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Evaluation of Anti-epileptic activity of *Unmad Gaja Kesari Rasa II* in Animal Models

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ABSTRACT

Apasmara can be correlated with epilepsy, In Ayurveda, *Unmada Gaja Kesari Ras II* is advocated in the treatment of *Apasmara.* Present study was done to evaluate Antiepileptic activity of UGK II a herbo-mineral drug. In Electro shock model total 24 albino mice were classified into 4 groups each group containing 6 mice. Human dose was extrapolated with extrapolating factor 0.0026 & drug dose was given to control, standard, test x and 2x group. The duration of tonic hind limb extension (THLE) and mortality was observed for duration of 15min. The complete inhibition of THLE was considered as positive criteria. In PTZ induce convulsion model total 24 albino mice were classified into 4 groups each group containing 6 mice. Human dose was extrapolated with extrapolating factor 0.0026 and drug dose was given to control, standard, test x and 2x group. The time required for clonic convulsion, incidence and mortality was observed for duration of 30 mins. *UGK II* at 2 drug dose level proves equally effective to standard drug Phenytoin to abolish THLE. *UGK II* at 2 drug dose level is effective when compare to control group and Sodium valproate prove effective to reduce onset of time of convulsion when compare to test drug.

Key words: *Apasmara, Epilepsy, Unmada Gaja Kesari Rasa II, Electric shock model, PTZ induce convulsion model.*

INTRODUCTION

In the recent era there is competition in each and every field, so there is lot of mental stress or mental disorder seen. World-wide depression is ranked as the leading cause of disability and affecting 120 million people.[1] Mental and neurological disorders are common in all countries and cause immense suffering.

Recently published reports say that 50 million people suffer from epilepsy and 24 million from Alzheimer and dementia. So there is necessary to focus on this area.[2] Epileptic seizures are etiological deforms of both morphological and functional changes within brain imparting cognitive and neuropsychological alterations. Antiepileptic drugs are intended to reduce seizure frequency and severity within the frame work of safety. However, regular treatment with most antiepileptic conventional drugs may produce profound side-effects like cognition impairment (i.e. memory attention, mental speed and learning).[3] Further, most of the antiepileptic drugs have some neurotoxic effects and cognitive deficits which diminish their clinical utility in current medicinal practices.[4,5]

Epilepsy in the Indian context becomes inevitable as India is the largest populated country next to China. Apart from this, treatment of this disorder under the advanced medication system is not extended as it was expected. The treatment under traditional system was proved to be far more applied. In Ayurveda, mental disorders are mentioned as *Unmada* and *Apasmara.* Total 112 number of herbal as well as herbomineral

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formulation advocated to treat this disorders.[6] Herbal medicines have their limitations as these should be administered for long duration for expected effect. *Unmada Gaja Kesari Rasa II*[7] is one of herbomineral preparation explained in *Rasakamadhenu* is one among those formulations which is exclusively indicated in *Apasmara* having minimum ingredients, which is unique combination in which herbs like *Vacha, Shankhpusphi* and animal product like *Gomutra* and minerals like mercury and sulphur are used. *Vacha* have anti convulsant,[8] CNS depressant, *Apasmarnashak* and *Kaphaj Unmadhara* activity. *Shankpusphi* have antistress,[9] *antianxiety, Medhya, Manasroghar* and *Apasmarhara* activity. Mercury has *Smrutishaktikar* activity[10] sulphur has *Manasroghara* activity.[11] All these drugs are mixed and triturated with *Gomutra*. The dried mixture is kept in crucible and sealed crucible is heated in *Laghuputa*[12] in 8 cow dung cakes. Due to this process particle size of the formulation may be reduced which is helpful to increase the faster absorption of drug. *Unmadgajkesari Rasa II* may be helpful to treat convulsion and to enhance learning and memory.

Materials and Methods

Preparation of *Unmada Gaja Kesari Rasa II (Ras kamdhenu i.e.; RK Unmadchikitsa / 9-12)*[13] *Shodhan* (Detoxification) of *Parad* (Mercury)[14] and *Gandhak* (sulphur)[15] was done as per standard method. Preparation of *Vacha Kwatha*[16] was done as per SOPs. Preparation of *Shankpusphi Swaras*[17] was done as per SOPs. Collection of *Gomutra* was done. (A). Trituration of *Shuddha* (detoxified) *Parad* with *Vacha Kwatha* was done. (B).Trituration of *Shuddha* (detoxified) *Gandhak* with *Shankhpushpi Swaras* was done. Part (A) and (B) were mixed with each other and *Gomutra* was added with mixture and trituration was done to prepare black colored lusterless mixture.

Black colored lusterless mixture was analyzed by *Ayurvedic* parameters The black coloured lusterless mixture was placed in *Musha* (crucible), the mouth of the *Musha* was sealed with the help of cloth coated with fullars earth (*Multani mati*) seven times. Then that *Musha* will be placed in *Bhudar Yantra* (16 cm x 24 cm) and *Laghu puta* (8 cow dung cakes) was given. The finished product was stored in air tight glass container and utilized for study.

