

**IN-VITRO TRANSDERMAL DRUG DELIVERY ANALYSIS OF ZIMAD-E-KHARDAL: AN ANTI-EMETIC UNANI FORMULATION****Mohd Nauman Saleem<sup>1\*</sup> and Mohammad Idris<sup>2</sup>**<sup>1</sup>Post Graduate Student, M.D. (Ilm-us-Saidla).<sup>2</sup>Professor & Head, Departments of Ilm-us-Saidla & Ilm-ul-Advia, Ayurvedic & Unani Tibbia College & Hospital, Govt. of Delhi, Ajmal Khan Road, Karol Bagh, New Delhi.Article Received on  
10 Feb 2015,Revised on 05 March 2015,  
Accepted on 30 March 2015**\*Correspondence for****Author****Dr. Mohd Nauman Saleem**Post Graduate Student,  
M.D. (Ilm-us-Saidla)**ABSTRACT**

*Zimad-e-Khardal* (ZK) is one of the Unani classical formulations used to inhibit nausea and vomiting. In the present study, the formulation based on *Khardal* (*Brassica nigra*) and *Sirka* (Vinegar) was prepared in the form of *zimad* (paste) to evaluate its transdermal permeation. The *in-vitro* permeation study was carried out using the Franz diffusion cell. The permeation study of ZK confirmed the presence of glycosides, carbohydrates, alkaloids and flavanoids as bioactive constituents in the receptor compartment. Also gradual increase with time in the qualitative parameters such as intensity of colour, amount

of precipitates showed the permeation of bioactive agents from the designed formulation. The quantitative analysis was also done by using UV-Visible Spectrophotometer at 316 nm, and the collected samples were analyzed for concentration. The observations and result led to the scientific basis of transdermal delivery of ZK as well as validated its claim as a transdermal anti-emetic Unani formulation.

**KEYWORDS:** Unani system, transdermal delivery, *Zimad-e-Khardal*, antiemetic Unani formulation.

**INTRODUCTION**

Vomiting or emesis (*Qai*) is an abnormal indication of gastrointestinal tract. (Kasper et al., 2005) This situation warrants an urgent medical attention. Conventionally the medication in general is provided orally or en- route parenterally, but has certain limitations and drawbacks. (Latha et al., 2011) Also, there is no provision of parenteral drug delivery in Unani system of

medicine. Thus, a need arises to explore a possible alternate route through skin, i.e. transdermal.

The Transdermal Drug Delivery System (TDDS) is one of the novel routes for systemic delivery of drugs through the intact skin. The ultimate goal of this dosage design is to maximize the flux through the skin into the systemic circulation and simultaneously minimize the retention and metabolism of the drug in the skin and at the same time ensure that compounds are delivered, preferably at a specific rate to the systemic circulation. TDDS can deliver certain medications to systemic circulation in a more convenient and effective way than conventional dosage form. (Gupta et al., 2011) The potential of skin as a path of drug administration has been amply demonstrated by the acceptability of marketed therapeutic systems. Transdermal delivery offers a better route of delivery, reported to have better patient compliance. (Patel & Patel, 2012)

Though the concept of TDDS appears to be new in conventional pharmacology emerging in the 20<sup>th</sup> century, (Wilson, 2011), but Unani classical literature has ample evidence of it. According to the literature, it had been conceptualized, devised and put into practice by the Unani physicians in various pharmaceutical forms, such as *marham*, *zimaad*, *tila*, *roghan* etc. They used these forms for both local as well as systemic delivery of drugs. (Kabiruddin, 2006)

*Zimad-e-Khardal* (ZK) is one of the classical formulations used to inhibit nausea and vomiting (Kabiruddin, ynm). Use of *Khardal* (*Brassica nigra*) in the form of mustard plaster for severe chest congestion and vomiting is perhaps one of the oldest remedies. Commercially manufactured mustard plasters were also sold at pharmacies. (Gupta et al., 2011) In spite of the fact that ZK had been used as an anti emetic formulation, but the scientific data regarding its permeation through the skin is not available. An effort has been made to evaluate the *in-vitro* permeation analysis of ZK and revalidate the Unani pharmacopoeial formulation.

