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TOXICITY STUDY OF UNMADGAJAKESARI RASA [UGK] AND ITS ANTIPSYCHOTIC ACTIVITY IN ANIMAL MODELS

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ABSTRACT

Schizophrenia is a severe, chronic, and generally disabling brain and behavior disorder that disrupt normal thoughts, speech, and behavior. *Unmadgajakesari Rasa*[UGK], a potent herbomineral formulation claimed to be useful in Schizophrenia in traditional medicine. Due to lack of scientific evidences of its use in Schizophrenia lead to objective of the present work. After doing the acute toxicity study of UGK, it was evaluated for its antipsychotic activity in 5HTP induced head twitches model and extra pyramidal adverse effect in catalepsy model in albino mice. For each study animals were divided into 4 groups, each group comprising of 6 animals. Group I- Vehicle Control group (*ghrita*), Group II- Standard Drug Group. In test group UGK was administered in the doses of 7.8 mg and 15.6 mg orally.Acute toxicity study revealed that UGK was nontoxic at 2000mg/kg dose. It shows statistically significant [$p<0.001$] antipsychotic activity in 5HTP induced head twitches model. It does not produce catalepsy [$p< 0.001$] which is a known adverse effect of antipsychotic drug.

Keywords: Schizophrenia, *Unmadgajakesari rasa*, acute toxicity study, 5HTP, catalepsy.

INTRODUCTION

Mental health is an essential component of health according to *Ayurveda*[1]as well as Modern science[2]. According to *Ayurveda* intellect and mind both are equally important for thinking and decision making; yet thinking is especially the task of mind. When mind and intellect are in their normal status the behavior of an individual is healthy for him, his family, and society.

The modern science too describes a group of mental disorders. Psycho neurological disorders are marked by predominant disturbances of emotions rather than detectable organic abnormalities of the brain. *Unmad*[3] is co-related with the disease Schizophrenia in modern times.

Schizophrenia is a severe divesting mental disorder in which behavioral, emotional and cognitive disturbances follow a chronic course often with relapses. Schizophrenia affects around 0.3-0.7% of people at some point in their life[4]or 24 million people worldwide[5]. It causes approximately 1% of worldwide disability-adjusted life years[6]. It is diagnosed 1.4 times more frequently in males than females, and typically appears earlier in men[6].

Therefore scientists all over the world are searching for effective medicines to treat mental disorders.

Here *Ayurveda* has a lot to offer. There are approximately 112 formulations [*kalpas*] for *Unmad* listed in *BBR*. These are 6 in Powders [*Churna*], 9 Decoctions [*Kashaya*], and 34 *Ghritas*, 5 *Oils*, 14 *Anjanas*, 2 *Avlehas*, 2 *Dhupas*, 10 *Aasavaarishta* formulations, 3 *Gutikas* and 25

herbo-mineral formulations. Also approximately 30 single herbs are used to treat *Unmad* according to *BhavprakashNighantu*. Herbs are processed with mineral drugs to prepare herbo-mineral compounds whereby reduction in dose, time period of first effect and increase in efficacy is attained.

Unmadgajakesari rasa[7]is a herbo-mineral formulation recommended for *Unmad* and *Apasmar*[8]. UGK is prepared from the detoxified metal Mercury and detoxified Sulfur along with *Vacha*[9][*Acoruscalamus*], *Shankhapushpi*[10][*Convolvulus pluricaulis*], and cow's urine.

Shankhapushpi and *Vacha* are reported as "*Manasroghar"* in *Ayurveda*. The claim is supported by some scientific studies showing anticonvulsant[11], anti-stress[12] and anti-anxiety[13]activities. *Ayurveda* propounds that Mercury when added to herbs enhances their actions. However; Mercury is known to possess some toxic effects. Therefore '*Rasashastra'* advocates that each and every metal and mineral must be subjected to specific processing for detoxification which as *Samskaras*.

Though many works have been done on formulations which contain *Vacha* and *Shankhapushi* like "*Bramhi ghrita*"[14], no study is reported for toxicity profile and evaluation of pharmacological action of a compound of these two ingredients that contain Mercury and Sulphur.