Experimental Study

Animal Ethics Committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA New Delhi, India. Animals (Swiss Albino mice and Wistar rats) of either sex from our breeding stock were used in this study. They were in-housed at the institute animal house in groups of six animals per cage at standard laboratory conditions at a temperature of 24^0 C±1^0C, relative humidity of 45-55% and 12:12 h dark and light cycle. Animals had free access to standard pellet laboratory animal diet and water ad libitum. The experimentation was carried out in noise free area.

Anti-epileptic activity

A. Electro shock model.

B. Pentelyenetetrozole induce convulsion in mice.

A) Electro shock model

Groups: Animals were grouped randomly. (By stratified random sampling)

4 Groups were classified each group containing of 6 mice.

Animal identification

Animals were marked on head, back, tail using picric acid. Appropriate labels were attached to the cages indicating study number, test substance, group number, sex and dose and cage number.

Instrument: Electro shock model, feeding needle, cotton, water.

Drug Dose

The therapeutic dose of Test drug X and 2X- *UGK II* is 1.5 gm in the formulation addition of equal quantity of Mustard powder (Brassica compestris) was advocated hence the final drug dose was 3gm[18] in human. This dose was extrapolated using extrapolating factor (E.F) 0.0026 the dose of Test X

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UGK II was 0.0078mg and the dose of Test 2X of *UGK II* was 0.0156mg and administered to mice orally.

The therapeutic dose of Standard drug - Phenytoin is 300mg[19] it was extrapolated with 0.0026 (E.F). The dose was 0.78mg which was administered in mice orally.

The dose of Control group - *Goghruta* was 40ml it was extrapolated with 0.0026 (E.F) and dose was administered to rats in 0.1ml orally.

Procedure

Test was started 60mins after administration of control, standard and test drug. Electric stimuli of 33 mA (0.2sec) was given using ear electrode which produced tonic hind limb extension, clonic jerky and unconsciousness sequentially. The duration of tonic hind limb extension (THLE) was observed for duration of 15 min. The complete inhibition of THLE was considered as positive criteria.

Observation

The duration of tonic hind limb extension (THLE) and mortality was observed for duration of 15 min. The complete inhibition of THLE was considered as positive criteria.

B) Pentelyenetetrozole induce convulsion in mice

Animal: Swiss albino mice of either sex of 18 - 20gm.

Groups: Animals were grouped randomly. (By stratified random sampling)

4 Groups were classified each group containing of 6 mice.

Animal identification

Animals were marked on head, back, tail using picric acid. Appropriate labels were attached to the cages indicating study number, test substance, group number, sex and dose and cage number.

Instrument: Syringe, feeding needle, spatula.

Drug Dose

The therapeutic dose of Test drug- *UGK II* is 1.5gm in the formulation addition of equal quantity of Mustard powder (Brassica compestris) was advocated hence the final drug dose was 3gm in human. This dose was extrapolated using extrapolating factor (E.F) 0.0026 the dose of Test I (x) *UGK II* was 0.0078mg and the dose of Test II (2x) of *UGK II II* was 0.0156mg and administered to mice orally.

The therapeutic dose of Standard drug - Sodium valproate is 600mg[20] it was extrapolated with 0.0026 (E.F). The dose was 0.78mg which was administered in mice orally.

The dose of Control group - *Goghruta* was 40ml it was extrapolated with 0.0026 (E.F) and dose was administered to rats in 0.1ml quantity orally.

Procedure

PTZ (60mg/kg) was injected to all mice 60min after control, test and standard drug were administered. Control, test and standard drug (sodium valproate 600mg/kg) was administered orally. The time required for clonic convulsion, incidence and mortality was observed for duration of 30 mins.

OBSERVATIONS AND RESULTS

Anti-epileptic activity

Electro shock model (ESM)

Table 1: Statistical Analysis of Es Model

Type of Treatment	Number of Animals	Duration (Mean ± SD)	95% Confidence Interval for Mean		p-value
			Lower Bound	Upper Bound	
Control	6	94.00 ± 10.67	82.80	105.20	< 0.001
Phenytoin	6	41.67 ± 10.39	30.77	52.57	
Test I	5	42.00 ± 7.87	33.74	50.26	
Test II	5	45.20 ± 9.36	33.57	56.83	

Analysis of data was structured by applying ANOVA test followed by tukey's test for comparison between all four groups. Mean difference (p value) is less than 0.05 i.e., 0.001 it specify that there is significant difference between the mean duration of tonic hind limb extension with respect to type of treatment.

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Table 2: p-value table for pair wise comparison of treatment by using Tukey's test.

p - Value				
	Control	**Phenytoin**	**Test I**	**Test I**
Control	-	< 0.001*	< 0.001*	0.04*
Phenytoin		-	0.99	0.929
Test I			-	0.924
Test I				-

Penteylenetetrozle (PTZ) induced convulsion in mice

Table 3: Statistical analysis of PTZ induced convulsion in mice model.

