Characterization and Comparative Chemical Analysis of Kushta-e-Faulad (Iron Calx) Prepared by Conventional as well as Modern Method

[1]Tajuddin, [2*]K.S. Siddiqi and [1]Aziz ur Rahman

[1]Department of Saidla, Faculty of Unani Medicine, Aligarh Muslim University, Aligarh-202002, India

[2]Chemistry Department, P.B. 196, Gondar University, Gondar, Ethiopia

Abstract

Kushta-e- Faulad is a common herbo-metallic preparation of Unani medicine used in anemia, liver disorders, as a tonic in general debility and convalescence. In this work the kushta-e-faulad was prepared in laboratory according to the method given in National Formulary of Unani Medicine (NFUM) and modern method to make a comparative study of the physical properties of Kushta- e- Faulad. In this study we are reporting the preparation and characterization of the finished kushta by Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM) and Energy Dispersive X-ray Analysis (EDAX). It was observed that the Kushta-e-Faulad contained nano-particles of iron-oxide and metallic iron in the range of 06 to 49 nm. Its LD_{50} and the toxic dose was determined and was found to be 660 mg/kg b.w.

Keywords: Kushta-e-Faulad, SEM, TEM, Nanoparticle, LD_{50}

Introduction

Despite being important for the body, all metals and minerals cannot be used as therapeutic agent due to pharmacokinetic inconvenience and potential toxicity. They are therefore, first converted to carbonates or oxides. These oxides of metals or minerals are technically known as Kushta (Calx) and the process by which kushtajats are prepared is known as Taklees (Calcination). Many metals and minerals are effectively used to cure various ailments. Probably the Roman physician, Pliny and Greek philosopher, Dioscrides were the first to use gold in medicine while Hippocrates, explained the beneficial effect of silver in human system (Sudha *et al.,* 2009)

Islamic philosophers made great contributions to pharmacy. Probably the art of calcination was mainly derived from the alchemical techniques used during the Arabian era of Unani medicine (Holmyard, 1924). Jabir ibn Hayyan and Al-Razi set the foundation of modern science. Jabir described the preparation of many chemical substances such as oxides (kushta), sulphide of mercury and arsenic etc. He gave an exact description of calcination, crystallization, solution, sublimation and reduction (Anawati, 1970). Abu Bakr Mohammad Bin Zakaria Razi, in his book Sirr-al-Asrar (*Liber Seceretorum bubacaris*) described calcination, distillation and crystallization (Holmyard, 1931). Also he gave a comprehensive list of apparatus employed in alchemical work (Clagett, 1961). Razi coined the term taklees for calcination. Chemically, kushta may be defined as the calcined product of any desired metal or mineral while literally kushta means “to kill” (Sudha *et al.,* 2009). In medical terms, it is defined as the

[2*]Author for correspondence; Email: ks_siddiqi@yahoo.co.in

detoxification of the harmful properties of a metal. The calcined metals are termed as bhasmas, parpams and kushta in Ayurveda, Siddha and Unani systems of medicine, respectively (Mishra, 2003). In Unani system of medicine kushta-e-faulad is used as a tonic in general debility and in the treatment of anemia (Anonymous, 2006 and 2003). It also cures impotency if it occurs due to anaemia (Said, 1970). The normal prescribed dose of most of the kushta is about one grain of rice which is equivalent to about 10 mg. The high efficacy of kushta may be due to its very small particle size which has high propensity of absorption (Brown *et al.,* 2007). Although, kushta of various metals and minerals are in use for decades no scientific parameters have been developed for the preparation and characterization of these age old remedies (Cardarelli, 1986). It was therefore, essential to develop a standard protocol for the preparation and characterization of kushta-e-faulad by both traditional and modern techniques. We have also explored the toxic dose of kushta- e- faulad by LD_{50}.