UGK being a promising compound of Mercury and Sulphur along with *Vacha* and *Shankhpushpi* with a peculiar "*puta"*[15]method of processing it is worth studying to evaluate its toxicity, pharmacological action and adverse effect if any.

Materials and methods

Test Drug- *Unmadgajakesari rasa*[UGK] *is prepared* in the Department of Rasashastra&BhaishajyaKalpanavigyan of BharatiVidyapeeth College of Ayurved, Pune, as per classical reference of Rasakamdhenu. The finished product was stored in air tight glass container and utilized for study.

Animals: 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.

Acute toxicity

The acute toxicity of UGK was determined as per internationally accepted protocol drawn under Organisation for Economic Co-operation and Development (OECD) guideline no.423[16].UGK was found to be safe even at 2000mg/kg dose.

Experimental Study

Animals were divided into 4 groups, each group comprising of 6 animals. Group I- Vehicle Control group (*ghrita*), Group II- Standard Drug Group. The therapeutic dose of UGK in humans is 1.5 gm. In the formulation addition of equal quantity i.e.1.5 gm. of *Sarshap*[17] powder (Brassica campestris) was done. Final drug dose was 3gm[7]. This dose was extrapolated to animal doses[18] as X dose. In test groups (III-IV), UGK was administered in doses 7.8 mg (X dose), 15.6 mg (2X-dose) in mice along with goghrita[19](cow ghee). All the doses were given orally.

Antipsychotic activity using 5HTP induced head twitches in mice model[20]

Goghrita, Olanzapine, UGK in 7.8mg and 15.6mg dose for 20gm mice were administered orally into their respective groups I, II, III and IV. 60 min later, 5HTP (100mg/kg i.p.) was injected to all animals in each group. After 5 min of 5-HTP injection head twitches were counted for the duration of 15 min.

Evaluation of extra pyramidal adverse effect using catalepsy in mice model[21]

Goghrita, Haloperidol, UGK in 7.8mg and 15.6mg dosefor 20gm mice were administeredorally into respective groups I, II, III, and IV. After a gap of 30 minutes, assessment of catalepsy was started. It was done by placing forepaw of mice on horizontal bar (0.9 cm in diameter) kept at a height of 2.5 cm from platform. The time span for which the mice retained its forepaw on bar during the observation was recorded. Observations were made at 5, 10, 15, 30, 60, 90, 120, and 150 minutes after drug administration.

Statistical analysis: The data was analyzed by using ANOVA test following by Tukey's mean difference test. P<0.001 was considered statistically significant.

Results: In acute toxicity study UGK was found to be safe up to 2000mg/kg body weight. There were no changes in normal behavior pattern and no signs and symptoms of toxicity were observed. Adverse effects such as sedation, loss of righting reflex or mortality was not observed. In our experimental study, significant antipsychotic activity was observed in 5HTP head twitches model and UGK does not produce extra pyramidal side effect in catalepsy model.

Table1: Values obtained on applying ANOVA test for number of head twitches

Type of treatment	Number of animals	(Mean ± SD)	95% Confidence Interval for Mean		p-value
			Lower Bound	Upper Bound	
Goghrita	6	16.83 ± 1.47	15.29	18.38	< 0.001
Olanzapine	6	1.00 ± 0.632	0.34	1.66	
UGK 7.8 mg	6	11.83 ± 1.33	10.44	13.23	
UGK 15.6 mg	6	7.67 ± 1.86	5.71	9.62	

Table 2: P-value table for Pair wise comparison of treatment by using Tukey's mean difference test

P-value				
	Goghrita	Olanzapine	UGK7.8 mg	UGK15.6 mg
Goghrita	-	< 0.001*	< 0.001*	< 0.001*
Olanzapine		-	< 0.001*	< 0.001*
UGK 7.8 mg			-	< 0.001*
UGK 15.6 mg				-

