HEPATOPROTECTIVE ACTIVITY OF *MACROTYLOMA UNIFLORUM*. SEED EXTRACT ON PARACETAMOL AND D-GALACTOSAMINE INDUCED LIVER TOXICITY IN ALBINO RATS

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Abstracts
Hepatoprotective activity of methanolic extract of *Macrotyloma uniflorum* seed was investigated against D-Galactosamine and paracetamol induced hepatotoxicity in wistar albino rats. Silymarin was used as the reference standard at 50mg/kg orally. Rats were divided in 5 groups each containing 6 animals. Group-I and Group-II were administered saline and Group-III was administered Silymarin (50mg/kg). Group-IV (200 mg/kg b.w) and Group-V (400 mg/kg) were administered MEMUS. The degree of protection was determined by the estimation of biochemical parameter like Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT), alkaline phosphate (ALP), Bilirubin (Direct and Total). The 95% methanolic extract of fruit of *Macrotyloma uniflorum* (MEMUS) at the dose of (200mg/kg and 400mg/kg) produced a dose dependant reduction in biochemical parameters like SGPT, SGOT, ALP, Bilirubin (Direct & Total) as well as in morphological parameters in D-Galctosamine and paracetamol induced hepatotoxicity in rats. The histopathological study further supported the hepatoprotective activity of the test extract. Maximum protection was seen at 400mg/kg MEMUS. t *Macrotyloma uniflorum* seed showed significant hepatoprotactive properties in wistar albino rats.
Keywords: *Macrotyloma uniflorum* seed, D-galactosamine, paracetamol, silymarin, hepatoprotection.

1. Introduction

Liver is the second largest organ in the human body after skin. It removes chemical, alcohol, toxin and medicine from the blood and important for the metabolism, bile secretion, elimination of many substances, blood detoxifications, synthesis, and regulation of essential hormones[1]. Certain toxic chemicals and medicines can cause liver damage[2]. A large number of xenobiotics are reported to be potentially hepatotoxic. Some examples are acetaminophen, tetracycline, ethanol and carbon tetrachloride. Hepatotoxins may react with the basic cellular constituents like proteins, lipids, RNA and DNA and induce almost all types of lesions of the liver. Acetaminophen is commonly used antipyretics. Liver is among the organs most susceptible to the toxic effects of acetaminophen due to over dosage[3]. Medicinal plants play a key role in the human health care. About 80% of the world population rely on the use of traditional medicine which is predominantly based on plant materials. The traditional medicine refers to a broad range of ancient natural health care practices including tribal practices as well as Ayurveda, Siddha, Amchi and Unani[4]. One such plant is *Macrotyloma uniflorum* has been used in traditional system of medicine for treating haemorrhoids, tumours, bronchitis, cardiopathy, nephrolithiasis, urolithiasis, splenomegaly, strangury, hiccough, ophthalmopathy, verminosis, vitiated condition of *vata*, remove kidney stone, inflammation, liver trouble[5]. The hepatoprotective activity of plant of *Macrotyloma uniflorum* has been not reported. An attempt was made to investigate the hepatoprotective activity of *Macrotyloma uniflorum* in wistar albino rats.

2. Materials and methods

2.1 Animals: Albino wistar rats of either sex weighing between 150 to 200 gms were provided by Shri Dhanvantary pharmacy college, Kim. After randomization into various groups, animals were acclimatized for period of 10 days under standard husbandry conditions. The animals were housed under standard conditions of temperature (25 ± 30°C) and relative humidity 50 ± 20% with a 12:12 light-dark cycle as per CPCSEA guide line. All the animals were fed with rodent pellet diet and water was allowed *ad-libitum* under strict hygienic condition. Ethical clearance for

performing experiments on animals was obtained from Institutional Animal Ethics Committee (IAEC) number SDPC-AFC/2012/124.