In this paper we are reporting, for the first time, the preparation and characterization of kushta-e-faulad by Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Energy Dispersive X-ray Analysis (EDAX), Integrated Coupled Plasma Atomic Emission Spectroscopic (ICPAES) and animal toxicity studies. The particle size and solubility of the calx has also been determined. The present study conforms to the guidelines laid down by the Institutional Animal Ethics Committee (IAEC) and WHO for the testing of finished herbo-metallic drugs to restrict the heavy metal content within permissible limit.

Material and Methods

General consideration

Physicochemical tests like gravimetric analysis, HCl digestion were done in accordance with the reported methods (Vogel, 1986) whilst traditional tests were also carried out to standardize the final drug in accordance with classical parameters given in official pharmacopeias (Rasheed *et al.,* 2011 and Tariq *et al.,* 2013).

Collection of raw material

Iron powder procured from Merck (India Ltd.) was used. Sheer-e-Madar [Latex of *Calotropis procera* Ait (R.Br.)] was collected from the wild plant and Loab-e-Gheekawar (Exudate of *Aloe barbadensis* Mill) was obtained from the herbal garden of department of Ilmul Advia. Roghan-e-zard (clarified butter) was purchased from local dairy in Aligarh, India.

Preparation of Kushta-e-Faulad

Initially the iron powder was subjected to hot and cold immersion (metal quenching) in water after heating it at 600^0C in Muffle furnace. This process was repeated twenty times to make the iron brittle. Processed iron powder was used for making Kushta-e- Faulad by conventional as well as modern method.

Preparation by conventional method

The processed iron powder was ground with Sheer-e-Madar in a traditional mortar and small cakes were made. These cakes were then put in earthen discs and sealed with the process of Gil-e-Hikmat (mud coating) and subjected to a fire of 5 kg of cow dung cakes. It was heated three times each with Loab-e-gheekawar and Raughan zard. The temperature was monitored regularly by Digital Pyrometer at an interval of 15 minutes. The temperature was maintained at 800°C - 900°C for up to 2 hours (Fig.1, 2, 3). The whole material was allowed to cool on its own. The calcined material was ground and sieved through mesh number160 to get microfine powder. It was recommended that kushta should not be prepared in open to maintain the constant temperature in the traditional furnace (portable tandur).

Preparation by modern method

The iron was processed in the same way as described earlier and was then sealed in a silica crucible. It was heated in a muffle furnace. Heating rate was kept at 10°C/min. The maximum temperature was maintained at 900 °C for two hours then slowly cooled (Fig.4) to room temperature. For this we had divided the furnace combustion into three steps.

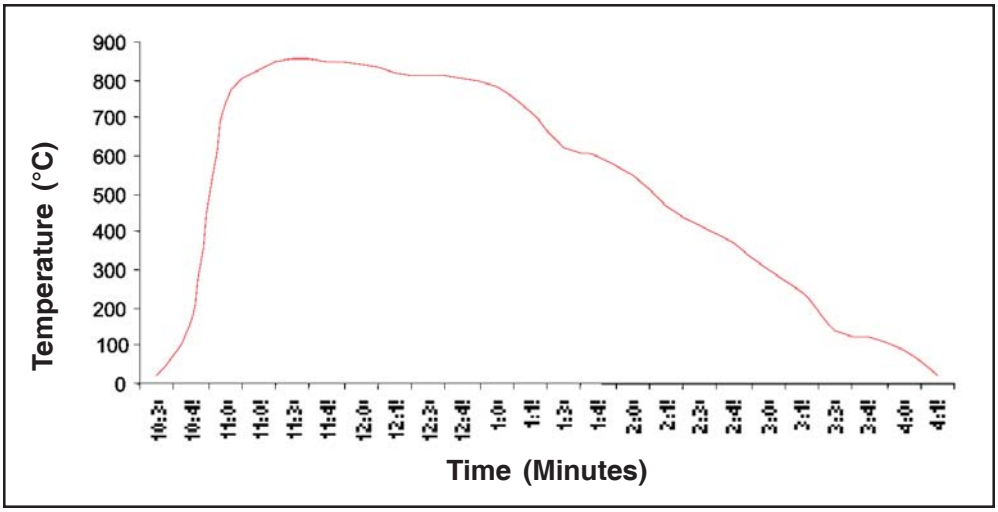


Figure 1: Heat quantification graph of kushta-e-faulad prepared by conventional method