*Significant

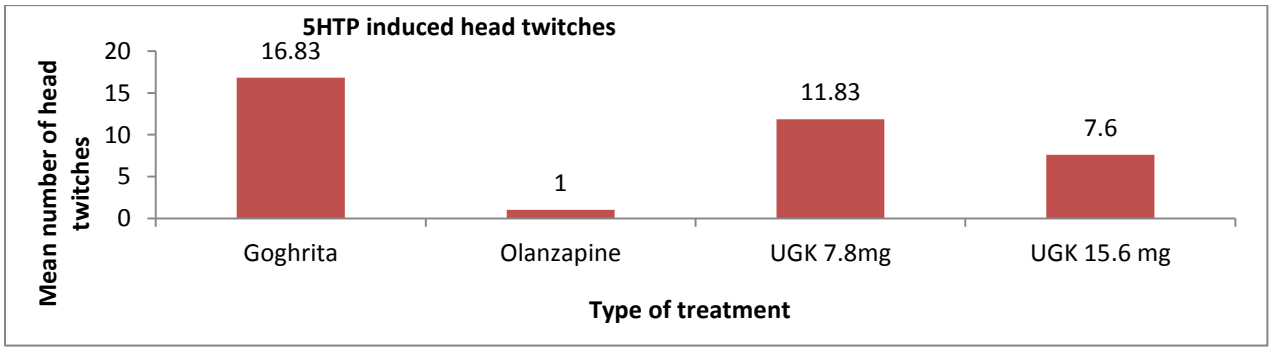

Figure1. Effect of UGK on 5HTP induced head twitches in mice. Each column represents mean ± SD of head twitches. P<0.001 when compared to vehicle control *ghrita* group

There is significant difference in mean number of head twitches. The standard drug Olanzapine showed significant reduction in mean number of head twitches as compared to control and test drug groups, as expected. UGK at dose of 7.8mg

and 15.6mg for 20gm mice per oral significantly reduced the mean number of twitches when compared with *Goghrita* treated group .

Table 3: Values obtained on applying ANOVA test

Time interval	Number of mice	Mean ± SD for treatment				p-value
		Goghrita	Haloperidol	UGK 7.8 mg	UGK 15.6 mg	
5 min	6	7.17 ± 1.72	25.33 ± 12.99	6.17 ± 2.56	9.00 ± 2.68	< 0.001
15 min	6	9.83 ± 1.17	43.67 ± 12.37	9.83 ± 3.43	10.50 ± 3.15	< 0.001
30 min	6	11.17 ± 1.60	83.67 ± 23.12	9.00 ± 2.37	12.33 ± 7.87	< 0.001
60 min	6	14.33 ± 1.63	155.17 ± 47.31	12.50 ± 1.76	9.50 ± 1.87	< 0.001
90 min	6	16.17 ± 3.31	189.67 ± 27.51	14.67 ± 1.51	16.50 ± 2.81	< 0.001
120 min	6	15.17 ± 4.79	208.00 ± 7.01	19.50 ± 1.05	21.33 ± 1.75	< 0.001
150 min	6	18.50 ± 2.81	239.50 ± 25.13	21.00 ± 1.26	24.17 ± 2.86	< 0.001

Table 4: P-value table for pair wise comparison of treatment by using Tuckey's test

		Goghrita	**Haloperidol**	**UGK 7.8 mg**	**UGK 15.6 mg**
5th min	*Goghrita*	-	0.001*	0.994	0.966
	Haloperidol		-	< 0.001*	0.003*
	UGK 7.8 mg			-	0.888
	UGK 15.6 mg				-
15th min	*Goghrita*		< 0.001*	1	0.998
	Haloperidol			< 0.001*	< 0.001*
	UGK 7.8 mg				0.998
	UGK 15.6 mg				
30th min	*Goghrita*	-	< 0.001*	0.999	0.984
	Haloperidol		-	< 0.001*	< 0.001*
	UGK 7.8 mg			-	0.996
	UGK 15.6 mg				-
60th min	*Goghrita*	-	< 0.001*	0.999	0.984
	Haloperidol		-	< 0.001*	< 0.001*
	UGK 7.8 mg			-	0.996
	UGK 15.6 mg				-
90th min	*Goghrita*	-	< 0.001*	0.998	1
	Haloperidol		-	< 0.001*	< 0.001*
	UGK 7.8 mg			-	0.996
	UGK 15.6 mg				-
120th min	*Goghrita*	-	< 0.001*	0.341	0.101

