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Hepatoprotective activity of herbadict tablets on alcohol-induced mortality and liver lesions in mice

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Abstract
Objective of the current research is to study the possible protective effects of Herbadict tablet a Polyherbal Ayurvedic product against alcohol-induced mortality and liver lesions in mice. Healthy Female Swiss albino mice were divided into six groups containing 8 animals each. Herbadict tablet was suspended in 0.5% carboxy methyl cellulose (CMC) and administered at two doses i.e.30mg/kg and 100 mg/kg body weight. Vehicle control, positive control and reference control (Silymarin) were used for comparison. Alcoholic intoxication was induced using ethanol. Treatment with Herbadict significantly (p<0.01) decreased SGOT, SGPT and γ-GT at 100 mg/kg when compared to the vehicle treated alcohol intoxicated group. A dose dependant significant decrease in total bilirubin was observed at 30 and 100 mg/kg of Herbadict when compared to vehicle treated alcohol intoxicated group. Treatment with Herbadict at 30 and 100 mg/kg *p.o.* showed hepatoprotective effect against alcohol induced damage in mice model

Keywords: Herbadict, ayurveda, alcoholism, liver disease

1. Introduction
Addiction- like alcohoilism, drugs, tobacco chewing and smoking are psychosomatic and social problem throughout the world[1]. Alcohol consumption is responsible for 3.8% of global mortality and 4.6% of disability-adjusted life-years (DALYs) lost due to premature death. Alcoholic liver disease, a direct result of chronic alcohol abuse, insidiously destroys the normal functions of the liver. The end result of the disease, cirrhosis, culminates in a dysfunctional and diffusely scarred liver. Cirrhosis is the final phase of alcoholic liver disease. Alcoholic liver disease (ALD) is the most prevalent cause of advanced liver disease in Europe. In the United States, approximately 100 000 deaths are attributed to alcohol abuse each year. In 2009, the World Health Organization listed alcohol use as one of the leading causes of the global burden of disease and injury.
Hence, it is essential to have a safe and effective drug to treat addiction like alcoholism. Herbadict an Ayurvedic polyherbal tablet designed and developed by Muniyal Ayurveda Research Centre is safe (safety is established by the toxicity study conducted as per the OECD guidelines) and prepared out of natural ingredients having hepatoprotective activities.

2. Objectives: To study the possible protective effects of Herbadict tablets against alcohol-induced mortality and liver lesions in mice

3. Materials and Methods [2, 3]
3.1 Test item: Herbadict is a polyherbomineral tablet manufactured by Muniyal Ayurveda Research centre Manipal as per the GMP guidelines following strict quality control procedures. It is licensed under AYUSH, Government of Karnataka. Composition and quality standards are given in Table1 and Table 2 respectively.

Table 1: Composition of Herbadict Tablet Each 500 mg tablet contains

S. No	Sanskrit Name	Part used	Latin/English name	Quantity
1.	Bhumyamalaki	Dried whole plant	*Phyllanthus niruri*	40 mg
2.	Guduchi	dry stem	*Tinospora cordifolia*	40 mg
3.	Bhringaraja	Dried whole plant	*Eclipta alba*	35 mg
4.	Punarnava	Dried root	*Boerhavia diffusa*	30 mg
5.	Shatavari	dry roots	*Asparagus racemosa*	30 mg
6.	Katuki	Dry root	*Picrorhiza kurroa*	25 mg
7.	Shilajatu	Mineral	*Asphaltum punjabicanum*	25 mg
8.	Vidanga	Dried fruit	*Embelia ribes*	20 mg
9.	Nagabala	Dried root	*Grewia tenax*	20 mg
10.	Ashvagandha	Dried root	*Withania somnifera*	20 mg
11.	Haridra	Dried rhizome	*Curcuma longa*	20 mg
12.	Chitraka	Dried root	*Plumbago zeylanica*	20 mg
13.	Muktasukti bhasma	Mineral	*Incinerated Pearle oyster*	20 mg
14.	Mandoora bhasma	Mineral	*Incinerated Iron*	20 mg
15.	Khadira	Dried heartwood	*Acacia catechu*	10 mg
16.	Amalaki	Dried fruit	*Emblica officinalis*	10 mg
15.	Hareetaki	Dried fruit	*Terminalia chebula*	10 mg
16.	Pippali	Dried fruit	*Piper longum*	10 mg
17.	Maricha	Dried fruit	*Piper nigrum*	10 mg
18.	Shunthi	Dried rhizome	*Zingiber officinale*	10 mg
19.	Arjuna	Dried stem bark	*Terminalia arjuna*	10 mg
20.	Kirata tikta	Dried whole plant	*Swertia chirata*	10 mg
21.	Rajata Bhasma	Incinerated metal	*Argenti oxidum*	5 mg
22.	Excipient	Gum	Gum acacia	50 mg