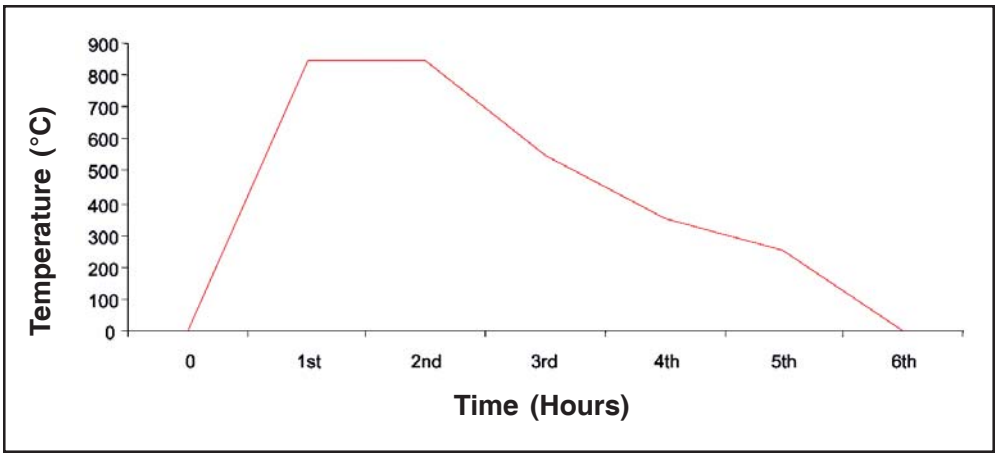


Figure 2: Heat quantification graph of kushta-e-faulad prepared by modern method

Ist step: Initial rise in temperature (25°C to 900°C in 55min)

IInd step: Maintenance of temperature (900°C for2 hours)

IIIrd step: Slow drop in the temperature (900°C to 25°C for 3 hours),

- 4th hour 900°C to 550°C.
- 5th hour 550°C to 250°C
- 6th hour 250°C to 30°C

Results and Analysis

The kushta-e-faulad is mainly iron metal in nano particulate form. The larger percentage loss in kushta-e-faulad may be attributed to more number of combustions (4 times) and many numbers of grinding steps.

Physico-chemical Tests

It includes chemical analysis of raw material (Table 1) organoleptic characters (Table. 2) and physical properties (Table.3). Loss in weight was found to be 35% and 32% prepared by conventional and modern methods, respectively. Kushta-e-faulad prepared by both the methods passes the classical physical tests of true kushta (Table 4).

Table 1: Chemical analysis of raw material

Raw Material	Density (g/ml)	Gravimetric Estimation (iron - %)
Iron powder (Merck, India Ltd.)	7.86	99.5%

Table 2: Organoleptic characters

Kushta-e-Faulad	
Colour	**Reddish Brown**
Appearance	Fine Powder
Taste	Tasteless

Table 3: Physical properties of Kushta-e-Faulad

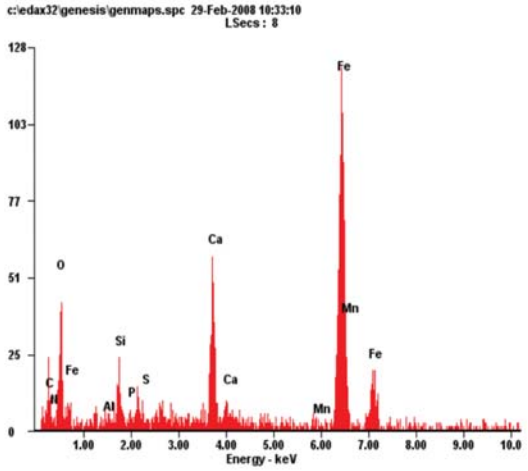
pH	6.95
Appearance	Reddish brown amorphous powder
Solubility	Light brown colour in water
% Iron (conventional)	56
% Iron (modern)	54.15

