# Hepatoprotective Activity of a Unani Polyherbal Formulation "Kabideen" in Paracetamol Induced Liver Toxicity in Rats

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## Abstract

*Kabideen* (Syrup) is a Unani polyherbal formulation manufactured and marketed by a reputed Unani Pharmaceutical establishment Dawakhana Tibbiya College, Aligarh Muslim University Aligarh, India. This preparation is being prescribed by the physicians of traditional medicines in the management of liver disorders since last many years. Although the reports of physicians suggest that it is clinically very effective hepatoprotective drug, but scientific studies have not been conducted so far on this product to validate the claims of Unani physicians. Therefore the present study was designed to investigate the self prepared (SP) and market sample (MS) of Kabideen for hepatoprotective activity against paracetamol induced liver damage in albino rats of either sex at a dose of 5.25 ml/kg body weight. Various biochemical parameters of liver function including serum total bilirubin, total protein, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), total cholesterol and lipid peroxidation were measured to assess the effect of the test drug on paracetamol induced hepatic damage. The histopathological study of liver tissue was also conducted. Results of the study revealed elevated level of marker enzymes in the animals treated with paracetamol (750 mg/kg intraperitoneally), indicating severe hepatic damage, whereas significant reduction in the serum markers were seen in the animals treated with two samples of Kabideen indicating significant hepatoprotective effect possessed by the test drug. However, the effect produced by the self prepared sample was more striking.

**Keywords:** Kabideen, Hepatoprotective activity, Paracetamol damage.

## Introduction

Liver has a pivotal role in regulation of physiological processes. It is involved in several biochemical pathways related to growth, nutrient supply, metabolism, secretion and storage. Liver diseases are mainly caused by chemical intoxicants (certain antibiotics, carbontetrachloride, chemotherapeutics, peroxidised oil, aflatoxins, excess consumption of alcohol, high doses of paracetamol and infections). Nearly 150 phytoconstituents from 101 plants have been claimed to possess liver protecting activity (Doreswamy and Sharma, 1944 and Handa *et al*., 1989). Only some of the plants with hepatoprotective property and few formulations used in Unani medicines have been evaluated pharmacologically for their hepatoprotective and associated effect on experimental and clinical models (Farooq *et al.,* 1997; Katuria and Singh, 1974). A number of polyherbal formulations both pharmacopoeial and non pharmacopoeial are available in the markets which

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are being used successfully to treat liver diseases, but most of them have still not been scientifically evaluated for the effects these are being used for. Kabideen (syrup) is a proprietary preparation of Dawakhana Tibbiya College, Aligarh Muslim University, Aligarh; it has been described to be effective in different types of liver disorders. It comprises of 21 ingredients (Table 1) of Unani herbal drugs that are attributed to have either hepatoprotective effect or are able to induce a response that complement the hepatoprotection directly or indirectly. According to the literatures of Unani medicine most of the ingredients of this formulation are used as Muhallil (anti-inflammatory), Muffatteh (deobstruent), Mudir (diuretic) and Muhafiz kabid (hepatoprotective), and are prescribed effectively in liver diseases by the physicians of traditional medicine.

**Table 1:** Ingredients of Kabideen

| S.No. | Unani Name | Botanical Name | Part used | Quantity |
|---|---|---|---|---|
| 1 | Biranjasif | *Achellia millefolium* | Top of Flowers | 10 gm |
| 2 | Barg-e-Shahattara | *Fumaria officinalis* | Leaves | 6 gm |
| 3 | Barg-e-Kasaundi | *Cassia occidentalis* | Leaves | 6 gm |
| 4 | Tukhm-e Kasni | *Cichorium intybus* | Seeds and root | 10 gm |
| 5 | Tukhm-e-Bathua | *Chenopodium alba* | Seeds | 6 gm |
| 6 | Tukhm-e-Kasoos | *Cuscuta reflexa* | Seeds | 6 gm |
| 7 | Tukhm-e-Khayarein | *C.sativa and c.melo* | Seeds | 6 gm |
| 8 | Mako | *Solanum nigrum* | Fruits | 10 gm |
| 9 | Rewand Chini | *Rheum emodi* | Rhizomes | 7 gm |
| 10 | Sumbullut Teeb | *Nordostachys jatamansi* | Rhizomes | 6 gm |
| 11 | Ood Hindi | *Aquillaria agallocha* | Roots | 6 gm |
| 12 | Narmusk | *Ocrocorpus longifolius* | Buds | 6 gm |
| 13 | Satar Farsi | *Zataria multiflora* | Leaves | 6 gm |
| 14 | Ushba | *Smilax regelli* | Roots | 6 gm |
| 15 | Khulanjan | *Alpinia galanga* | Roots | 6 gm |
| 16 | Chiraita Shireen | *Swertia chirata* | Leaves | 3 gm |
| 17 | Gul-Surkh | *Rosa demascena* | Flowers | 3 gm |
| 18 | Gul-e- Nilofar | *Nymphaea alba* | Flowers | 6 gm |
| 19 | Gul-e-Tisoo | *Butea frondosa* | Flowers | 10 gm |
| 20 | Gul-e-Ghafis | *Agrimonia eupatorium* | Flowers | 10 gm |
| 21 | Bekh-e-Kasni | *Cichorium intybus* | Roots | 6 gm |