2.2 Plant resources and preparation of crude drug extracts: The seed of *Macrotyloma uniflorum* were collected from Hakimchichi, Surat. The plant was Authentified at the Department of botany, The Maharaja Sayajirao University, Vadodara. A voucher specimen has been deposited at the museum of Shri Dhanvantry Pharmacy college, Kim. Herbarium No. SDPC/2011-12/10. The seeds were dried in direct sunlight. The dried seeds were powdered by using grinder, to coarse powder and were packed into Soxhlet column and extracted with 95% methanol for 24 hrs. The solvent was removed using rotatory flash evaporator. The extract was concentrated by using hot water bath (70 – 800). The dried extract was stored in airtight container.

2.3 Phytochemical studies: Preliminary phytochemical study was screened for methanolic extract of MEMUS for the presence of carbohydrates, glycosides, alkaloids, flavonoids, tannins[6].

2.4 Acute toxicity studies: Preclinical toxicity studies were carried our as per OECD guideline no. 425[7]. The methanolic extracts of MEMUS were studied for acute toxicity female albino rats. All the animals were observed for 24 hrs to 14 days for acute toxicity.

2.5 D- galacctosamine induced hepatotoxicity:

Experimental procedures: Albino rat of wistar strain (either sex) weighing 150 – 200 g were selected and divided into five groups of each containing six animals.

Group I: Control (Saline 5 ml/kg, p.o).
Group II: D-galactosamine (400mg/kg, i.p).
Group III: Standard (Silymarin 50 mg/kg, p.o).
Group IV: MEMUS (200 mg/kg, p.o).
GroupV: MEMUS (400 mg/kg, p.o).

Wistar albino rats of either sex, weighing 150-200 g, were maintained in standard environmental conditions and fed with standard laboratory diet and water *ad libitum* was used for the experiment. Group-I and Group-II were administered saline and Group-III was administered Silymarin (50mg/kg) for 10 days. Group-IV (200 mg/kg b.w) and Group-V (400 mg/kg) were administered MEMUS orally for 10 days at different dosage level. On 9th and 10th day single dose per day of D-galactosamine (400mg/kg, i.p) was administered in rats of all the Groups except control. After 24 hrs, blood was collected by retro orbital plexus under mild ether anesthesia. Blood samples were collected for evaluating the serum biochemical parameters (SGPT, SGOT, ALP, Total Bilirubin level and Direct Bilirubin level) and liver was dissected out, blotted off blood, washed with saline and stored in 10% formalin and preceded for histopathology to evaluate the details of hepatic architecture in each group microscopically[8].

2.6 Paracetamol induced hepatotoxicity:

Method: Albino rat of wistar strain (either sex) weighing 150 – 200 g were selected and divided into five groups of each containing six animals.

Group I: Control (Saline 5 ml/kg, b.w).
Group II: Paracetamol (2g/kg, p.o).
GroupIII: Standard (Silymarin 50 mg/kg, p.o).
Group IV: MEMUS (200 mg/kg, p.o).
GroupV: MEMUS (400 mg/kg, p.o).

Wistar albino rats of either sex, weighing 150-200 g, were maintained in standard environmental conditions and fed with standard laboratory diet and water *ad libitum* were used for the experiment. Group-I and Group-II were administered saline and Group-III was administered Silymarin (50mg/kg) for 7 days. Group-IV (200 mg/kg) and Group-V (400 mg/kg) were administered MEMUS orally for 7 days. On 7th day single dose of Paracetamol (2g/kg, p.o) was administered in rats of all the groups except control[9]. After 18 hrs blood was collected by retro orbital plexus under mild ether anesthesia. Blood samples were collected for evaluating the serum biochemical parameters (SGPT, SGOT, ALP, Total Bilirubin level and Direct Bilirubin level) and liver was dissected out, blotted off blood, washed with saline and stored in 10% formalin and preceded for histopathology to evaluate the details of hepatic architecture in each group microscopically[10].