Table 4: Classical Physical tests

Kushta-e-Faulad	Metallic luster	Finger-thumb test	Still Water test
Conventional Method	Absent	+	+
Modern Method	Absent	+	+

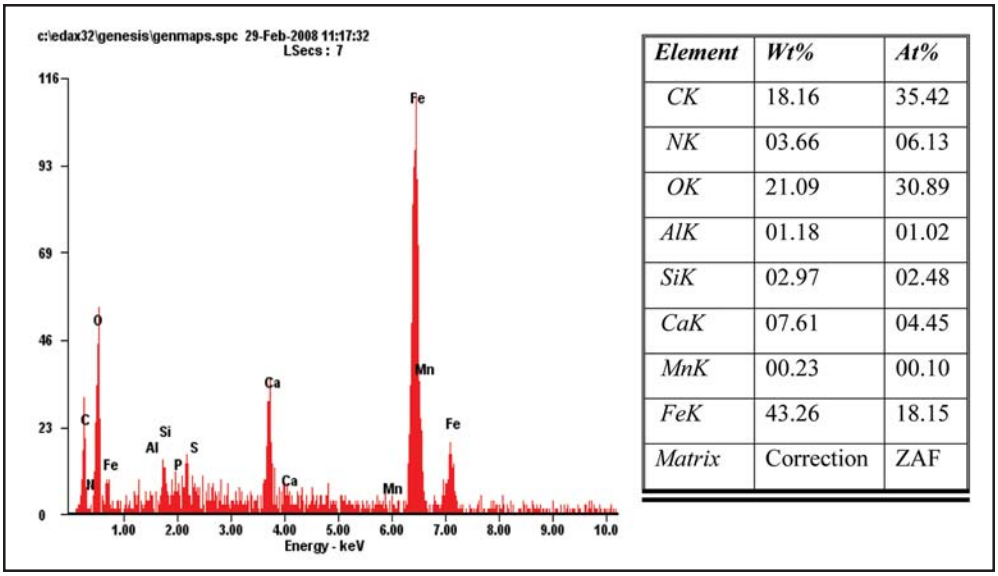
Energy Dispersive X-ray Analysis (EDAX) of Kushta-e-Faulad

Metal contents were determined by EDAX which showed that all constituents are present in the test sample. Some toxic elements have also been detected in trace amounts which are given below in the tabular and graphical form (Fig. 3 and 4).



Element	*Wt%*	*At%*
CK	04.02	10.59
OK	23.50	46.47
CuL	00.00	00.00
AsL	01.32	00.56
AlK	01.15	01.35
SiK	03.07	03.46
CaK	01.22	00.96
FeK	63.09	35.73
Matrix	Correction	ZAF

Figure 3: EDAX pattern of Kushta-e-Faulad prepared by conventional method.



Element	*Wt%*	*At%*
CK	18.16	35.42
NK	03.66	06.13
OK	21.09	30.89
AlK	01.18	01.02
SiK	02.97	02.48
CaK	07.61	04.45
MnK	00.23	00.10
FeK	43.26	18.15
Matrix	Correction	ZAF

Figure 4: EDAX pattern for Kushta-e-Faulad prepared by modern method (Muffle Furnace).

Atomic Absorption Spectroscopic (A A S) Analysis of Kushta-e-Faulad

The results show that the metals are not completely converted to oxide but major part of it remains in the elemental form. The solubility of Kushta-e- Faulad was determined by dissolving 20 mg of sample in 25 ml of aqueous hydrochloric acid at pH 4. It was found that the solubility of kushta is very poor at this pH(Table 6 and 7).