## MATERIALS AND METHODS

*Khardal* (*Brassica nigra*) seeds and *Sirka* (Vinegar) were procured from open market. *Khardal* seeds were grounded in mortar and pestle and simultaneously *Sirka* was added. Fine grinding was done to obtain paste like consistency of *zimaad* (Kabiruddin, ynm; Chughtai & Chughtai, 2004; Hafeez, 1931). In this process, 1 gram seeds and 2.5 ml of *sirka* were taken.

From the prepared *zimad*, *in-vitro* permeation analysis was done by using the Franz diffusion cell. The outer water jacket was filled with water and kept over hot plate with magnetic stirrer. The temperature was maintained at  $37 \pm 1$  °C. Phosphate buffer pH 7.4 was used as dissolution medium in receptor compartment and a teflon coated mini magnetic bead was kept in the receiver compartment for agitating the solution. The dialysis membrane was carefully mounted in between the receiver compartment and the donor compartment and both compartments were held tight with the help of spring clamps. The *zimad* equivalent to 125 mg was applied on the releasing surface i.e. the upper side of membrane. The experiment was continued for 6 hours and 1ml sample was withdrawn at regular interval. The same volume of phosphate buffer pH 7.4 was added to receptor compartment to maintain the sink conditions. The samples were analyzed qualitatively by chemical methods to observe the permeation profile of different bioactive constituents present in ZK, as well as quantitatively by UV-Visible Spectrophotometer (Suryadevara, 2010).

### **Qualitative analysis**

#### **(i) Test for glycosides**

One ml of sample was taken in a test tube and a few drops of aqueous sodium hydroxide solution were added to it and observed. Yellow colour formation confirmed the presence of glycosides (Anonymous, 1987).

#### **(ii) Test for carbohydrates**

One ml of collected sample was taken in a test tube and one ml of a mixture of equal parts of Fehling's solution 'A' and Fehling's solution 'B' was added. The contents of the test tube were boiled and observed. Formation of brick red precipitate confirmed the presence of carbohydrates (Anonymous, 1987)

#### **(iii) Test for alkaloids**

One ml of sample was taken in a test tube and 2-3 drops of Dragendorff's reagent were added to the test tube containing the sample and observed. Brownish yellow precipitate confirmed the presence of alkaloids (Anonymous, 1987).

#### **(iv) Test for flavanoids**

One ml of sample was taken in test tube and treated with few drops of 20% sodium hydroxide solution and colour change was noticed. Later on a few drops of dilute HCl were added and

again noted for the change in colour. Formation of yellow colour, which becomes colourless on addition of dilute HCl, indicates the presence of flavanoids (Anonymous, 1987).

### Quantitative analysis

- **Determination of  $\lambda_{\text{max}}$ :** Dilution of 250 $\mu\text{g}$  of Khardal was made using phosphate buffer pH 7.4. UV spectrum was recorded in the wavelength range 200-600 nm.
- **Preparation of calibration curve for Khardal:** A standard curve was prepared by serial dilution method. A stock solution of 50mg in 10ml of phosphate buffer pH 7.4 was used for making different concentrations of 500, 250, 125, 62.5, 31.25 and 15.625 $\mu\text{g}/\text{ml}$  respectively. The absorbances of these solutions were determined spectrophotometrically at  $\lambda_{\text{max}}$ .
- ***In-vitro* permeation study:** The quantitative analysis of the samples collected at time intervals of 0, 15, 30, 60, 120, 180, 240, 300 and 360 minutes was done using UV-Vis spectrophotometer. The different samples were analysed at  $\lambda_{\text{max}}$  and respective absorbances were noted. The concentration from each absorbance was calculated using the regression equation and then the percentage cumulative drug release (%CDR) was calculated.

## RESULTS

### Qualitative Analysis

The qualitative analysis was done by various chemical methods. Four different samples were tested. The observations of tests are shown in figures 1-4.

