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PHARMACEUTICAL PREPARATION, PHYSICOCHEMICAL CHARACTERIZATION, AND IN VITRO HEPATOPROTECTIVE EVALUATION OF ARKA YAVA SAINDHAVA KSHARA IN HEPG2 CELLS

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ABSTRACT

Background: Arka Yava Saindhava Kshara is a classical Ayurvedic alkaline formulation described in Rasatarangini. Its pharmaceutical characteristics and biological activity require experimental evaluation. **Objective:** To prepare and characterize Arka Yava Saindhava Kshara and evaluate its protective activity against carbon tetrachloride (CCl_4)-induced toxicity in HepG2 cells. **Methods:** The formulation was prepared using Arka Kshara, Yava Kshara, and Saindhava Lavana according to the classical method. Organoleptic and physicochemical parameters were assessed. In vitro protective activity was evaluated using the MTT assay in CCl_4-exposed HepG2 cells at formulation concentrations of 50, 100, and 200 µg/mL, with silymarin as the reference treatment. **Results:** The formulation exhibited a pH of 9.75, loss on drying of 3.51%, total ash content of 98.22%, and water-soluble extractive value of 91.16%. Cell viability in the CCl_4-exposed group was

33.64% relative to the untreated control. Treatment with the formulation increased cell viability to 42.68%, 56.92%, and 61.24% at 50, 100, and 200 µg/mL, respectively. Corresponding viability values for silymarin were 53.16%, 65.47%, and 82.70%. Thus, the formulation showed a concentration-dependent increase in cell viability, with a smaller protective response than silymarin. **Conclusion:** Arka Yava Saindhava Kshara demonstrated preliminary in vitro protective activity against CCl_4-induced injury in HepG2 cells. Further studies using additional cellular assays, normal-cell controls, and in vivo models are required to clarify its mechanism and safety. These findings do not establish anticancer efficacy.

KEYWORDS: Arka Yava Saindhava Kshara; Kshara Kalpana; HepG2 cells; hepatoprotective activity; carbon tetrachloride; MTT assay; physicochemical characterization.

INTRODUCTION

Liver injury and chronic liver disease are major health concerns. Persistent hepatic damage can contribute to fibrosis, cirrhosis, and hepatocellular carcinoma. The association of liver disease with metabolic disorders, obesity, and fatty liver highlights the importance of investigating agents that may protect hepatic cells against injury.[1] Experimental assessment of traditional formulations can provide preliminary evidence of their biological activity and guide further research.

Ayurveda describes several pharmaceutical preparations for the management of hepatic and digestive disorders. Among these, *Kshara Kalpana* comprises alkaline preparations obtained by processing the ash of medicinal plants. Classical texts describe Kshara as possessing *Lekhana*, *Chedana*, and *Bhedana* properties and discuss its use in various conditions.[2] These traditional applications provide a basis for experimental investigation of individual Kshara formulations.

Arka Yava Saindhava Kshara is a classical formulation consisting of Arka Kshara, Yava Kshara, and Saindhava Lavana, described in *Rasatarangini*.[3] Arka (*Calotropis procera*) is an important medicinal plant in Ayurveda, with several reported pharmacological activities.[4] Yava (*Hordeum vulgare*), commonly known as barley, is traditionally valued for its nutritional importance and *Lekhana* and *Medohara* properties.[5] Saindhava Lavana, or rock salt, is described in Ayurvedic literature for its digestive and therapeutic applications.[6]

However, the biological properties of the finished alkaline formulation require direct evaluation, as processing may substantially alter the composition of the original ingredients.

