Research Article

Interaction of herbal formulation and conventional drug on anxiolytic potential in mice

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Abstract

Objective: The present study was designed to evaluate the interaction among the herbal formulation with the conventional drugs in the treatment of anxiety in rodents. Present study deals with the pharmacodynamics interaction of conventional Diazepam with Ashwagandha churna. **Material and Methods:** Interaction was studied by the influence on the anxiolytic effect of the standard drug in Elevated plus Maze apparatus and light/dark model induced anxiety in Swiss albino mice (male) weighing between (28-35gm). The study also includes the standardization of selected herbal formulation with various parameters like Ash value, extractive value, moisture content, pH and phytochemical investigation. Acute toxicity studies (50mg/kg, 300mg/kg and 2000 mg/kg p. o.) were conducted to determine the safe dose as per OECD-423 guidelines. Four Groups of animals (n=6) were used in this study. Group I was disease control group, Group II received Ashwagandha churna, Group III received Diazepam and Divya ashwagandha churna and Group IV kept as standard drug (Diazepam) treated. **Results and Conclusion:** No sign of toxicity was observed. The test sample increases the time spent and number of entries in open arms but the combination of Diazepam and test herbal formulation decreases the time spent and number of entries in open arms in E.P.M. Data of this method was analysed by annova and dunnetts multiple comparison at $p<0.01$. In conclusion, the results suggest that there exist an interaction between conventional Diazepam and herbal formulation which was a negative type.

Keywords: Anxiolytic, Ashwagandha churna, diazepam, light/dark arena

Introduction

When two or more drugs are given to a patient simultaneously a drug interaction occurs. The effects of the drugs may increase, decrease or a new effect may be produced that neither produces on its own. As drug-drug and drug-food interactions, drug- herb interactions are also very common. Herbal medicines can inhibit, exaggerate or cancel the actions of a prescription drug. As herbs can affect body functions drug interactions are possible when herbs are taken concurrently with drugs. If an interaction between a drug and an herb does occur, conventional drugs are usually thought to be responsible because they are more pharmacologically active. Unlike conventional drugs, herbal products are not regulated for purity and potency. Thus, some of the adverse effects and drug interactions reported for herbal products could be caused by impurities (e.g., allergens, pollen and spores) or batch-to batch variability. In addition, the potency of herbal product may increase the possibility of adverse effects. Physicians must be alert for adverse effects and drug interactions associated with herbal remedies, and they should ask all patients about the use of these products (Sandozi, 2012; Barrett, 2004). Pharmacokinetic Interaction; Herb-drug interactions which disrupt the absorption, distribution, metabolism and excretion of a drug are considered to be pharmacokinetic interactions (Table 1). Ingested agents decrease or increase the absorption of other drugs from the gastrointestinal tract (GIT). In other cases, the metabolism, distribution and excretion of a compound may be affected. The pharmacokinetic properties (such as Tmax, Cmax or AUC) of these agents are modified through drug-herb interactions, 62 such that drug efficacy and ultimate toxicity is

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subsequently affected. This occurs more frequently for drugs possessing narrow therapeutic windows, such as digoxin (Brahmanker and Jaiswal, 2009; Laurence et al., 2008). The present study was designed to evaluate the interaction among the herbal formulation with the conventional drugs in the treatment of anxiety in rodents.

Material and methods

Drugs and chemicals

Drug- Diazepam, Ashwagandha churna and chemical-methanol, iodine, potassium iodide, conc. Sulphuric acid, chloroform, hydrochloric acid, acetic acid, Sodium hydroxide, Hagner's reagent, Wagner's reagent, Mayer's reagent, Dragendroff's reagent, gelatin, molisch reagent, glacial acetic acid, ferric chloride, sodium bicarbonate, acetic acid, gum acacia. Alls drugs and all chemicals were of analytical grade.

Animals

Swiss albino mice (28-35gm) were used in the present study. The animals were procured from disease free small animal house. They were provided normal diet and tap water and were exposed to 12 hour Light and 12 hour dark cycle. The animal was acclimatized to the laboratory condition before experiments. Experimental protocol was approved by Institution (IPS-College of pharmacy) animal ethics committee. Care of animal was taken as per guideline of the committee for the purpose of control and supervision of experiments on animal (CPCSEA), ministry of environment and forest government of India. Experimental protocol was approved by Institution animal ethic committee. The animals were kept in polypropylene cages under standard laboratory condition. The animal house was maintained at 27°c ±2°c temperature and 50 to 60% humidity. The animals were obtained from animal house of institute (IPS-College of pharmacy, Gwalior).