Table 2: Quality testing report of Herbadict Tablet

Test Parameters	Specifications
Description	Dark brown colored biconvex discs
Identification	Positive for Iron, Calcium, silver
Average weight	500 mg ± 12.5 mg
Uniformity of weight	± 2.5% of actual average weight
Tablet hardness	4.3 kg/cm^2
Loss on drying	3.68% w/w
Methanol soluble extractive	28.81% w/v
Chloroform soluble extractive	7.0% w/v
Ash value	17.44% w/w
Average Disintegration time	18 minutes
ASSAY	Each tablet contains, Iron –2.84 mg, Calcium –7.76 mg, and silver-2.42 mg. TLC of alcoholic extract of the drug on silica gel 'G' plate using Toluene: Ethyl acetate(9:1) shows under U.V. light(366 nm), eight spots a Rf. 0.04(yellow), 0.12(light green), 0.25(green), 0.31(light green),0.36(light green), 0.53(light green), 0.65(green) and 0.97(blue)

3.2. Ethical clearance: Approval was taken from Institutional Animal Ethical Committee before commencing the study (IAEC approval number: 219/2017 and study no.206/2018)

3.3. Acclimatization and Grouping: Female Swiss albino mice aged between 9 to 10 weeks were acclimatized for six days under laboratory conditions. Randomization was performed on last day of acclimatization. Mice were maintained at ideal macro and micro environment, standard laboratory diet and reverse osmosis water ad libitum. Healthy mice were selected and grouped based on the stratified body weight. (Test system source: CPCSEA Reg No: 971/bc/06/CPCSEA). Animals were grouped as under: Animals were divided into six groups containing 8 animals each as shown in table 3.

Table 3: Grouping of test animals

S. No.	Group	Treatment	No. of animals / group
I	Vehicle control	0.5% CMC (10 ml/kg, *p.o*)	8
II	Positive control	Alcohol in drinking water + 0.5% CMC(10ml/kg, *p.*o)	8
III	Reference control	Alcohol in drinking water + Silymarin (100 g/kg, *p.*o)	8
IV	Test item	Alcohol in drinking water + Herbadict (30 mg/kg, *p.o*)	8
V	Test item	Alcohol in drinking water + Herbadict (100 mg/kg, *p.o*)	8
VI	Test item control	Herbadict (100 mg/kg, *p.o*)	8

3.4. Dose formulation: Test item was suspended in 0.5% carboxy methyl cellulose (CMC). Test item was freshly prepared in vehicle prior to dosing. Test item was weighed, transferred to mortar and grinded with pestle. A small quantity of the vehicle was added to test item and triturated. This was transferred to a measuring cylinder. A small quantity of the vehicle was added to motor again, triturated and transferred to the measuring cylinder. Sufficient quantity of vehicle was added to make up the required volume of formulation. The formulation prepared was then transferred to

motor and again triturated. Following trituration, the formulation was transferred to a labelled beaker for dosing. The dose volume of the formulation was 10 ml/kg body weight.

3.5. Induction of hepatotoxicity

Alcoholic intoxication was induced using ethanol. Concentration of ethanol was progressively increased in the drinking water viz 10% (v/v) alcohol in the first week, 20% in the second, 30% in the third, and 40% in the fourth week. Animals were treated with vehicle or test item simultaneously (10.00 am to 11.00 am) every for 28 days. On day 29, the animals were fasted for 4 h and blood samples were collected for biochemical analysis. The animals were then euthanized (ketamine 100 mg/kg, i.p) and liver sample were harvested for histopathological evaluation.

3.6. Parameters

3.6.1. Liver function test

Plasma Biochemistry - ALT, AST, ALP, γ-GT and total bilirubin using diagnostic kit (Spinreact, Spain) in Semiautomatic biochemical analyzer (Labmate, India).