In view of the above therefore the present study was designed to investigate the hepatoprotective effect of Kabideen against paracetamol induced hepatic damage in experimental model. Paracetamol (acetaminophen) is a widely used antipyretic and mild analgesic drug which produces acute liver damage if overdoses are consumed. It is mainly metabolized in liver to excreteable glucuronide and sulphate conjugates (Jollow *et al*., 1974 and Wong *et al*., 1981). Two samples of Kabideen were used during the study. One sample was prepared by us (self prepared, henceforth SP) and other was procured from the company that prepares it (Market sample, henceforth MS).

## Materials and Method

### Collection and Authentication of Plant

The ingredients of Kabideen were procured from local market of Aligarh. The samples were identified and authenticated by NISCAIR, New Delhi and the Pharmacognosy section of the Department of Ilmul Advia, Aligarh Muslim University. The ingredients were used to prepare the sample of Kabideen (SP).

Kabideen syrup (MS) was procured from Dawakhan Tibbiya College, Aligarh Muslim University, Aligarh.

### Method of Preparation of Kabideen Syrup (SP)

The decoction of the ingredients was poured into a tin-coated vessel and added 2.5 parts of sugar, and then the vessel was kept on low fire and waited till it attained the required consistency (Anonymous, 2006).

### Experimental Animals

Wistar albino rats of either sex weighing 150- 250 g were used for the study. The animals were procured from Central Drug Research Institute (CDRI), Lucknow. The animals were placed in polypropylene cages with paddy husk as bedding. They were fed on standard diet and water *ad libitum* and housed at a temperature of 24±2 $^{0}$C and relative humidity of 30-70%.

### Experimental Design

All the animals were divided into five groups consisting of 6 animals each. They received the treatment as follows:

Group I received normal saline in the dose of 1ml/kg/p.o for 7 days and served as control

Group II treated with Paracetamol in the dose of 750 mg/kg i.p. on 7th day and served as model for paracetamol toxicity and it was

Group III treated with SP (5.25ml/kg/day) for 7 days and paracetamol (750mg/kg i.p.) on 7th day.

Group IV received MS (5.25ml/kg/day) for 7 days and paracetamol (750mg/kg i.p.) on 7th day.

Group V received Silymarin (50mg/kg/day,p.o) for 7 days and paracetamol (750mg/kg i.p.) on 7th day.

All the animals were sacrificed after 36 hours and biochemical tests on blood sample and histopathological studies on liver tissues were performed (Vivek *et al*., 1994).

### Biochemical Estimation

After sacrificing the animals, the blood was collected and centrifuged at 7000 rpm for 15 minutes and stored at $4^0$C. AST (Moss, 1994), ALT (Moss, 1994), ALP (Kaplan and Lavernel, 1983), Bilirubin (Malloy and Evelyn, 1937), Total protein (Kingsley, 1939), Total cholesterol (Abell *et al*., 1952) and lipid peroxidation (Ohkawa *et al*., 1979) were estimated in serum.

Histopathological examination: The liver of all the animals was removed and preserved in 10% formalin solution for histopathalogical investigations (Luna, 1966).

Statistical Analysis: All the data were expressed as Mean ± S.E.M and analyzed statistically using one way ANOVA and compared with respective control group by graph pad instat. A value of $P<0.05$ was considered significant.

