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Development and Evaluation of Hydroalcoholic Extract of Alhagi Camelorum as Hepatoprotective Agent in NDDS

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Abstract: Despite use of Alhagi camelorum extract against liver disorders, the clinical use of it is limited due to their poor solubility and instability. Moreover, the available therapies are facing the challenges of efficacy and safety. Hence, Alhagi camelorum extract loaded nanoemulsion has been formulated to improve its hepatoprotective activity. The animals were segregated in 6 groups (6 male rats each) weighing 250–290 g. Group I animals were treated with normal saline, Group II animals were treated with paracetamol at the dose of 2g/kg while Group III were treated with Alhagi camelorum hydroalcoholic extract at the dose of 400mg/kg, Group IV and V were the test formulation and were treated at the dose equivalent to 400mg/kg and half dose or equivalent dose to 200mg/kg. Group VI was treated with standard silymarin at the dose of 25 mg/kg for seven consecutive days, respectively. On 5th day 30 minutes after respective treatments, paracetamol 2g/kg orally was administered. After seven consecutive days, blood serum samples and liver tissues were collected for biochemical and histopathological analysis. Phytochemical screening was carried out to screen for phytochemical classes and HPLC analysis was conducted to screen polyphenols. The administration of AC extract showed hepatoprotection at the doses of 400 mg/kg. However, nanoemulsion loaded AC (dose 400 mg/kg) significantly reduces the elevated serum levels of liver biomarkers compared to the paracetamol- induced hepatotoxic group. These findings were confirmed with histopathological changes where nanoemulsion was capable of reversing the toxic effects of paracetamol acid on liver cells.

Keywords- Alhagi Camelorum; Novel Drug Delivery System; Nanoemulsion; Hepatoprotection; Particle Size

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INTRODUCTION: The advances in combinatorial chemistry and screening strategy resulted in new chemical compounds with higher molecular weight and enhanced lipid solubility [1-2]. Thus, the successful formulation of most of the newly discovered drugs has become a challenge because of their inherent low aqueous solubility that generally leads to low oral availability, increased variation between and within subjects, and lack of dose-response proportional relationship [3-4]. Recently, lipid-based formulations have been evolving as an effective approach for enhancing oral bioavailability of lipophilic drugs. This approach comprises loading of drugs into lipid carriers such as surfactant or oils dispersions [5], liposomes [6], solid lipid nanoparticles, lipid nanocarriers [7] micro- and nanoemulsion [8-9]. Liver plays a vital role in our body by regulating metabolic conversion inside our body. It is involved in almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction. More than 900 drugs have been implicated in causing liver injury and it is the most common reason for a drug to be withdrawn from the market [10-12]. More than 75 percent of cases of idiosyncratic drug reactions result in liver transplantation or death. One of the major causes suggested for hepatotoxicity of drugs is presence of oxidative stress in liver. Medicinal plants are considered to very good source of many bioactive components that are very good antioxidants. Even crude extracts were found to provide synergistic approach to fight against various oxidative stresses. One of such reported medicinal plants is *Alhagi camelorum* (AC) which has been observed to show hepatoprotection. It's histopathological and biochemical analysis validated the hepatoprotection. However, extracts when taken orally some constituents may be destroyed in the gastric environment. As standardized extracts are established, poor bioavailability often limits their clinical utility due to above said reasons. It has been observed that complexation with certain other clinically useful nutrients substantially improves the bioavailability of such extracts and their individual constituents. Hence, phytotherapeutics needs the scientific approach to deliver the components in a sustained manner, which can be achieved by designing novel drug delivery systems for herbal extracts. [13-14]

During the past few decades, natural compounds have been considered as one of the promising therapeutic agents against cancer, cardiovascular diseases, aging, diabetes, and especially neurodegenerative disorders due to their wide variety of modes of action, efficiency, accuracy, and fewer side effects [15-16]. *Alhagi camelorum* (AC) belongs to the family Leguminosae and is a traditional herb used to treat metabolism, digestive and hepatic problems, autoimmune diseases, headaches, and infections. AC was used to treat stomach disorders, heartworm prevention, and temperature. The herb is regarded as a laxative, diuretic and purgative, and antipyretic. Proteins, glycosides, coumarins, flavonoids, phenolic, resin, saponins, steroids, terpenes, ascorbic acid, essential oils, salicylic acid, ascorbic acid, and gallic acid are the plenteous phytonutrients found in AC. [17]