Diazepam: 1mg/kg, Ashwagandha churna (DAC): 520mg/kg. Dose of Ashwagandha churna and diazepam calculated for rats in the present study from the available therapeutic dose for the human use. The therapeutic dose was calculated by using dose conversion table (Paget and Barnes, 1964).

Extraction of plant material

Powder was taken and weigh for the further evaluation. Hydro alcoholic solvent system was the preferable solvent system for the study as per the available literature. Simple maceration technique of extraction was followed to get the purified drug for further investigations (Paget and Barnes, 1964).

Preliminary Phytochemical study

Determination of ash value, Loss on drying, determination of extractive value, determination of pHvalue.

Quantitative studies

T.L.C. (Thin layer chromatography) (Khandelwal, 2006)

Thin layer chromatography is the separation of mixture into individual components using stationary phase and mobile phase. Mobile phase prepared by taking methanol + water (distilled water) in the ratio 6:4. Sample A (Ashwagandha Churna powder) and Sample B (Ashwagandha extract) were dissolved in methanol and the sample were spotted on the chramatoghraphic plate.

Retardation factor R_f is defined as:

$$Rf = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

High performance liquid chromatography (HPLC) **(**Kathleen and Gregory, 2009)

HPLC mainly utilizes a column that holds packing material (stationary phase), a pump that move the mobile phase(s) through the column, and a detector that shows the retention time of the molecules.

HPLC of Ashwagandha churna

Preparation of mobile phase:

Acetonitril : water (6:4)

Wave length : 215 nm

Flow rate : 1ml/min

Preparation of Standard solution/Sample solution: Taken 100mg (std) drug/Sample dissolved in 100ml of mobile phase, prepared the solution of concentration 1000µg/ml. Taken1 ml of 1000 µg/ml of solution then volume was make up to 10 ml, prepared the solution of concentration 100 µg/ml. Taken 0.5 ml of 100 µg/ml of solution then volume was make up to 10 ml and thus prepared the solution of concentration 50 µg/ml.

Acute oral toxicity study

Acute toxicity study was carried out to determine the safe dose by acute toxic class method of oral toxicity as per Organization for Economic Co-operation and Development (OECD) 423 guidelines(Anonymous, 2001).

In vivo study

The animal was divided into 4 groups, and each group contains five animals. The cages were labeled properly with number, age and sex and weight of animals. The animals were marked with permanent marker on tail for identification. The groups were as follows:

Animal groups and protocol

- Diseased control group.
- Ashwagandha churna (test drug) treated group.

- Diazepam and Ashwagandha churna treated group.
- Diazepam (standard drug) treated group.

Elevated plus maze apparatus

Elevated plus maze is the simplest apparatus to study anxiolytic response of almost all type of antianxiety agents. Exposure of the animals to novel maze alley evokes an approach avoidance conflict which is stronger in open arm as compared to enclosed arm.. Plus maze consisted of two open arms (50×10cm) and two enclosed arms (50×10×40cm) with an open and is elevated to a height 50cm, stop watch.

Weigh and number the animals. Divided them each group each consist of one group used as control and other for drugs treatment. Animals was placed individually in the center of the maze, head facing towards open arm and start the stop watch and note following parameters for five minutes. (a) First preference of mouse to open or enclosed arms, (b) Number of entries in open or enclosed arm (An arm entry defined as the entry of four paws into arm). (c) Average time each animal spends in each arm (Average time = total duration in the arm/number of entries).

Drug was injected to the test group. After 30 minutes rats were placed individually in the center of the maze and noted all parameters as described under step 2. Finally, it was compared the preference of the animal to the open/enclosed arms, average time spent in open arm and number of entries in open arm in each group(Kulkarni, 2005).

Light and dark model

Crawley and Goodwin described a simple behavior model in mice to detect compounds with anxiolytic effects. In a two chambered system, where the animals can freely move between a brightly light open field and dark corner, they show more crossing between the two chambers and more locomotor activity after treatment with anxiolytics. The numbers of crossings between the light and dark sites are recorded. A polypropylene animal cage, 44×21×21cm, is darkened with black spray over one third of its surface. A partition containing a 13 cm long×5cm high opening separates the dark one third from the bright two thirds of the cage. The cage rests on an animal activity monitor which counts total locomotor activity. Male mice are place in to the cage. The animals are treated 30 min before the experiment with the test drugs or vehicle intraperitoneally and are then observed for 10 min. Groups of animals are used for each dose. The number of crossing through the partition between the light and the dark chamber are compared with activity counts during the 10 min(Kulkarni, 2005).

Statistical Analysis

The results were expressed as the mean ± SEM for each group. Statistical differences were evaluated using a One-way analysis of variance (ANOVA) followed by Tukey's t-test. Results were considered to be statistically significant at $P<0.05$.