Pharmaceutical preparation and physicochemical characterization are essential for describing the quality of a traditional formulation. Evaluation of moisture content, ash value, water-soluble extractive value, and pH provides baseline information for subsequent quality assessment. In vitro cell-based studies offer an initial approach to examining biological responses under controlled conditions. The MTT assay estimates cell viability through cellular reduction of a tetrazolium compound and can be used to assess changes in viability following experimental injury and treatment.[7]

The present study aimed to prepare and characterize Arka Yava Saindhava Kshara and evaluate its protective activity against carbon tetrachloride-induced injury in HepG2 cells using the MTT assay, with silymarin as the reference treatment. The findings may provide preliminary evidence for further investigation of the formulation's hepatoprotective potential, mechanism of action, and safety.

AIM: To prepare and characterize Arka Yava Saindhava Kshara and evaluate its in vitro hepatoprotective activity against CCl_4-induced injury in HepG2 cells.

METHODOLOGY: The formulation was prepared according to the classical Ayurvedic method and assessed for organoleptic and physicochemical parameters. Cell viability was evaluated using the MTT assay at concentrations of 50, 100, and 200 µg/mL, with silymarin as the reference treatment.

Details of the Study

The present study involved the pharmaceutical preparation, physicochemical characterization, and in vitro evaluation of Arka Yava Saindhava Kshara. The experimental work assessed the formulation's protective effect against carbon tetrachloride (CCl_4)-induced injury in HepG2 cells.

Pharmaceutical Preparation

Arka Kshara and Yava Kshara were prepared separately from dried Arka wood and Yava plant material. The materials were burned to obtain ash, which was mixed with water and filtered to collect the alkaline liquid known as *Ksharajala*. The filtrate was evaporated to

obtain Kshara. The prepared Arka Kshara and Yava Kshara were combined with Saindhava Lavana to obtain the final formulation.

Processing of 2 kg of dried Arka wood yielded 22 g of ash and approximately 2 g of Arka Kshara. Similarly, 3 kg of dried Yava plant material yielded 25 g of ash and approximately 2.5 g of Yava Kshara.

Physicochemical Evaluation

The final formulation was examined for its organoleptic characteristics and physicochemical parameters. It appeared as a grey, fine powder with a salty taste, burnt odour, and soft texture. The recorded loss on drying was 3.51%, total ash content was 98.22%, water-soluble extractive value was 91.16%, and pH was 9.75. Qualitative analysis indicated the presence of sodium, potassium, and iron. These observations provide baseline characteristics for future assessment of the formulation.

Experimental Evaluation

HepG2 cells were maintained in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum. Cells were seeded in 96-well plates and incubated for 24 hours at 37°C in a humidified atmosphere containing 5% CO_2 before treatment.

The experimental groups included untreated control cells, CCl_4-exposed cells, CCl_4-exposed cells treated with silymarin, and CCl_4-exposed cells treated with Arka Yava Saindhava Kshara. Both the formulation and silymarin were evaluated at concentrations of 50, 100, and 200 µg/mL.

Cell viability was assessed using the MTT assay. Following incubation with MTT, the resulting formazan crystals were dissolved in propanol, and absorbance was measured at 540 nm. Cell viability was calculated relative to the untreated control. The reported readings were obtained in triplicate.

RESULTS

CCl_4 exposure reduced cell viability to 33.64% of the untreated control. Treatment with Arka Yava Saindhava Kshara increased viability in a concentration-dependent manner.

Treatment	50 µg/mL	100 µg/mL	200 µg/mL
Arka Yava Saindhava Kshara	42.68%	56.92%	61.24%
Silymarin	53.16%	65.47%	82.70%

The formulation improved cell viability compared with the CCl_4-exposed group, although the response was lower than that observed with silymarin at all tested concentrations.

Interpretation and Limitations

The results suggest preliminary protective activity against CCl_4-induced cellular injury. However, the study was limited to one cell line and a single viability assay. Normal-cell toxicity, mechanisms of protection, and in vivo effects were not evaluated. The document also does not clearly establish whether the triplicate readings represent independent experiments.

Although the original dissertation title refers to anticancer activity, the reported experiment demonstrates increased survival of injured HepG2 cells. These findings support investigation of hepatoprotective potential but do not establish anticancer activity or clinical efficacy.