The nanoemulsion approach is a versatile technique that has been utilized to increase the solubility of poorly aqueous-soluble drug molecules for better oral bioavailability and therapeutic activity [18-19]. In the present investigation, an attempt has been made to develop an optimal stable nanoemulsion formulation of *Alhagi camelorum* containing sefsol-218 as an oily phase, Tween 80 as a

surfactant, ethanol as a co-surfactant and double distilled water as an aqueous phase. The hepatoprotective activity of the developed and optimized stable nanoemulsion formulation was compared with extract of AC (42 mg/kg body weight) [20] against paracetamol (PCM) induced hepatotoxicity in Wistar albino rats. Biochemical examinations of serum and liver tissue, as well as histopathological studies were carried out to evaluate the hepatoprotective activity of nanoemulsion formulation of *Alhagi camelorum*.

MATERIALS AND METHODS

Materials

Alhagi camelorum were procured from local market. Chitosan (medium molecular weight) and alginate were also procured from Sigma Aldrich (New Delhi, India). Tween 80 and calcium chloride were purchased from CDH Laboratory Limited (New Delhi, India). Acetic acid was purchased from Thomas Baker Pvt. Ltd (Mumbai, India). Double distilled millipore water was used for formulation and evaluation. All the chemicals and reagents were of analytical grade and were used as received.

Preparation of *Alhagi camelorum extract* loaded Nanoemulsion *via.* ultrasonication

The multilayer nanoemulsion was prepared in two steps with method described elsewhere with a slight modification [4]. Firstly, AC extract (250 mg) was dissolved in sufficient amount of ethanol (oil phase) and then mixed with tween 80 in proportion 1:2 of oil phase. The oil phase-surfactant mixture was then added to 0.7% alginate solution (pH 4.9, 117.5 mL) and gently mixed using a stirrer for 30 min at 40°C. To produce an alginate pre-gel, 18 mM (1.5 %, 7.5 mL) calcium chloride solution was added drop wise for 60 min under gentle stirring into a beaker to form alginate modified NE. Subsequently, 0.7% chitosan in 1% acetic acid (pH 4.6, 25 mL) solution was added drop wise to alginate modified NE over 90 min. A colloidal dispersion formed on addition of polycation chitosan. The formed NE was sonicated to control particle size. The ultra-sonication vibrations could be fixed with the amplitude of 30% with each cycle consisted of 30s on/off pulse.

Pharmacological activity

Animals

Male Wistar Albino rats weighing 250–290 g was obtained from the animal house of School of Pharmacy, Monad University, Hapur. The animals were kept in polycarbonated cages covered by raw dust and changed after three days under standard laboratory conditions (27 ± 2 ◦C) 12 h day and night cycle. The rats were fed with water and standard diet pallets ad libitum. The experiments were performed and approved by Institution Animal Ethical Committee of School of Pharmacy, Monad University, Hapur.

Paracetamol-Induced Acute Hepatotoxicity in Rats

Albino rats of either sex weighing between 150 – 200 g were randomly assigned into 6 groups of 6 animals each. Group-I (Negative control) received 1ml/kg normal saline, Group-II (positive control), Group- III (extract 400 mg/kg), Group IV (formulation 200 mg/kg) Group-V (formulation 400 mg/kg) and Group- VI (standard silymarin 25 mg/kg) were treated with respective treatments for 7 days, p.o. On 5th day 30 minutes after respective treatments, paracetamol 2g/kg orally was administered. After 48 hours of paracetamol challenge, blood samples were collected under

mild ether anesthesia; later the animals were sacrificed and liver tissues were collected. The blood samples were analyzed for biochemical markers of hepatic injury and liver samples were subjected for study of histopathology [21-24].

Biochemical studies

The blood samples were drawn from all the animals by puncturing retro-orbital plexus on 7th day of the treatment. The blood samples were centrifuged immediately to get clear serum and subjected for estimation of various biochemical parameters namely SGPT (serum glutamic pyruvic transaminase), SGOT (serum glutamic oxaloacetic transaminase), ALP (alkaline phosphatase) and serum bilirubin (total and direct). The liver was dissected out for estimation of liver antioxidant profile (LPO, SOD, GSH and CAT). The liver tissues of the all groups of rat were immersed in 10% formaline to study the histopathological changes using haematoxylin–eosin staining [25-27].

Results

Effect on liver function test: Serum liver function test parameters like SGOT, SGPT, ALP and bilirubin were found to be increased in paracetamol induced hepatotoxicity group in rats which were significantly decreased (*P<0.05,**P<0.01,* **P<0.001) in rats on pretreatment with extract (400 mg/kg) and formulation (200 and 400 mg/kg) and silymarin (25 mg/kg).