2.7 Biochemical evaluation: Serum enzymes namely serum glutamate pyruvate transaminase (SGPT)[11], Serum glutamate oxaloacetate transaminase (SGOT)[12], serum alkaline phosphatase (ALP) and (total and direct) bilirubin[13] were assayed using kits (Span Diagnostic, Surat).

2.8 Histopathological studies: The liver tissue was dissected out and fixed in 10% formalin,

dehydrated in isopropyl alcohol of increasing strength (70%, 80% and 90%) for 12 hrs. Then the final dehydration is done using absolute alcohol with about three changes for 12 hrs. cleared in chloroform, and embedded in paraffin. Sections were prepared and then stained with hematoxylin and eosin (H–E) dye for photomicroscopic observation, including cell necrosis, fatty change, hyaline regeneration, ballooning degeneration[14].

2.9 Statistical analysis: The statistical analysis was performed using a software graphpad prism 5 significant difference among various groups were analysed using one way ANOVA, dunnet's test. The data were expressed as mean ± S.E.M. The differences among means were analyzed by one-way ANOVA. A value of $P < 0.001$ was considered as statistically significant.

3. Results

3.1 Phytochemical study: MEMUS subjected for phytochemical study showed the presence of alkaloids, carbohydrates, saponin glycosides, tannin and flavonoids.

3.2 Acute toxicity studies: No mortality or any gross behavioral changes were observed during and after the treatment of MEMUS for 24 hrs to till 14 days. The MEMUS was found to be safe up to dose of 2000 mg/kg body weight in wistar albino rats..

3.3 Effects of extracts on SGOT, SGPT, ALP and (total and direct) bilirubin

3.3.1 Effect of methanolic extract of *Macrotyloma uniflorum* seed on biochemical markers in Paracetamol induced hepatotoxicity: Rats subjected to the D-Galactosamine challenge alone (group II) developed significant liver injury as evident from a significant elevation in the biochemical markers, like SGPT, SGOT, ALP, TB and DB when compared with group I (Table No.1). Oral administration of the test extract exhibited significant reduction in the D-Galactosamine induced increase in the biochemical levels. However there were decrease in the levels of SGPT, SGOT, ALP, TB and DB were found with the 200 mg/kg and 400 mg/kg of the test extract. Treatment with the reference standard, silymarin (50 mg/kg p.o.) also reversed the hepatotoxicity significantly. Hepatoprotective potency of the test extract at the dose 400mg/kg was found closer to that of standard silymarin.

3.3.2 Effect of methanolic extract of *Macrotyloma uniflorum* seed on biochemical markers in Paracetamol induced hepatotoxicity: Rats subjected to the paracetamol challenge alone (group II) developed significant liver injury as evident from a significant elevation in the biochemical markers, like SGPT, SGOT, ALP, TB and DB when compared with group I (Table No.2). Oral administration of the test extract exhibited significant reduction in the paracetamol induced increase in the biochemical levels. However there was decrease in the levels of SGPT, SGOT, ALP, TB & DB were found with the 200 mg/kg and 400 mg/kg of the test extract. Treatment with the reference standard, silymarin (50 mg/kg p.o.) also reversed the hepatotoxicity significantly. Hepatoprotective potency of the test extract at the dose 400 mg/kg was found closer to that of standard silymarin.

3.4 Histopathological observations

3.4.1D-Galactosamine induced hepatotoxicity in wistar albino rats: Histopathological examination of liver section of normal rats showed normal hepatic cells with cytoplasm and nucleus whereas D-Galactosamine treated group showed various degree of fatty degeneration like ballooning of hepatocytes, infiltration of lymphocytes and the loss of cellular boundaries. Administration of MEMUS at higher doses (400 mg/kg, p.o.) normalized these defects significantly in the histological architecture of the liver (Figure 1).