- Sample A was drawn from the donor compartment.
- Sample B was taken from plain Phosphate buffer solution pH 7.4.
- Sample C was drawn from the receptor compartment after 1 hour.
- Sample D was drawn from the receptor compartment after 6 hours.

All the tests performed were positive. The results are given in table I.

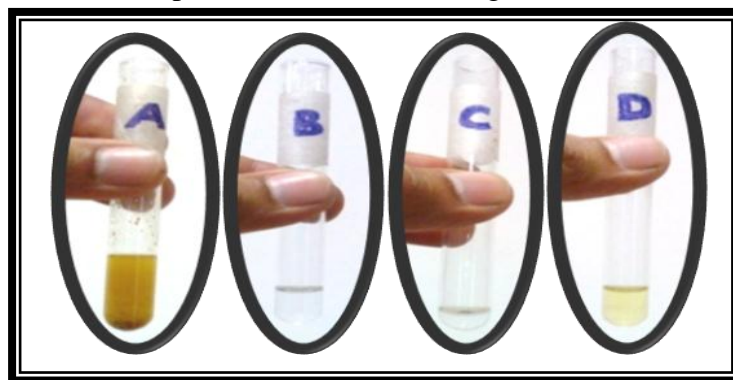


Figure 1. Test for glycosides

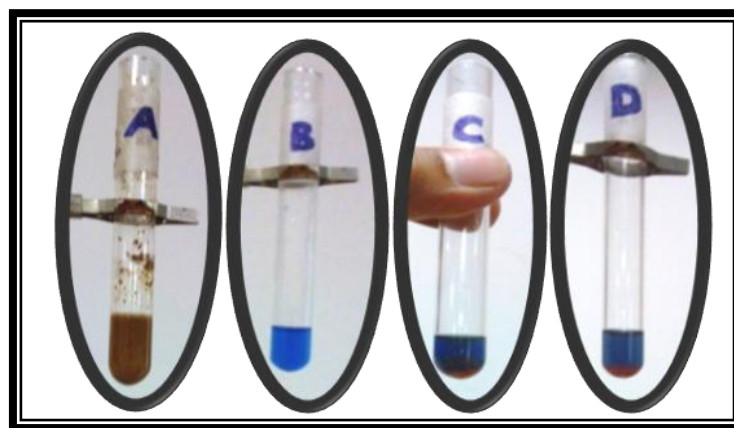


Figure 2. Test for carbohydrates

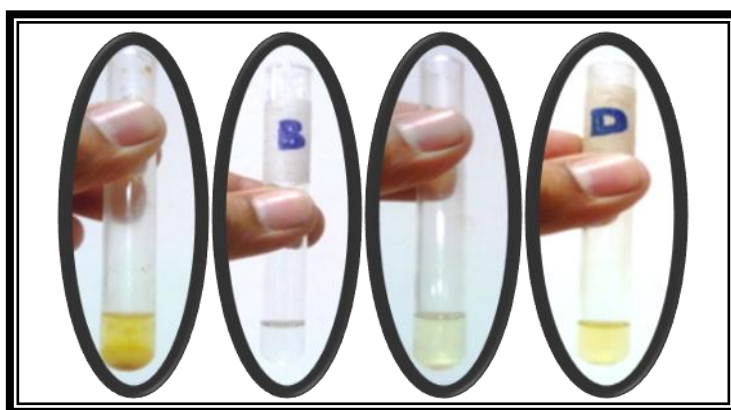


Figure 3. Test for alkaloids

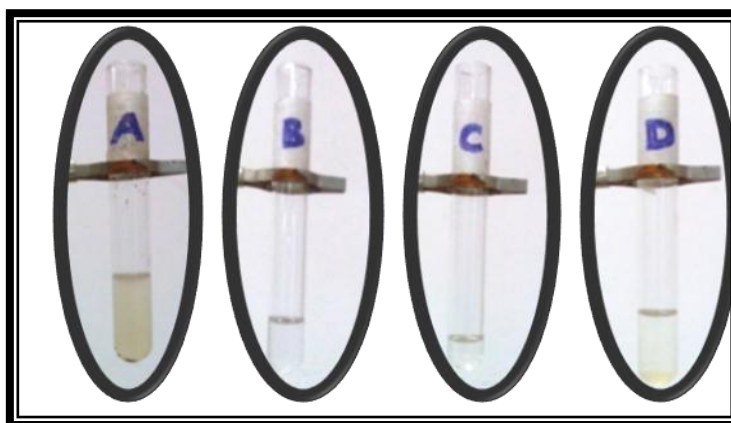


Figure 4. Test for flavanoids

Table I. Results of the qualitative analysis

S. No.	Sample	Test for Glycosides	Test for Carbohydrates	Test for Alkaloids	Test for Flavanoids
1	Sample A	Positive (Yellow colour)	Positive (Brick red ppt.)	Positive (brownish yellow ppt.)	Positive (colourless)
2	Sample B	Negative	Negative	Negative	Negative