	Haloperidol		-	< 0.001*	< 0.001*
	UGK 7.8 mg			-	0.885
	UGK 15.6 mg				-
150th min	*Goghrita*	-	< 0.001*	0.986	0.867
	Haloperidol		-	< 0.001*	< 0.001*
	UGK 7.8 mg			-	0.973
	UGK 15.6 mg				-

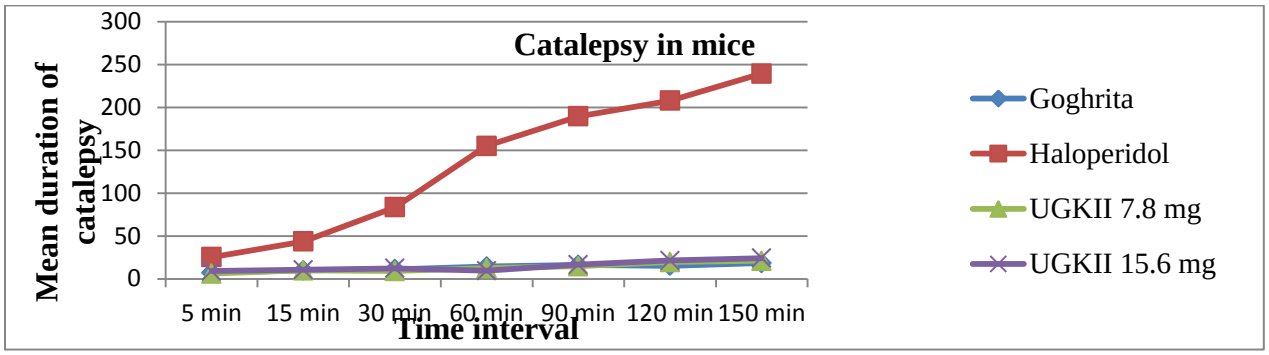

Figure1. Effect of UGK in catalepsy in mice model

The calculated P value of all the 4 groups at 7 time interval is less than 0.001. There is a significant difference in mean duration of catalepsy among all four groups. In the other words UGK at the doses of 7.8mg and 15.6mg did not produce catalepsy at any of the time intervals. As expected; Haloperidol showed highly significant ($p < 0.001$) catalepsy at all time intervals. Haloperidol ($p < 0.001$) produced significant catalepsy in comparison to *Goghrita*, UGK 7.8mg and 15.6mg. It is evident from the statistical analysis that UGK does not produce catalepsy.

DISCUSSION

Unmadgajakesari rasa [UGK] is a herbomineral formulation; having minerals like mercury, sulfur and herbs like *Vacha*[Acoruscalamus], and *Shankhapushpi*[*Convolvulus pluricaulis*]. In our study UGK was found to be safe upto 2000mg/kg. No signs and symptoms of toxicity or mortality were observed.

UGK is used clinically to treat Psychosis and epilepsy. In present study, antipsychotic activity of UGK and its extra pyramidal adverse effect if any was evaluated.

The most widely used in-vivo models to test antipsychotic activity has been the 5HTP induced head twitches and for extra pyramidal adverse effect catalepsy model in normal mice/rats. Hence the antipsychotic activity of UGKand its extra pyramidal adverse effect were evaluated in these models in our study. In 5HTP induced head twitches model the results obtained in all the 4 groups were compared that indicates there is significant difference between the mean numbers of head twitches. Standard drug Olanzapine showed significant reduction in number of head twitches when compared to all other groups.UGK 7.8mg and 15.6 mg for 20gm mice per oral showed significant ($p<0.001$) reduction in the mean number of head twitches when compared with *Goghrita*treated group. Though in comparison with Olanzapine, UGK 7.8mg and 15.6 mg shows more number of head twitches, they are significantly less when compared with control group *goghrita*. Thus it can be said that UGK has moderate antipsychotic activity.Catalepsy in mice model in our study it was essential to administer Haloperidol through oral route to avoid bias in route of drug administration. But oral dose of Haloperidol that produces catalepsy was not known. Pilot study was done to find out oral dose. The first step 1mg/kg,

Haloperidol was given orally to three animals and assessment of catalepsy was done. 30 minutes later, not a single animal showed catalepsy. In the second step another three animals were given Haloperidol in a dose of 1.5mg/kg (orally).