Table 5: AAS analysis of Kushta-e-Faulad prepared by conventional method

Kushta-e-Faulad	Metal	Soluble Metal Content at pH 4		Total Metal Content		Oxygen Content
		ppm	%	ppm	%	
Fe	169	0.69	514564	62.13	17.50	

Table 6: AAS analysis of Kushta-e-Faulad prepared by modern method

Kushta-e-Faulad	Metal	Soluble Metal Content at pH 4		Total Metal Content		Oxygen Content
		ppm	%	ppm	%	
Fe	203	0.76	514967	62.98	12.11	

Integrated Coupled Plasma Atomic Emission Spectroscopic (ICPAES) of Kushta-e-Faulad

ICPAES analysis shows that the samples prepared by conventional method contain impurities like Al, Mg and Ca in larger quantities as compared to the raw material. It may be due to the mixing of small parts of mud (buta) during the processing. The analysis is in agreement with the AAS analysis (Table 8).

Table 7: ICPAES results of Kushta-e-Faulad

Elements Measured	Kushta Faulad Prepared by Conventional method (in ppm)	Kushta Faulad Prepared by Modern method (in ppm)	Raw material (in ppm)
Al	4414.3	1922.95	2527.83
As	BDL	BDL	BDL
Ca	19451.52	2434.22	2817.66
Cu	561.21	414.14	406.91
Fe	481946.56	524662.81	561165.05
Hg	.....	.....	.....
Mg	7812.93	411.47	590.98
Mn	4091.09	4133.23	4333.97
Pb	254.54	216.52	249.33
Zn	688.54	682.39	1591.94

Scanning Electron Microscopic (SEM) Analysis of Kushta-e-Faulad

The surface of the particles of the Kushta-e-faulad appears to be rough and porous which increases the solubility and absorption in the living system. This is well reflected from the SEM images obtained at room temperature (Fig. 5 and 6). It has been observed that micro cracks are developed at the particle boundaries during processing. It is believed that microfine product quickly reacts with digestive juices and absorbed.

Transmission Electron Microscope (TEM) Analysis of Kushta-e-Faulad

The particle size of Kushta-e-Faulad prepared by conventional method was found to be smaller (6-11 nm) than those prepared by modern method (14-49 nm) (Fig. 7 and 8).

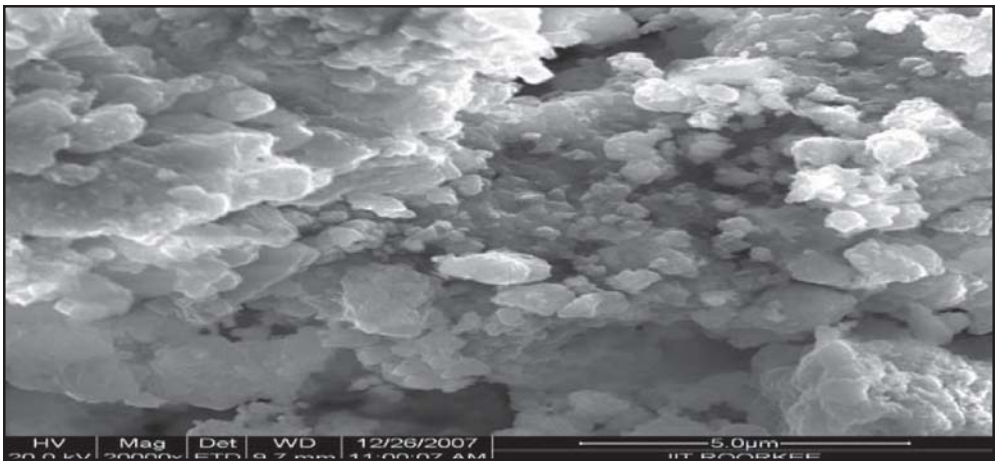


Figure 5: Scanning electron micrograph of Kushta-e-Faulad prepared by conventional method