3.6.2 Histopathology: Liver collected from mice were fixed in 10% neutral buffered formalin solution, dehydrated in graded alcohol and embedded in paraffin. Paraffin sections of 3-5 micron were mounted on glass slides and counter-stained with Hematoxylin and Eosin (H&E) for light microscopic analyses.

3.7. Data analysis; Data were expressed as mean ± SEM. Mean difference between the groups were analysed by One way anova followed by Tukey's multiple comparison test as post hoc. P value ≤ 0.05 was considered as statistically significant.

4. Results and Discussion

4.1. Plasma Biochemistry (Figures 1-5)-Liver function test: Alcohol intoxicated mice showed significant (p<0.01) increase in SGOT, SGPT, ALP, γ-GT and total bilirubin levels when compared to vehicle control mice. Treatment with Herbadict significantly (p<0.01) decreased SGOT, SGPT and γ-GT at 100 mg/kg when compared to the vehicle treated alcohol intoxicated group. A significant (p<0.01) decrease in ALP was recorded at both 30 and 100 mg/kg doses of Herbadict when compared to the vehicle treated alcohol intoxicated group. A dose dependant significant decrease in total bilirubin was observed at 30 and 100 mg/kg of Herbadict when compared to vehicle treated alcohol intoxicated group. Effect of Herbadict was comparable with that of the reference drug, Silymarin.

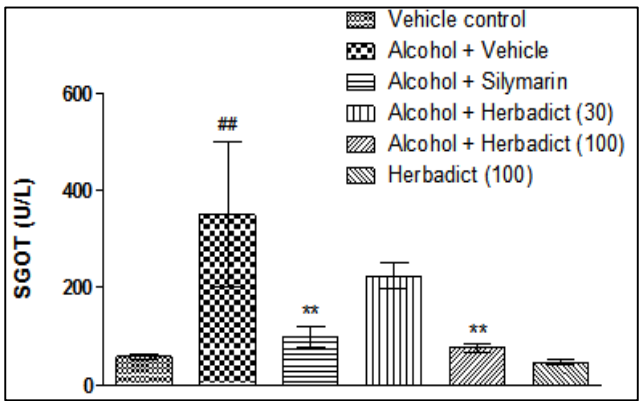

Fig 1: Effect of HERBADICT on plasma SGOT activity in alcohol intoxicated mice

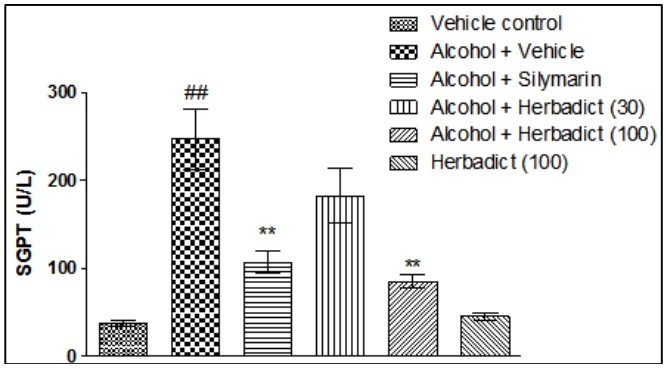

Fig 2: Effect of HERBADICT on plasma SGPT activity in alcohol intoxicated mice

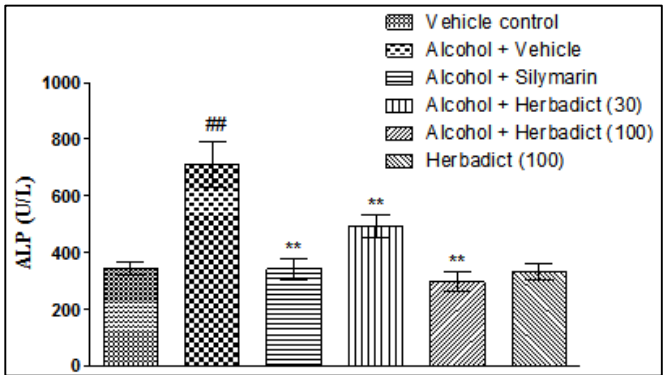

Fig 3: Effect of HERBADICT on plasma alkaline phosphatase (ALP) activity in alcohol intoxicated mice