Animals got catalepsy in 15 minutes. Hence the study was conducted at the dose level of 1.5 mg oral administration instead of intra peritoneal.In catalepsy model, UGK 7.8 mg and 15.6mg did not produce significant catalepsy at all intervals. Haloperidol showed highly significant ($p < 0.001$) catalepsy at all time intervals. Test drug UGK for 20gm mice per oral, shows similar values with that of *Goghrita* e. g. at the interval of 5 minutes, *goghrita* is at 7.17 ± 1.72, UGK 7.8mg is at 6.17 ± 2.56 and UGK 15.6 mg is 9.00 ± 2.68 as against that of haloperidol 25.33 ± 12.99. There is a significant difference [$p < 0.001$] in both the dose levels of UGK. However, the drug does not produce catalepsy as compared to that of Haloperidol [standard drug] ($p < 0.001$) at both dose levels.

UGK formulation contains *Kajjali* (combination of mercury and sulfur). Mercury affects Central nervous system (CNS) as it easily crosses the blood- brain barrier, gets accumulated in the brain thus affecting multiple cellular functions.[22]Mercury readily forms covalent bond with sulphur. This property accounts for most of the biological properties of the metal. It is reported that addition of sulfur counteracts the toxicity of mercury.[23]*Kajjali* own properties as *yogavahi* (catalyst) which helps in carrying other drugs to CNS and enhance the efficacy and potency of the formulation.[24]

Herbs like *Vacha* [Acoruscalamus] is used in the treatment of insomnia, melancholia, neurosis, epilepsy and other mental disorders either alone or as a component of *Ayurvedic* preparations[25]. Its active constituent being α and β asarone.[26]Recently it has been reported that *Vacha* has anti-stressor activity and prevents stress induced changes in the rat brain by its antioxidant activity.[25] It is also used as a sedative, tranquillizer, anxiolytic, nervine tonic and memory enhancer. Its rhizome is used as an intellect-promoting agent against depression, mental disorders and general debility. [25]

Shankhapushpi[Convolvulus pluricaulis] has been widely screened for its various pharmacological activities. It has relatively well documented neuro-pharmacological actions such as antioxidant[27,] psychotropic effect[28], anxiolytic[29], antidepressant[30], anticonvulsant[31], tranquilising and sedative activities which justify its use in central nervous system diseases in the *Ayurvedic* system of medicine. Whole plant is used to treat various brain disorders like insomnia, loss of memory, mental as well as physical fatigue anxiety, stress and neurodegenerative disorders.[32]

According to *Ayurved*cow's urine is *Medhya* but no reported study was found that single cow's urine shows activity towards CNS system. Cow's urine as an ingredient of formulation shows activity towards CNS system.[33]The plant material i.e. *Vacha* and *Shankhapushpi*used in UGK are shown to possess antioxidant activity.

Goghrita(cow's ghee)is *medhya* according to *Ayurved* and reported studies shows that different medicated *ghritas* are memory enhancer, anti-depressant[34] and anticonvulsant[34]. There is correlation between *snehakalpana*and liposome from this correlation we can conclude that fat-encapsulateddrug can absorbed and distributed better.[35]

Though the exact mechanism of UGK is not clear, it appears to produce antipsychotic effect mainly mediated through blocking 5HT receptors. Antioxidant action of herbs present in UGK would impart additional neuro-protection.

UGK is a combination of minerals, herbs and animal product processed in traditionally validated methods. The probable action of this formulation could be by improving the therapeutic properties of each other with the increase in bioavailability of the formulation.

CONCLUSION

UGK appears to have significant antipsychotic activity after prolonged administration. The minerals, herbs and animal product together

probably balance the excitatory and inhibitory neurotransmitters in CNS, the main action being serotonin pathway and additional antioxidant activity of herbs. The combination of mineral with herbs seems rational. This study also supports the claim in *ayurveda*, of UGK being useful in treatment of psychosis. However research is still needed to clarify the development of antipsychotic activity only after prolonged use of UGK.

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