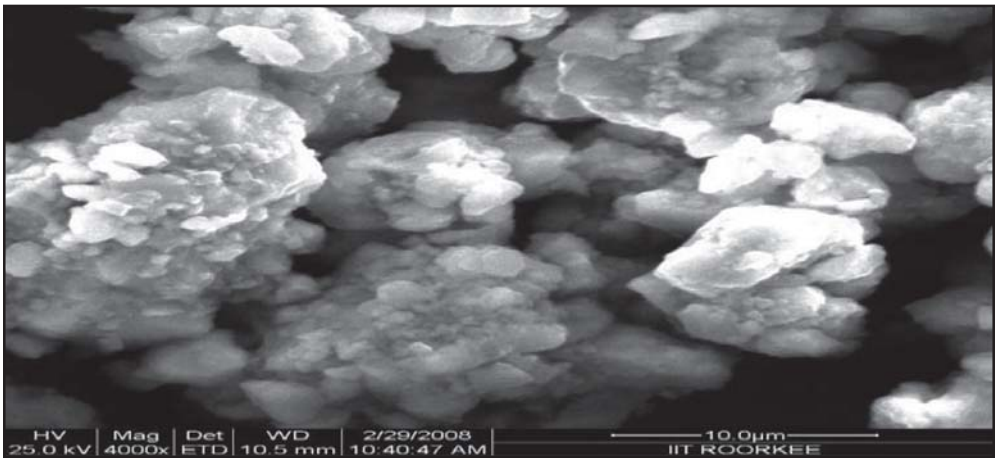


Figure 6: Scanning electron micrograph of Kushta-e-Faulad prepared by modern method

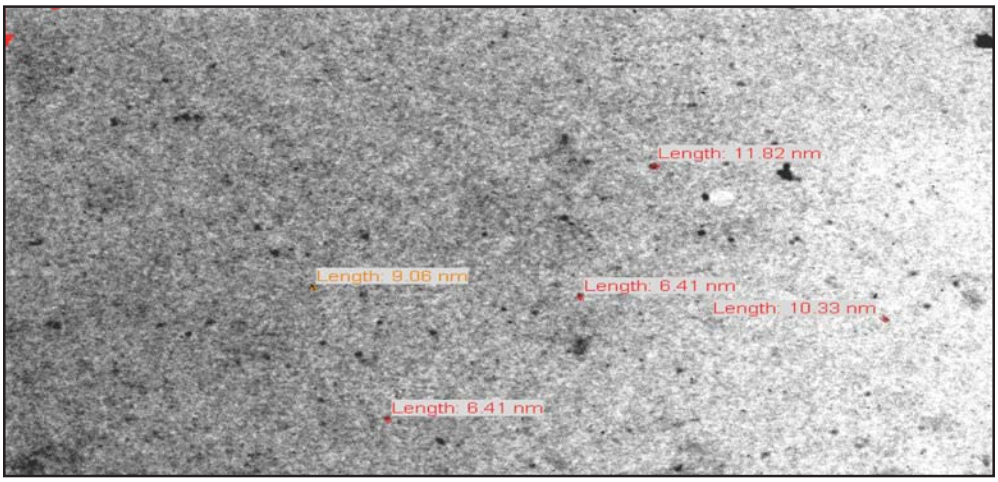


Figure 7: TEM micrograph of Kushta-e-Faulad prepared by conventional method, magnification of 11K. (Particle size: 6-11nm)