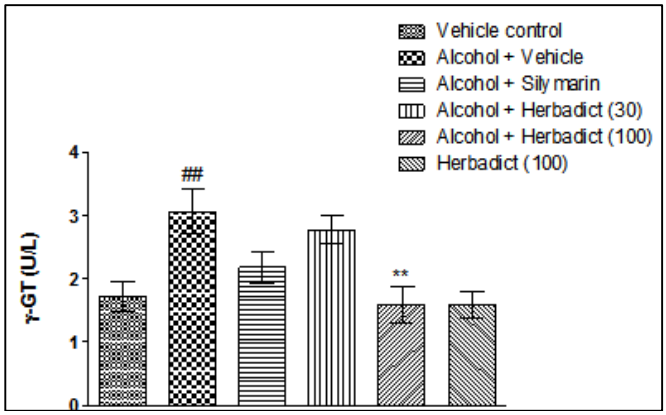

Fig 4: Effect of HERBADICT on plasma γ-GT activity in alcohol intoxicated mice

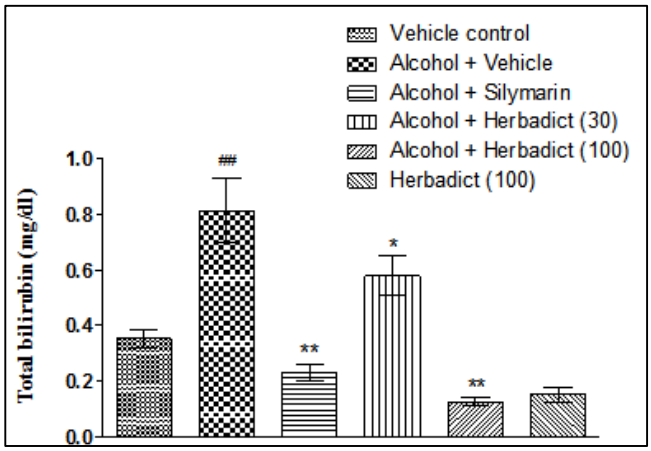

Fig 5: Effect of HERBADICT on total bilirubin content in alcohol intoxicated mice

4.2. Histopathology (Figure 6)

- **Group I: Vehicle control:** Liver section showed normal architecture with central vein, hepatocytes radiating from the central veins and the portal triads.
- **Group II: Positive Control:** Liver sections revealed moderate to severe degree of hepatocellular vacuolations multifocal areas of ballooning degeneration of the hepatocytes. Few hepatocytes showed granular cytoplasm. Dilatation of sinusoidal spaces containing erythrocytes was also noticed. Focal areas of perivascular lymphocytic infiltration and centrilobular necrosis
- **Group III: Reference control:** Liver sections revealed moderate degree of vacuolations within the hepatocytes, mild degree of ballooning degeneration in hepatocytes and mononuclear cells infiltration in the necrotized area.
- **Group IV:** Treatment with Herbadict at 30 mg/kg p.o. Liver sections revealed mild degree of hepatocellular vacuolations, minimal degree of ballooning degeneration, absence of sinusoidal dilatation and necrosis. Focal area of restoration of normal parenchyma was evident.
- **Group V: Pre-treatment with Herbadict at 100 mg/kg p.o.:** Liver sections revealed moderate degree of vacuolations and ballooning degeneration of hepatocytes, focal areas of perivascular infiltration and centrilobular necrosis.
- **Group VI: Pre-treatment with Livokot at 300 mg/kg p.o.:** Liver lesions were similar to the positive control.