3	Sample C	Mildly positive (Very light yellow colour)	Positive (Brick red ppt.)	Positive (Light yellow colour ppt.)	Positive (colourless)

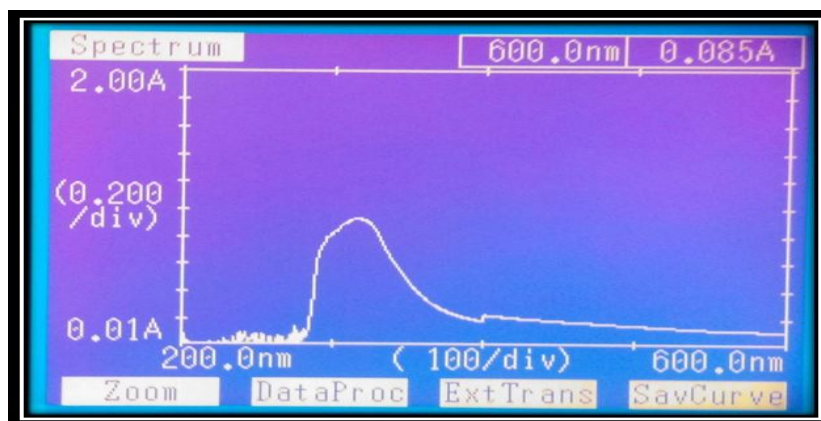
4	Sample D	Positive (Light yellow colour)	Positive (Brick red ppt.)	Positive (Yellow colour ppt.)	Positive (colourless)
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### Quantitative Analysis

- Determination of  $\lambda_{\max}$  for Khardal drug**

The spectrum obtained for Khardal drug in phosphate buffer pH 7.4 is shown in the figure 5.

The peak of maximum wavelength ( $\lambda_{\max}$ ) is obtained at **316 nm**.



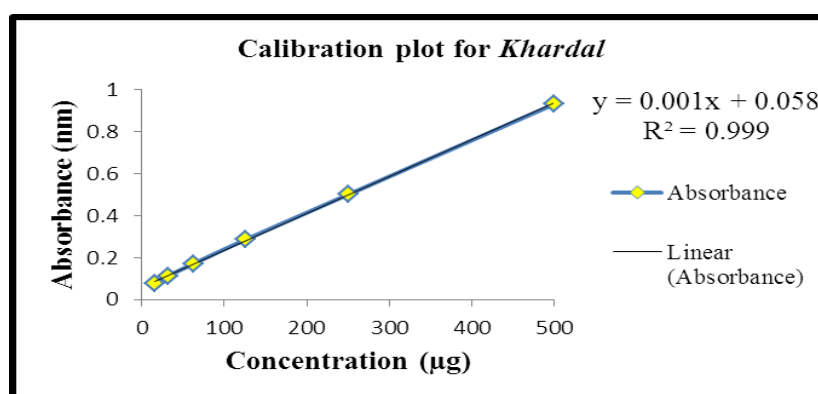
**Figure 5. UV spectrum of Khardal drug**

- Preparation of calibration curve for *Khardal***

The absorbance values obtained are given in table II. Using concentration and absorbance data, a Beer and Lambert's plot was obtained. The plot is shown in the figure 6.