3.4.2 Paracetamol induced hepatotoxicity: Histopathological examination of liver section of normal rats showed normal hepatic cells with cytoplasm and nucleus whereas paracetamol treated group showed various degree of fatty degeneration like ballooning of hepatocytes, infiltration of lymphocytes and the loss of cellular boundaries. Administration of MEMUS at higher doses (400 mg/kg, p.o.) normalized these defects significantly in the histological architecture of the liver (Figure 1).

4. Discussion and conclusion

In present study of MEMUS showed remarkable hepatoprotactive activity in wistar albino rats. As review of literature this *Macrotyloma uniflrum* used in treatment of different liver disorders and the hepatoprotactive activity of *Macrotyloma uniflorum* has not reported. So this hepatoprotactive activity of MEMUS in wistar

albino rats was investigated in present study. D-galactosamine induced hepatitis is a useful model to study hepatic injury. It has been shown to produce liver damage resembling human viral hepatitis[15]. D-galactosamine induces a decrease in liver uracil nucleotides which causes a rapid inhibition of both RNA and protein synthesis[15].

In the D-galactosamine induced acute hepatotoxic model pretreatment with methanolic extract of *Macrotyloma uniflorum* offered hepatoprotection as evidenced by the inhibition of the increase in SGOT, SGPT, ALP, Total Bilirubin and Direct bilirubin levels. In addition, the absence of necrotic lesions in liver samples of the extract-treated group, suggests that the hepatoprotective action may be due to membrane stabilizing effects in hepatic cells[16].

Paracetamol is a common analgesic and antipyretic drug. Several studies have demonstrated the induction of hepatocellular damage or necrosis by acetaminophen higher doses in experimental animals and humans. For screening of hepatoprotective agents, Paracetamol-induced hepatotoxicity has been used as a reliable method. Paracetamol is metabolized primarily in the liver and eliminated by conjugation with sulfate and glucuronide, and then excreted by the kidney[17]. Moreover, Paracetamol hepatotoxicity has been attributed to the formation of toxic metabolites, when a part of Paracetamol is activated by hepatic cytochrome P-450 to a highly reactive metabolite N-acetyl-p-benzoquinoneimine (NAPQI). Toxic metabolites (N-acetyl-p-benzoquineimine) can alkylate and oxidise intracellular GSH, which results in liver GSH depletion subsequently leads to increased lipid peroxidation by abstracting hydrogen from a polyunsaturated fatty acid and ultimately, liver damage due to higher doses of Paracetamol[18]. A number of scientific reports indicated that certain flavonoids, triterpenoids and steroids,tannins have protective effect on liver due to its antioxidant properties[19].

Active compound flavanoids and tannins from *Macrotyloma uniflorum* might play key role in hepatoprotective activity. The hepatotoxic property was assessed by D-galactosamine and Paracetamol induced liver injury in wistar albino rats. The results of the present investigation indicated that methanolic extracts of *Macrotyloma uniflorum* seed has significant hepatoprotactive properties. Thus support the folklore use of the title plant in liver disorders.

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Table No.1 Effects of methanolic extract of *Macrotyloma uniflorum* seed on biochemical and morphological parameters in D- Galactosamine induced epatotoxicity.

Groups	Liver weight in gm	SGPT IU/l	SGOT IU/l	ALP IU/l	TB mg/dl	DB mg/dl
Group I	6.86± 0.130	40.83± 1.222	43.17± 1.515	94.67± 4.169	0.86± 0.021	0.22± 0.012
Group II	9.28± 0.090	194.0±8.817	106.7± 4.177	174.8± 14.28	2.38± 0.233	1.56± 0.192
Group III	7.083± 0.060	137.7± 6.771***	74.33± 3.169***	97.33± 5.296***	1.63± 0.1333**	0.43± 0.180***
Group IV	8.55± 0.117	150.8±7.125***	73.33± 1.909***	109.7± 4.602***	1.66± 0.102**	0.28± 0.144***
Group V	8.10± 0.106	146.7± 3.095***	57.67± 1.667***	108.8± 7.499***	0.88± 0.101***	0.25± 0.034***

Values are mean ± SEM (n = 6). * p < 0.05, ** p < 0.01, *** p < 0.001 as compared to positive control. One way ANOVA followed by Dunnett.'s multiple comparison test.