S. No	Treatment and Dose	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	Bilirubin (U/L)
1	Normal Control	61.83 ± 4.87	35.18 ± 2.96	77.23 ± 6.97	0.244 ± 0.04
2	Paracetamol Control 2 g/kg, po	189.93 ± 11.60	110.63 ± 8.75	191.3 ± 14.8	0.699 ± 0.02
3	Extract 400 mg/kg	101.25 ± 9.74*	76.49 ± 6.14*	111.86 ± 10.97*	0.379 ± 0.03*
4	Formulation 200 mg/kg	90.37 ± 8.73*	78.47 ± 5.79*	106.74 ± 8.34*	0.311 ± 0.02*
5	Formulation 400 mg/kg	82.69 ± 6.85**	56.70 ± 5.97**	88.65 ± 6.42**	0.281 ±0.02**
6	Silymarin 25 mg/kg	67.84± 5.94***	41.26 ± 3.87***	79.1 ± 5.14***	0.25 ± 0.04***

Values are mean ± SEM of 6 animals in each group. Group II compared with group-I, Group III-VI is compared with Group II (ONE WAY ANOVA) *P<0.05,**P<0.01,* **P<0.001

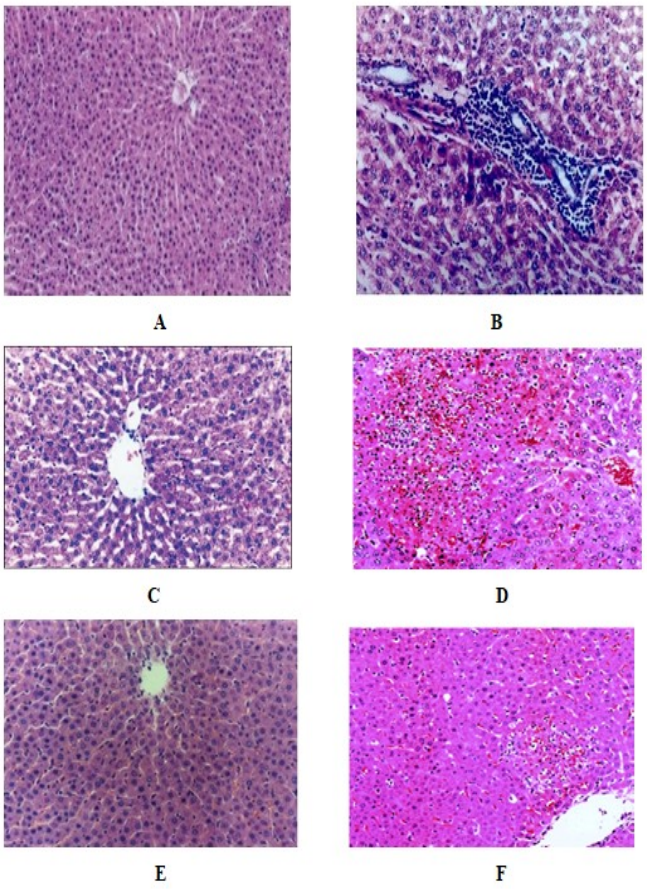
Effect of extract and formulation on antioxidant profiles in rats

Other biochemical parameters like LPO, SOD, GSH and Catalase altered levels to be found in paracetamol induced hepatotoxicity model in rats. Increased level of MDA was significantly reduced (*P<0.05, **P<0.01, ***P<0.00) on pretreatment with extract (200 mg/kg), formulation (200 and 400 mg/kg) and silymarin (25 mg/kg), whereas decreased of SOD, GSH and Catalase were increased on pretreatment with previous treatment.

S. No	Treatment and Dose	LPO (nmol/mg)	SOD (U/gm)	GSH (mmol/ g)	Catalyse (U/L)
1	Normal Control	15.633 ± 0.2	5.810 ± 0.27	10.28 ± 1.84	40.5 ± 2.8
2	Paracetamol Control 2 g/kg, po	28.34 ± 1.4***	3.204 ± 3.2***	3.89 ± 0.375***	26.9 ± 2.18***
3	Extract 400 mg/kg	20.84 ± 3.6*	3.91 ± 0.2*	7.55 ± 0.47*	34.12 ± 1.05*
4	Formulation 200 mg/kg	19.16 ± 1.8*	4.36 ± 0.2*	7.96 ± 0.53*	35.81 ± 1.21*
5	Formulation 400 mg/kg	17.94 ± 1.4**	4.93 ± 0.3**	8.96 ± 0.93**	36.71 ± 1.11**
5	Silymarin 25 mg/kg	16.74 ± 1.2***	5.170 ± 0.5***	9.17 ± 0.57***	39.8 ± 1.89***