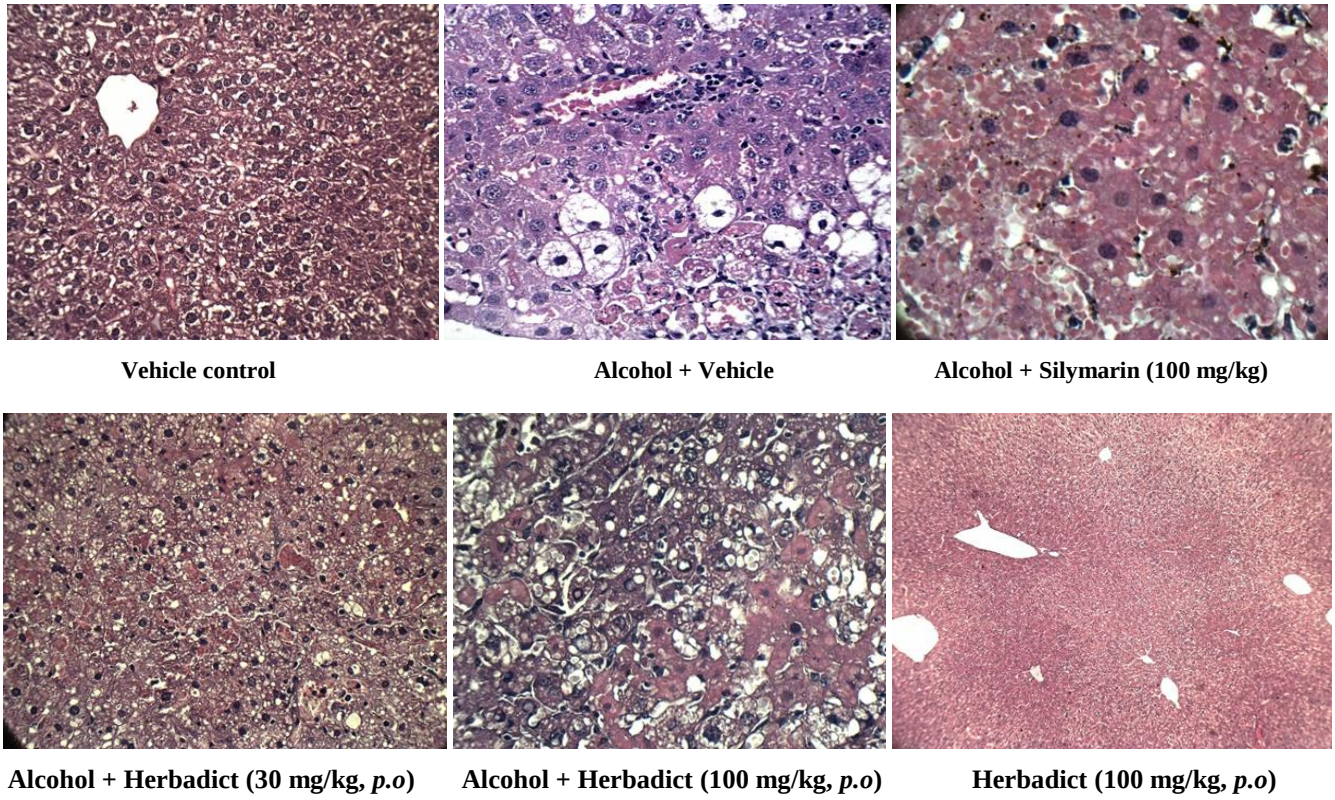

Fig 6: Representative Photo Graphs - Effect of HERBADICT against alcohol induced hepatic changes in mice

Test product contains the herbs having hepatoprotective and antioxidant activities. *In vivo* hepatoprotective effect of aqueous extract of *Phyllanthus niruri* on nimesulide-induced oxidative stress is established[4]. Vinay et.al, in their work have established the Hepatoprotective activity of combination of Phyllanthus niruri and Curcuma longa extracts against ethanol induced toxicity in wistar rats[5]. Harish R and Shivanandappa T. have proved antioxidant activity and hepatoprotective potential of phyllanthus niruri[6]. Picroliv active constituent of Picrorhiza kurrooa[7], is found to have protective activity against oxytetracycline induced hepatic damage. Rawat A.K. et.al. In their study have established hepatoprotective activity of Boerhaavia diffusa L. roots[8]. Hepatoprotective activity of Eclipta alba hassk against paracetamol induced hepatocellular damage[9] and carbon tetrachloride induced acute liver damage[10] are reported. Aqueous extract of Tinospora cordifolia has shown encouraging hepatoprotective activity[11]. Summative effect of these multiple ingredients might have contributed to the hepatoprotective activity of the product.

5. Conclusion

Treatment with Herbadict Tablet at 30 and 100 mg/kg *p.o.* showed hepatoprotective effect against alcohol induced damage in mice model. This indicates that Herbadict tablet possesses hepatoprotective activity against alcohol induced liver damage.

6. Acknowledgement

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