Type of Treatment	**Number of Animals**	**Seconds (Mean ± SD)**	**95% Confidence Interval for Mean**		**p-value**
			Lower Bound	**Upper Bound**	
Control	6	48.33 ± 22.51	24.71	71.96	< 0.001
Na Valproate	6	329.67 ± 48.79	208.47	450.86	
Test I	6	84.75 ± 17.98	56.13	113.37	
Test II	6	147.17 ± 28.33	117.44	176.90	

Analysis of data was structured by applying ANOVA test for comparison between all four groups. Mean difference (P value) is less than 0.05 i.e., 0.001 it specify that there is significant difference between the mean onset of convulsion with respect to type of treatment.

Table 4: Value table for pair comparison of treatment by using Tukey's test.

p - Value				
	Control	**Na valproate**	**Test I**	**Test I**
Control	-	< 0.001*	< 0.001*	< 0.001*
Na valproate		-	0.242	0.124
Test I			-	0.019
Test I				-

DISCUSSION

Anti-epileptic activity

For computing the anticonvulsant activity in mice animal models such as Electro shock induced convulsions (grand mal epilepsy) and PTZ induced convulsions (petit mal epilepsy) are utilized.

Electro shock model

1. Maximum electro shock model is used primarily as an indication for compounds which are effective in grandmal epilepsy, action of this drugs acting through this model they are termed as anti convulsant drugs.
2. We have found 15 research papers from which we concluded that current given in mA was not constant and varied from 40 mA to 150 mA in mice.
3. In this experimentation the modified method was use to assess anti epileptic drug was used i.e Kitano et al. (1996)
4. Initially pilot study was done all animals were screened from 2 mA to 30 mA but we observed that at 33 mA all animal showed THLE and this current was seizure threshold current by this current further experiment was carried out.
5. In this experiment Phenytoin was taken as standard drug, as all the drugs in this experiment were administered orally Phenytoin was administered orally to reduce bias.
6. As phenytoin is administered 300mg in 70 kg man, this human dose was extrapolated in 20gm mice ie 0.78mg. this drug dose was administered orally to mice, Phenytoin i.e. 0.2ml was administered for the concentration of 1mg by tuberculin syringe.
7. In this study four groups were made viz. Control, Standard and Test drug. Test drug U.G.K was administered at two drug dose levels (test X and test 2X).
8. Test was started 60mins after administration of vehicle, test drug and standard drug orally.

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9. An electrical stimulus of 33mA for 0.2sec was given using ear electrode which produces tonic hind limb extension, clonic jerking and unconsciousness sequentially to the animals.
10. Anti-epileptic activity was assessed with the following parameters, time required to abolish THLE was recorded and percent protection of convulsion recorded.
11. Mean time required for abolishing THLE was deliberated in all four groups. The data was analyzed by using ANOVA test followed by Tukey's test for Pair wise comparison for all four groups. The obtained P-value was less than 0.05 hence there was significant difference were found. This result indicates that *UGK II* is equally effective when compared with Standard drug and significant when compared with Control group.

Pentelyenetetrozole induce convulsion in mice

1. Pentyelenetetrazole induce convulsion model is used primarily as an indication for compounds which are effective in petitmal epilepsy, action of this drugs acting through this model they are termed as anti convulsant drugs.
2. In this experiment Sodium valproate was taken as standard drug, as all the drugs in this experiment were administered orally Sodium valproate was administered orally to reduce bias.
3. As Sodium valproate is administered 600mg in 70 kg man, this human dose was extrapolated in 20gm mice ie 0.78mg. this drug dose was administered orally to mice orally. Sodium valproate i.e 0.2ml was administered for the concentration of 1mg by tuberculin syringe.
4. In this study four groups were made viz. Control, Standard and Test drug. Test drug U.G.K was administered at two drug dose levels (test X and test 2X).
5. PTZ was administered i.p to all animals 60 mins after administration of Control, Test drug and Standard drug.
6. Animals were observed for 30mins after administration of PTZ for onset of clonic convulsion. Further animals were observed for mortality for 30 mins.
7. Mean time required for onset of convulsion was calculated in all four groups. The data was analysed by using ANOVA test followed by Tukey's test for Pair wise comparison for all four groups. The obtained P-value was less than 0.05 hence there was significant difference were found. This result indicates that *UGK II* is not effective when compared with Standard drug and significant when compared with Control group.

CONCLUSION

Manufacturing and authentication process of *UGK II* was standardized. *UGK II* in Electro shock model illustrate analogous effect with standard drug and showed significant activity when judge with control group. *UGK II* in PTZ induce convulsion in mice model does not illustrate significant effect when judged with standard drug and showed significant activity when judged with control group. Hence it is concluded that *UGK II* has highly significant Learning memory activity which established the study. Anti-epileptic activity of UGK II is established through Electro shock and PTZ induced convulsion in mice models.

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