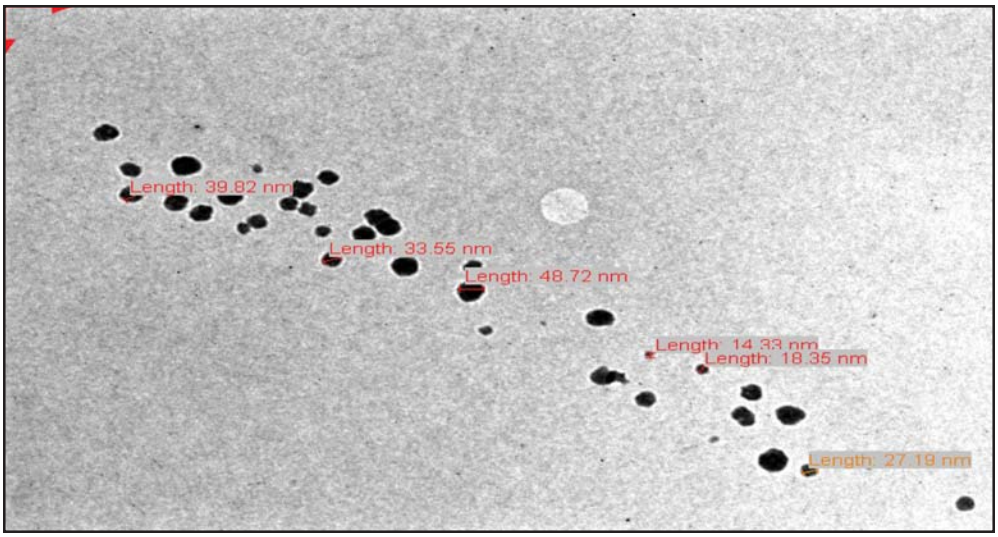


Figure 8: TEM micrograph of Kushta-e-Faulad prepared by modern method, magnification of 11K. (Particle size: 14-49nm)

Determination of LD_{50} of Kushta-e-Faulad prepared by conventional method

The results of LD_{50} of Kushta-e-Faulad prepared by conventional method were analyzed by graphical method of Miller and Tainter (1944). It was found to be 660 mg/kg body weight which means that Kushta-e-Faulad is 22 times more tolerable than its prescribed dose (Table-8 and Fig. 9).

Table 8: LD_{50} of Kushta-e-Faulad

Group	Dose (mg/kg)	Log Dose	Dead/ Total	Dead %	Corrected %*	Probit
1.	480	2.68	0/6	0	4.2	3.25
2.	540	2.73	1/6	16.7	16.7	4.05
3.	600	2.77	2/6	33.3	33.3	4.56
4.	660	2.81	3/6	50	50	5.00
5.	690	2.83	4/6	66.7	66.7	5.44
6.	840	2.92	6/6	100	95.8	6.75

* Corrected formula: for the 0% dead: 100 (0.25/n); for the 100% dead: 100[(n-0.25)/n], where n is the number of animals in the group.