Table no.2 Effects of methanolic extract of *Macrotyloma uniflorum* seed on biochemical and morphological parameters in paracetamol induced hepatotoxicity.

Groups	Liver weight in gm	SGPT IU/l	SGOT IU/l	ALP IU/l	TB mg/dl	DB mg/dl
Group I	6.83± 0.147	40.83± 1.222	43.17± 1.515	94.67± 4.169	0.86± 0.021	0.22± 0.012
Group II	8.95± 0.07	76.50± 1.668	72.50± 1.979	151.50± 5.993	1.70± 0.115	0.90± 0.073
Group III	6.95± 0.076	52.33± 1.542***	48.50± 2.825***	82.83± 5.003***	1.21± 0.054**	0.23± 0.021***
Group IV	7.61± 0.065	67.83± 2.040***	55.83± 1.078***	126.5± 2.232**	1.41± 0.047*	0.25± 0.022***
Group V	7.20± 0.057	54.33± 2.333***	49.80± 1.384***	99.83± 1.740***	1.25± 0.076**	0.21± 0.016***

Values are mean ± SEM (n = 6). * p < 0.05, ** p < 0.01, *** p < 0.001 as compared to positive. One way ANOVA followed by Dunnett.'s multiple comparison tests.

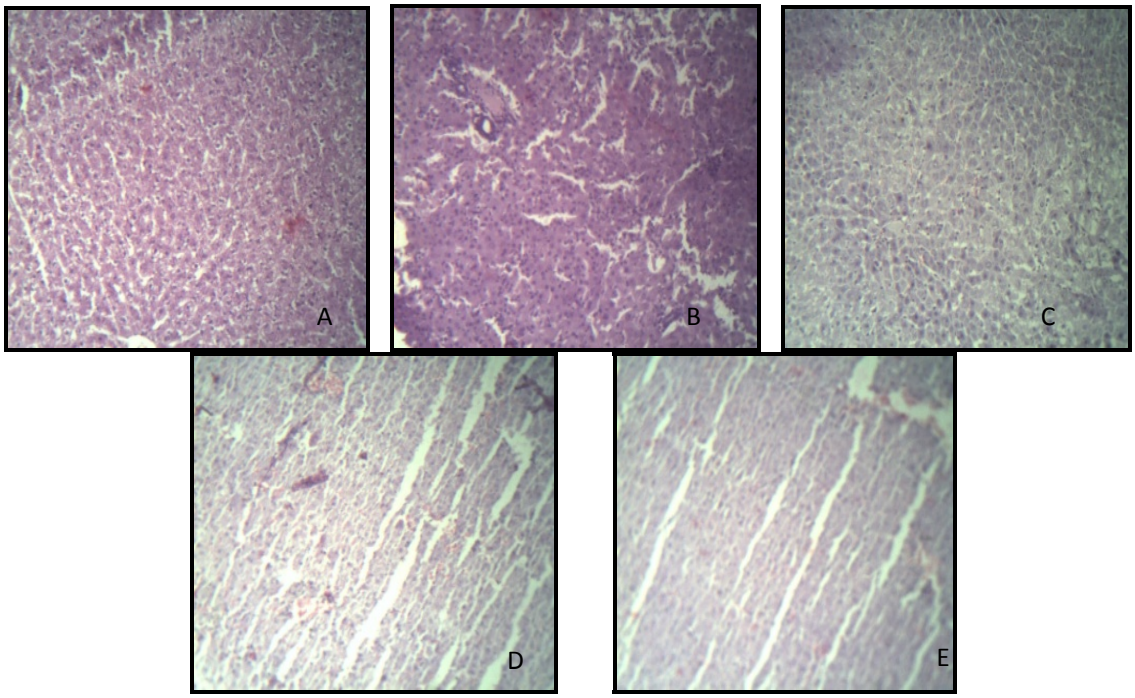