## Results

### Biochemical

Effect of the two samples of Kabideen i.e. SP and MS on paracetamol induced liver injury in rats with reference to biochemical changes in serum and lipid peroxidation are given in Table 2 and 3. Histological profile of liver tissue is depicted in Figure 1, 2, 3, 4 and 5. Blood samples of paracetamol treated animals collected at the end of the treatment showed significant increase in the serum level of total bilirubin, alanine amino transferase, aspartate amino transferase, alkaline phosphatase, lipid peroxidation and cholesterol as compared to the normal control group, while the total protein level decreased reflecting liver injury caused by paracetamol; whereas blood samples from the animals treated with SP and MS

**Table 2:** Effect of test drug Kabideen (SP and MS) on paracetamol induced toxicity

| Group | S. ALT/SGPT (Units/ml) (Mean ± SE) | S.AST/SGOT (Units/ml) (Mean ± SE) | Serum Bilirubin (mg/dl) (Mean ± SE) | S.Alk. Phosphatase (Unit/dl) (Mean ± SE) | Total Protein (gm/100 ml) (Mean ± SE) | Total cholesterol |
|---|---|---|---|---|---|---|
| Plain control | 26.77± 0.74 z*a*b* | 51.06± 0.94 z*a*b* | 0.74±0.02 z*a*b* | 65.48±1.76 z*a*b* | 6.01±0.12 z*a*b* | 78.46±2.31 z*a*b* |
| PCM 750 mg/kg | 91.71±1.31 x* | 91.50±2.06 x* | 2.23±0.07 x* | 170.2±0.94 x* | 4.40±0.01 x* | 162.13±2.31 x* |
| SP 5.25 ml/kg | 74.40±1.06 Y* | 78.32±2.13 Y* | 1.83±0.03 Y* | 134.51±2.31 Y* | 4.88±0.09 Y* | 149.93±0.57 Y* |
| MS 5.25ml/kg | 90.57±0.83 Y* | 85.99±1.60 Y* | 2.01±0.03 Y* | 157.22±0.09 Y* | 4.41±0.02 Y* | 156.26±3.14 Y* |
| Silymarin 50 mg/kg | 62.76±1.26 y* | 64.02±1.14 y* | 0.93±0.14 y* | 95.97±9.02 y* | 5.12±0.10 y* | 98.78±3.29 y* |

n=6 *P< 0.001

x = against plain control
y= against PCM (750mg/kg)
z= against standard (silymarin 50 mglkg)
a= against SP (5.25 ml/kg)
b= against MS (5.25 ml/kg)

**Table 3:** Effect of Kabideen on Lipid Peroxidation in Paracetamol induced hepatic damage

| Groups | Lipid peroxidation (n mole of MDA/mg of protein) (Mean ± SE) |
|---|---|
| Plain control | 1.99 ± 0.20 z*a*b* |
| PCM 750mg/kg | 7.38 ± 1.70 x* |
| SP 5.25 ml/kg + PCM 750 mg/kg | 2.64 ± 0.10y* |
| MS 5.25 ml/kg + PCM 750 mg/kg | 3.10 ± 0.07y* |
| Silymarin 50 mg/kg + PCM 750 mg/kg | 2.53± 0.29y* |

n=6 *P< 0.001

x = against plain control

y = against PCM (750mg/kg)

z = against standard (Silymarin 50mglkg)

a = against SP (5.25ml/kg)

b = against MS (5.25ml/kg)

at the dose 5.25 ml/kg showed significant decrease in the level of serum marker enzymes while a significant increase in the total protein was observed. The findings suggested that the test drugs protected the hepatic cells from the likely injury of paracetamol. The values in respect of SP were comparable to the values determined in the group treated with standard drug (Silymarin 50 mg/kg/day). Further, the response of SP was comparatively better than the MS.

## Histopatological

Fig 1. shows normal hepatic architecture with single plate of heptic cords and normal hepatocytes with central vesicular nucleus and prominent nucleoli. Fig 2. PCM treated liver showing condensation of nuclear chromatin, foci of necrosis around congested and dilated central vein with nuclear pyknosis and cytoplasmic eosinophilia with cloudy swelling. Fig 3. SP treated Group showing mild microvesicular degeration of hepatocytes around central vein with maintained central vein and hepatic cords. Fig 4 MS treated Group showing moderately bilated and congested central vein & portal vein with marked interportal and periportal fibrosis. Fig 5. Silymarin treated liver showing mildly congested sinusoids with centrilobular necrosis and mild fibrosis.