**Table II. Data of calibration plot of *Khardal***

S. NO.	CONCENTRATION ( $\mu\text{g}$ )	ABSORBANCE (nm)
1	15.625	0.077
2	31.25	0.112
3	62.5	0.171
4	125	0.285
5	250	0.501
6	500	0.932



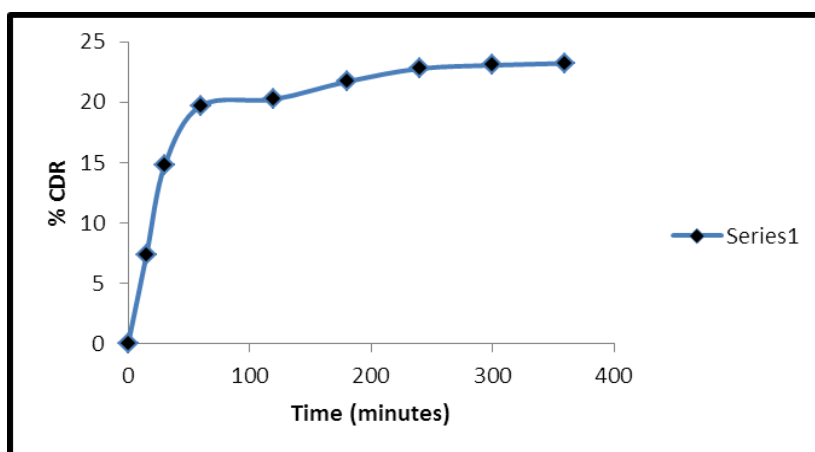
**Figure 6. Calibration curve of *Khardal***



The samples collected at different time intervals were analysed using UV- Visible Spectrophotometer at 316 nm and respective absorbances were noted. The concentration from each absorbance was calculated using the regression equation and then the percentage cumulative drug release (%CDR) was calculated.

**Table III. *In-vitro* diffusion study data of ZK**

S. NO.	TIME OF COLLECTION (minutes)	CONCENTRATION (mg/ml)	% CDR
1	0	0	0
2	15	0.366	7.32
3	30	0.738	14.76
4	60	0.986	19.72
5	120	1.012	20.24
6	180	1.085	21.70
7	240	1.139	22.78
8	300	1.153	23.06
9	360	1.161	23.22



**Figure 7. *In-vitro* diffusion study of ZK**

## DISCUSSION

The *in-vitro* qualitative analysis, through different chemical methods, clearly indicates the pharmacokinetics of ZK. The quantity of drug permeating through the membrane increased gradually with time, which is indicated by the increase in colour intensity and amount of precipitates formed. Furthermore, the quantitative analysis shows the exact quantification of the amount of drug release. The analysis shows that 7.32% of the drug permeates within 15 minutes, and the amount of release increased to 14.76% in 30 minutes. The cumulative drug release reached to 19.72% in 1 hour. After that the drug release was slowed down, though it showed an increasing pattern throughout the study. After 6 hours, the % CDR reached to

23.22%. It is worthwhile to mention that no additional permeation enhancer was added to the formulation in this study.

## CONCLUSION

This study is the first of its kind in which an effort has been made to validate the transdermal drug delivery of *Zimad-e-Khardal* (ZK), a Unani pharmacopoeial formulation, which is applied over the abdominal region in the form of paste to mitigate the condition of nausea and vomiting. This *zimad* is already a time tested Unani formulation, but the scientific data was not yet available. The outcome of study shows a new approach as a novel drug delivery system in Unani pharmaceuticals.

## ACKNOWLEDGEMENT

I gratefully acknowledge the faculty of Post Graduate Department of Ilm-us-Saidla, A & U Tibbia College, Karol Bagh, New Delhi, for their valuable cooperation and support.

## CONFLICT OF INTEREST

None declared.

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