum creatinine in *A. paniculata* treated rats after CCl_4 administration.

Singh *et al*. (2009) [22] also reported kidney damage as evident by increased levels of serum creatinine and BUN in

gentamicin-induced acute renal failure returns to normal after treatment with *A. paniculata*. Gautam (2012) [8] in her study observed significant decrease in BUN value in *A. paniculata* treated rats in cisplatin-induced nephrotoxicity.

The value of Hb in normal control and paracetamol treated groups were 14.65^{a} ±0.33 gm/dl and 13.40^{b}±0.38gm/dl respectively, depicting a fall in Hb concentration on administration of paracetamol (Table 02). Yousef *et al*. (2010) [27] also indicated that paracetamol caused a significant decrease in Hb concentration. AL-Harbi *et al*. (2015)1 also demonstrated that administration of paracetamol for 30 successive days caused a significant decrease ($P<0.05$) in Hb level. Bukye and Masbau (2011) [5] recorded significant increase in Hb count after administration of aqueous extract of *A. paniculata.*

In this study, reduction in RBC (7.49^{b}±0.14x10^{6} /cu.mm) count was observed in paracetamol treated group. El-Megharbel *et al*. (2014) [7] in their study also observed that administering paracetamol causes reduction in RBC concentration in male albino rats. However, administration of *A. paniculata* in the treatment group caused increase in RBC (8.22^{a}±0.08(x10^{6}/cu.mm) counts. Bukye and Masbau (2011) [5] also recorded significant increase in RBC count after administration of aqueous extract of *A. paniculata.* Ul-Ain *et al.* (2017) [25] reported the reversal in Oxaliplatin caused anaemia, by prophylactic treatment with *A. paniculata* and showed raised RBC and haemoglobin levels in albino rats.

Table 1: Effect of ethanolic extract of *Andrographis paniculata* against paracetamol induced toxicity on different biochemical parameters

Group	Treatment	SGOT (IU/L)	SGPT (IU/L)	Albumin (g/dl)	Globulin (g/dl)	Total Protein (g/dl)	Creatinine (mg/dl)	BUN (mg/dl)
I	Normal saline control	76.51^{d}±1.57	86.00^{d}±2.55	3.10^{a}±0.08	4.91^{a}±0.15	8.03^{a}±0.18	0.71^{b}±0.02	11.78^{b}±0.26
II	Paracetamol control (8^{th} day)	116.45^{a}±3.26	139.43^{a}±3.56	2.55^{c}±0.03	4.21^{c}±0.04	6.86^{c}±0.13	0.84^{a}±0.03	14.10^{a}±0.23
III	APE (1-7 day) + PCM (8^{th} day)	80.54^{cd}±1.82	88.44^{d}±3.33	2.81^{b}±0.06	4.56^{b}±0.09	7.38^{b}±0.11	0.73^{b}±0.03	11.77^{b}±0.18

Table 2: Effect of ethanolic extract of *Andrographis paniculata* against paracetamol induced toxicity on different haematological parameters

Group	Treatment	Hb (gm/dl)	RBC (x10^{6}/ cu.mm)	WBC (x10^{3} /cu.mm)	N (%)	L (%)	M (%)	E (%)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)
I	Normal saline control	14.65^{a}±0.33	8.34^{a}±0.16	4.30^{c}±0.31	26.55^{b}±0.88	74.16^{a}±0.54	1.83±0.30	1.66±0.42	46.07±1.20	37.32^{z}±1.16	17.74±0.26	32.63±0.37
II	Paracetamol control (8^{th} day)	13.40^{b}±0.38	7.49^{b}±0.14	6.35^{a}±0.33	31.50^{a}±0.92	68.71^{b}±0.85	1.33±0.21	2.00±0.36	42.96±1.37	32.98^{b}±0.67	18.28±0.32	31.76±0.49
III	APE (1-7 day) + PCM (8^{th} day)	14.52^{a}±0.31	8.22^{a}±0.08	5.62^{ab}±0.27	25.07^{bc}±0.73	73.66^{a}±0.84	1.66±0.33	1.83±0.30	45.23±0.85	37.23^{a}±0.16	17.52±0.34	32.48±0.44

Conclusions

Based on the data from the current investigation, it can be concluded that paracetamol on oral administration produces hepatotoxicity as suggested by alteration in haematological and biochemical parameters. On administration of *A. paniculata* ethanolic extract amelioration in the toxicity status was observed denoting hepatoprotective activity of APE against paracetamol-induced hepatotoxicity in albino rats.

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