Histology

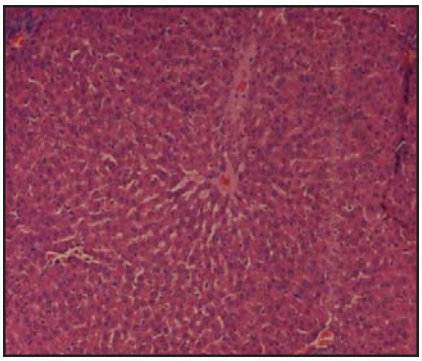

**Figure 1.** Normal

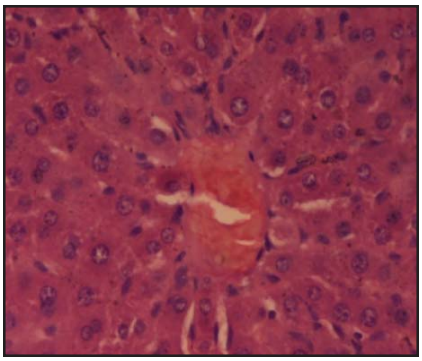

**Figure 2.** (Paracetamol treated)

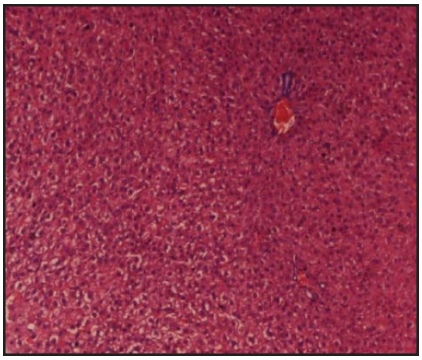

**Figure 3.** (SP treated)

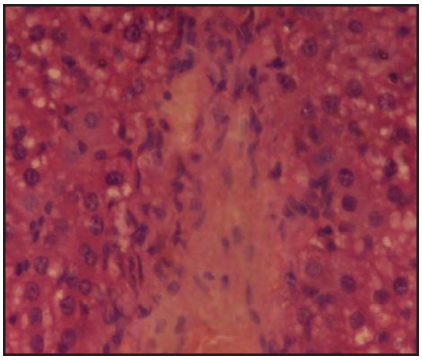

**Figure 4.** (MS treated)

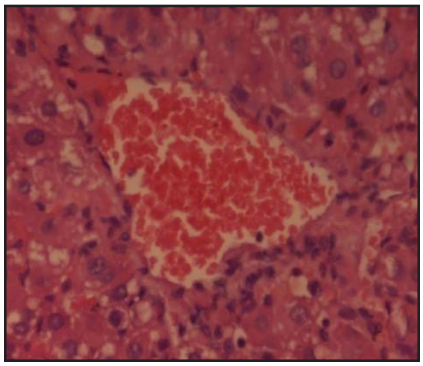

**Figure 5.** (Silymarin treated)

## Discussion

Paracetamol induced hepatic injury is an experimental model widely used for the screening of hepatoprotective drugs. Paracetamol undergoes a biotransformation by hepatic microsomal cytochrome p450 to produce trichloromethyl free radicals. These hepatotoxic metabolites can react with protein and lipid in the membrane of cells or organelles leading to necrosis of hepatocytes. As a result of hepatic injury, the altered permeability of the membrane causes the enzymes from the

cells to be released into the circulation. The magnitude of hepatic damage is usually assessed by measuring the level of released cytosolic transaminases including ALT and AST in the circulation. The rise in the serum levels of ALP, AST and ALT as observed in the present study could be attributed to the damaged structural integrity of the liver, because these are cytoplasmic in location and are released into circulation after cellular damage. Other researchers have reported that increased level of AST, ALT, ALP, bilirubin, cholesterol and decreased level of protein are due to PCM hepatotoxicity. The increase in level of serum bilirubin is an index of degree of jaundice. It could possibly be the result of increased production, decreased uptake by liver, decreased conjugation, decreased secretion from liver. Table 2,3 represents that the administration of paracetamol significantly increased the levels of AST, ALT, ALP, cholesterol, lipid peroxidation and bilirubin, and decreased the total protein level due to damaged structural integrity of the liver because these are cytoplasmic in location and are released into circulation after cellular damage. Kabideen prevented the paracetamol induced perturbations in the activities of AST, ALT, ALP, cholesterol, lipid peroxidation, total protein and bilirubin. The findings of the study were further complemented by histopathological observations (Fig 1-5) that suggested that the structural integrity of the liver tissue was maintained to a great extent in the groups treated with Kabideen. As compared to the control group, paracetamol intoxicated rats showed mild inflammatory cell infiltration and fatty changes, but the administration of Kabideen for 5 days attenuated these histopathological changes. Since paracetamol induces injury by stimulating the formation of free redicals therefore the hepatoprotective effect appears to be mediated through antioxidant property of Kabideen.

## Conclusion

In the light of the findings it can be concluded that the two samples of Kabideen possesses significant hepatoprotective effect in experimentally induced liver injury. However the effect produced by the SP was more prominent as compared to MS.


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