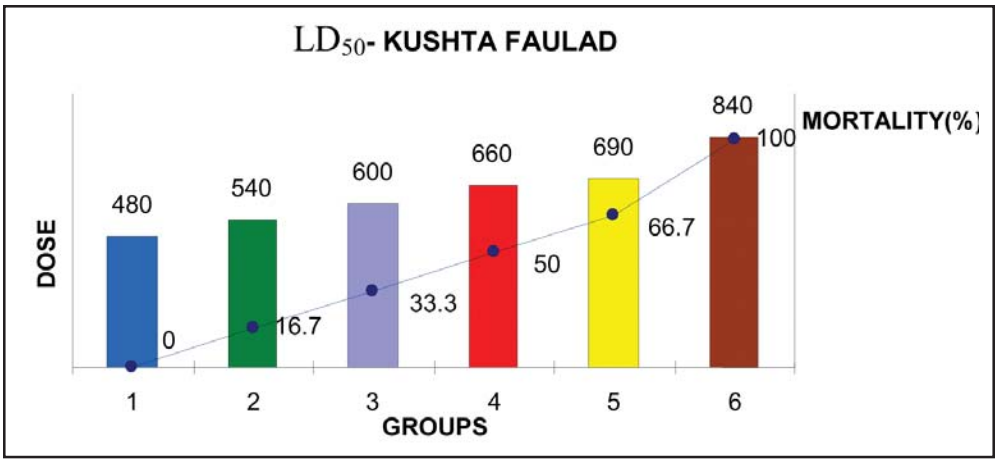


Figure 9: Depiction of LD_{50} of Kushta-e-Faulad

Discussion

Kushta-e-Faulad is a mixture of all oxides namely FeO, Fe_2O_3, Fe_3O_4 and elemental iron. The solubility also varies with pH of the solution. However, it was found that the conventional method is superior to modern method in this particular kushta preparation, as the particle size is smaller (6-11nm, Fig.8) than those prepared by modern method (14-49nm, Fig.9). It is therefore concluded that it may be more effectively absorbed when given to human subject for treatment. However, iron as such can be absorbed only if it dissolves in stomach although soluble iron salts like iron sulphate is highly soluble in biological system and easily absorbed.

Conclusion

Kushta-e-faulad prepared by both the conventional and modern method exhibit the same chemical and physical properties although there is a distinct difference in particle size. The kushta is a mixture of metal oxide and elemental metal in nano particulate form which has greater surface area due to which the absorption is enhanced.

Acknowledgement

Financial support from Central Council for Research in Unani Medicine (CCRUM), Ministry of Ayush, Govt. of India, New Delhi, is gratefully acknowledged.

References

Anawati, G.C., 1970. Science in the Cambridge History of Islam, Vol. 2. Cambridge University Press, pp. 741-779.

Anonymous, 2003. The Ayurvedic Pharmacopoeia of India (Part I). Ministry of Health and Family Welfare, Deptt. of AYUSH, Govt. of India, New Delhi, pp. 624-630.

Anonymous, 2006. National Formulary of Unani Medicine (Part I). Ministry of Health and Family Welfare, Deptt. of AYUSH, Govt. of India, New Delhi, pp. 63-67,70.

Boulanger, D., 2002. The Islamic Contribution to Science, Mathematics and Technology: Towards Motivating the Muslim Child, OISE Papers in STSE Education 3.

Brown, C.L., Bushell, G., Whitehouse, M.W., Agrawal, D.S., Tupe, S.G. *et al*., 2007. *Gold Bulletin* 40:250.

Burckhardt, T., 1997. Alchemy: Science of the Cosmos, Science of the Soul. Stuart and Watkins, US: 29.

Cardarelli, N.F., 1986. Tin as a vital nutrient: implications in cancer prophylaxis and other physiological processes. CRC Press, Florida.

Clagett, M., 1961. The Science of Mechanics in the Middle Ages. University of Wisconsin Press, US.

Holmyard, E.J., 1924. Maslama al=Majriti and the Rutbatu'l=Hakim. Isis 6: 293-305.

Holmyard, E.J., 1931. Makers of chemistry. Oxford at the Claredon Press

Miller, L.C. and Tainter, M.L., 1944. Estimation of LD50 and its error by means of log-probit graph paper. *Proc. Soc. Exp. Biol. Med*. 57:261.

Mishra, L.C., 2003. Scientific Basis for Ayurvedic Therapies. CRC Press, US: 86.

Ragai, J., 1992. The Philosopher's Stone: Alchemy and Chemistry. *Alif Journal of Comparative Poetics* 12: 58-77.

Rashid, A., Marri, A., Naik, M.M., 2011. Standardization of Bhasma – importance and prospectus. *Journal of Pharmacy Research* 4(6): 1931-33.

Said, M., 1970. Hamdard Pharmacopoeia of Eastern Medicine,; Hamdard Foundation, Karachi. Sri Satguru Publications Ed. IInd: 231

Sudha, A., Murty, V.S., Chandra, T.S., 2009. Standardization of Metal-Based Herbal Medicines. *American Journal of Infectious Diseases* 5: 200-206.

Tariq, M.; Chaudhary, S.S. and Imtiyaz, S., 2013. Introduction to kushta: A herbo-mineral Unani Formulation. *Journal of Pharmaceutical and Scientific Innovation* 2(1): 14-17.

Vogel, A.I., 1986. Quantitative Inorganic Analysis, Longman Green and Co., London, p. 405.