Values are mean ± SEM of 6 animals in each group. Group II compared with group-I, Group II-VI is compared with Group II (ONE WAY ANOVA) *P<0.05,**P<0.01,* **P<0.001

Histopathological Studies



(A) Liver of control rats presenting normal histology **B**) Paracetamol (2g/kg, p.o.) treated rats presenting severe fatty erosion and vacuolar degeneration of hepatocytes **C)** Liver of rats treated with 400mg/kg of extract and paracetamol suspension (2g/kg, p.o.) presenting mild fatty erosion and vacuolar degeneration of hepatocytes **D)** Liver of rats treated with 200mg/kg of formulation and paracetamol suspension (2g/kg, p.o.) showing mild congestion in central vein and abridged necrosis. **E**) Liver of rats treated with combination of 400 mg /kg of formulation and paracetamol suspension (2g/kg, p.o.) presentation congestion in central vein and mild abridged necrosis. Liver of rats treated with 25 mg/kg of silymarin and paracetamol suspension (2g/kg, po) presentation almost normal histology of liver lobule.

Discussion

When taken in therapeutic volumes, paracetamol (N-acetyl-p-aminophenol) is a frequently used antipyretic and analgesic medication that is nontoxic. Overdosage of paracetamol, on the other hand, is known to be hepatotoxic in humans and experimental animals [28]. In the present study the damage of liver due to paracetamol over dosage was confirmed by elevated levels of biochemical parameters like SGOT, SGPT, ALP and serum bilirubin (total and direct). This is due to the fact that hepatic cells possess a variety of metabolic activities and contain a host of enzymes. SGPT, SGOT found in higher concentration in cytoplasm and SGPT particularly in mitochondria. In liver injury the transport function of hepatocytes is disturbed, resulting in the leakage of plasma membrane [29], thereby causing leakage of such enzymes leading to the increased serum levels of them. The elevated activities of SGPT, SGOT in paracetamol induced When 400 mg/kg of formulation was compared to the paracetamol-induced hepatotoxicity group ($P<0.1$), significant reductions in SGPT, SGOT, ALP, and serum bilirubin levels were observed. At lower doses, about 80% of ingested paracetamol is eliminated mainly as sulfate and glucoronide conjugates before oxidation and at lower dosages, only 5% of ingested paracetamol is oxidised by hepatic cytochrome P-450 (CYP2E1) to a highly reactive and poisonous electrophile called N-acetyl-p-benzoquineimine (NAPQI), with the remaining 80% being removed mostly as sulphate and glucoronide conjugates. The glucoronidation and sulfation pathways become saturated after taking too much paracetamol, and as a result, paracetamol is progressively converted into NAPQI. The cytotoxicity of NAPQI is due to its one-electron reduction metabolite, the

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semiquinone radical. These semiquinone radicals can then interact directly with cellular macromolecules to cause toxicity or, alternatively, can be reoxidized back to their original quinones under aerobic circumstances by giving one electron to molecular oxygen. Following this donation of one electron, hydroxyl radical and reduced oxygen radical species are produced. The cytotoxic effects of quinones are known to be caused by both semiquinone and oxygen radicals [(30)]. However, at high paracetamol doses, NAPQI can alkylate and oxidise intracellular LPO, GSH, SOD, and CAT, depleting the liver's supply of these substances. Additionally, the reactive intermediate reacts with other nucleophilic centres of essential molecules in liver cells, resulting in hepatotoxicity. Additionally, it has been demonstrated that paracetamol directly inhibits cellular proliferation, causes oxidative stress that leads to lipid peroxidation, depletes ATP levels, and modifies Ca^{++} homeostasis; all of these modifications are thought to have the potential to be fatal to the cell [(30)].

The effect of formulation (dose 400 mg/kg) against paracetamol induced liver lipid peroxidation and alteration of level of SOD, GSH and Catalase was found to be dose dependent. Formulation at the dose of 400 mg/kg exhibited nearly similar effect to that of standard silymarin. Additionally, formulation 400 mg/kg dose were shown increased the regeneration of hepatocyte, central and portal vein with absence of necrosis when compared to paracetamol induced hepatotoxicity group.

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