Figure 1: Photograph of liver architecture in D-Galactosamine induced hepatotoxicity in rat, **Fig. No. A** (Liver architecture of Normal), Fig. No. B **(**Liver architecture of D-Galactosamine treatment), Fig. No. C (Liver architecture D-Galactosamine treatment + 50 mg/kg Silymarin treatment), Fig. No. D (Liver architecture D-Galactosamine treatment + 200 mg/kg of MEMUS), Fig. No. E (Liver architecture D-Galactosamine treatment + 400 mg/kg of MEMUS).

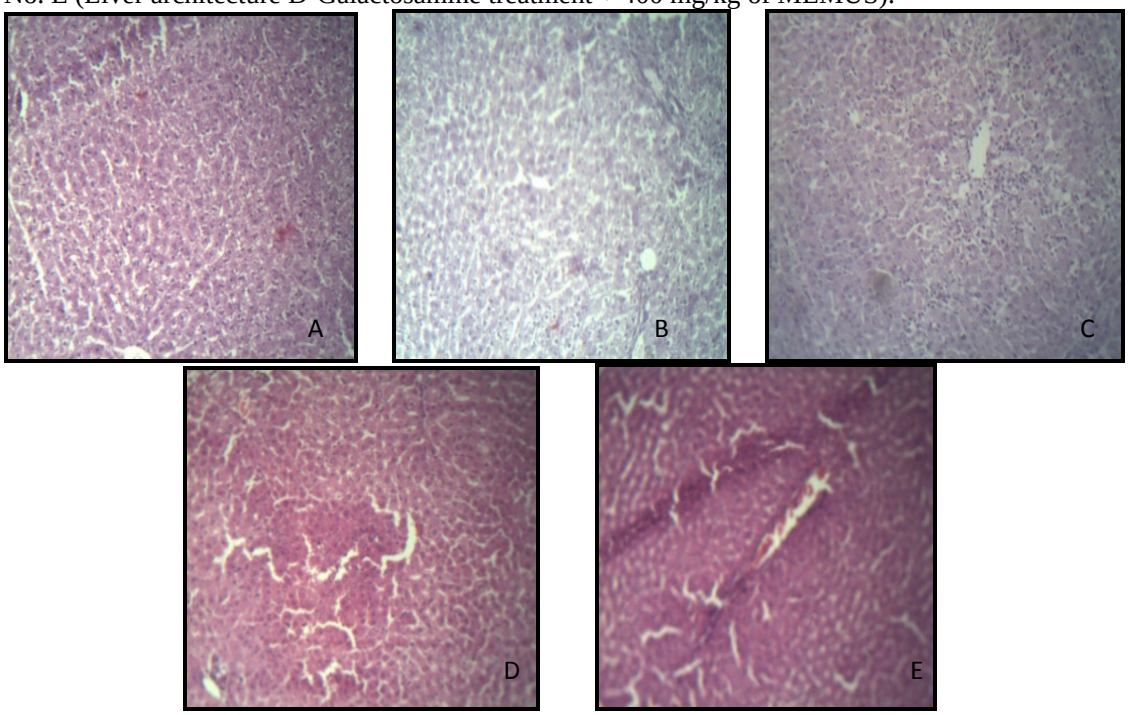

Figure 2: Photograph of liver architecture in paracetamol induced hepatotoxicity in rat,
Fig. No. A (Liver architecture of Normal), Fig. No. B **(**Liver architecture of paracetamol treatment), Fig. No. C (Liver architecture paracetamol treatment + 50 mg/kg Silymarin treatment), Fig. No. D (Liver architecture paracetamol treatment + 200 mg/kg of MEMUS), Fig. No. E (Liver architecture paracetamol treatment + 400 mg/kg of MEMUS).