DISCUSSION

The present study evaluated the pharmaceutical characteristics and in vitro protective activity of Arka Yava Saindhava Kshara against CCl_4-induced injury in HepG2 cells. The formulation produced a concentration-dependent increase in cell viability compared with the CCl_4-exposed group. These findings suggest preliminary cellular protection under the tested conditions, although the response was lower than that obtained with silymarin.

Cell viability was assessed using the MTT assay, which estimates cellular metabolic activity through the reduction of tetrazolium to formazan.[8] CCl_4 exposure reduced viability to 33.64% relative to the untreated control. Treatment with Arka Yava Saindhava Kshara increased viability to 42.68%, 56.92%, and 61.24% at concentrations of 50, 100, and 200 µg/mL, respectively. These represent increases of 9.04, 23.28, and 27.60 percentage points over the injury control. The progressively greater response suggests a relationship between formulation concentration and the measured protective effect.

However, an increase in MTT absorbance does not independently establish increased cell number or a specific mechanism of protection. Changes in metabolic activity and interference between the test material and assay reagents can influence the results.[9] Consequently, the observed response should be confirmed using complementary methods, such as direct cell counting, membrane-integrity assays, and appropriate cell-free formulation controls.

Silymarin produced viability values of 53.16%, 65.47%, and 82.70% at the corresponding concentrations. Its response exceeded that of the formulation at every concentration, with the

largest difference observed at 200 µg/mL. Thus, the formulation showed partial recovery of the measured viability endpoint but did not restore it to the level of the untreated control. The smaller increase between 100 and 200 µg/mL also indicates that further concentration-response studies would be useful.

Physicochemical examination showed a pH of 9.75, total ash content of 98.22%, water-soluble extractive value of 91.16%, and loss on drying of 3.51%. These findings describe an alkaline preparation with a substantial inorganic residue and a high water-soluble fraction. They provide baseline characteristics for quality assessment but do not establish batch reproducibility, storage stability, or biological safety. Because the formulation is alkaline, future experiments should measure culture-medium pH after its addition and include suitable controls to determine whether pH or ionic changes influence the cellular response.

The document reports significant treatment, concentration, and interaction effects in a two-way analysis of variance. Nevertheless, the interpretation of these findings depends on whether the triplicate readings represent independent biological experiments or repeated wells from the same experiment. Technical replicates assess measurement consistency and should not be treated as independent biological replication. Future studies should clearly report the experimental unit, number of independent experiments, and comparisons with the CCl_4 injury control.

An important distinction concerns the original anticancer objective. The reported experiment measured improved viability in injured HepG2 cells rather than inhibition of cancer-cell survival or proliferation. Therefore, these results cannot support rejection of the null hypothesis concerning anticancer activity. A separate anticancer evaluation would require treatment of cancer cells without the injury-induction model, assessment of concentration-dependent cytotoxicity, and comparison with nonmalignant cells.

Overall, Arka Yava Saindhava Kshara demonstrated preliminary protective activity in the reported HepG2 injury model. The study was limited by its use of a single cell line, one metabolic assay, and the absence of normal-cell safety and mechanistic evaluations. Further independent experiments, complementary viability assays, and subsequent in vivo investigations are required before therapeutic conclusions can be drawn.

CONCLUSION

Arka Yava Saindhava Kshara was prepared and physicochemically characterized, providing baseline parameters for future quality assessment. The formulation demonstrated a concentration-dependent increase in cell viability against CCl_4-induced injury in HepG2 cells, reaching 61.24% at 200 µg/mL compared with 33.64% in the injury control. Its protective response was lower than that of silymarin. These findings suggest preliminary in vitro hepatoprotective potential but do not establish anticancer activity or clinical efficacy. Further studies using complementary assays, independent biological replication, normal-cell controls, and in vivo models are required to clarify its mechanism, safety, and therapeutic relevance.

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