Results

Standardization of Divya Ashwagandha churna

It was standardized for its Ash content (Total ash), Acid insoluble ash, Water soluble ash, Ethanol soluble fraction, Water soluble, Moisture content (Loss on Drying) and pH (1% aqueous solution). Table 1 depicts the results of standardization.

Table 1. Values for various parameters for Standardization of Ashwagandha churna

Standardization Parameter	Value
Ash content (Total ash)	6.4%
Acid insoluble ash	2.6%
Water soluble ash	5.6%
Ethanol soluble	5%
Water soluble	2.5%
Moisture content (Loss on Drying)	4%
pH (1% aqueous solution)	5.44

Quantitative evaluation

T.L.C (Thin layer chromatography)

Thin layer chromatography was done and Rf value was calculated by the following formula:

$$\text{Rf} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

The Rf was calculated for sample A 0.87 and for sample B 0.85.

HPLC evaluation of Ashwagandha churna

High Performance Liquid Chromatography (HPLC) of sample (50µg/ml) and standard (50µg/ml) was analysed. Table 2 and 3 depicts the HPLC evaluations respectively.

Acute oral toxicity for Ashwagandha churna in Swiss albino mice (male)

Acute oral toxicity was observed for different doses such as 50 mg/kg, 300 mg/kg and 2000 mg/kg. No sign of toxicity was observed after 24 h. Table 4 shows the acute oral toxicity.

Anxiolytic effect

In elevated plus maze, total no. of entries and time spent in open arms were recorded for 05 minutes. Test sample treated group exhibited anxiolytic effect with increased time spent 3.024±0.23 and no. of entries 9.49±0.96 in open arms. It demonstrated a significant anxiolytic potential

Table 2. Analysis of sample (50µg/ml) by HPLC

S.No	Retention.Time (Min)	Area (mV.s)	Height (mV)	Area (%)	Height (%)	WOS (min)
1	2.187	22.424	2.938	16.5	31.1	0.12
2	2.397	26.410	1.340	19.5	14.2	0.35
3	3.057	44.349	2.395	32.7	25.4	0.33
4	3.420	40.066	2.581	29.6	27.4	0.26
5	4.367	2.324	0.182	1.7	1.9	0.21
	Total	135.573	9.435	100.0	100.0	

Table 3. Result analysis of standard (50µg/ml) by HPLC

S. N.	Retention Time (min)	Area (mV.s)	Height (mV)	Area (%)	Height (%)	W05 (min)
1	0.783	11.205	0.492	11.4	6.4	0.37
2	2.173	16.775	1.696	17.1	22.2	0.18
3	2.400	21.641	1.731	22.1	22.7	0.25
4	3.167	8.105	0.740	8.3	9.7	0.16
5	3.720	36.579	2.507	37.3	32.9	0.24
6	4.877	1.502	0.140	1.5	1.8	0.14
7	5.333	2.299	0.321	2.3	4.2	0.12
	Total	98.106	7.627	100.0	100.0	

Table 4. Acute oral toxicity for Ashwagandha churna in Swiss albino mice (male)

S. N.	Dose	Observation period (24 hr)	Observation period 14 days
1.	50 mg /kg	All animals survived	No sign of toxicity, normal diet and feeding.
2.	300 mg/kg	All animals survived	No sign of toxicity, normal diet and feeding.
3.	2000 mg/kg	All animals survived	No sign of toxicity, normal diet and feeding.

Table 5. Result of EPM showing the number of entries and time spent in both the arms

S. N.	Groups	Time Spent (mean±SEM)		No. of entries (mean±SEM)	
		Open arm	Closed arm	Open arm	Closed arm
1	Disease control group	0.175 ± 0.06	3.776±0.125	1.233±0.221	7.966±1.013
2	Test Sample treated group	3.024±0.23**	0.448±0.10**	9.499±0.96**	2.566±0.179**
3	Test sample and std drug treatred group	0.500±0.05*	2.900±0.12**	3.266±0.34*	8.66±0.48*
4	Standard drug	2.812±0.200**	0.567±0.75**	8.332±0.48*	2.931±0.201*

P< 0.0001 considered Extremely Significant* P> 0.05 - Non Significant; **P<0.01 - Compared to control

when compared to disease control group. Table 5 exhibits the anxiolytic effect.

In light dark arena model, total no. of entries and time spent was recorded in open arms same as above. Test sample treated group exhibited anxiolytic effect by increasing the time spent 6.588 ± 0.069** and no. of entries 13.12 ±0.120** in open arms. Table 6 depicts the anxiolytic effect.

Discussion

The physiochemical parameters viz. ash content, extractive value, moisture content and pH indicated that the formulation Ashwagandha Churna (AC) was intended for study of inquisite pharmacopoeial standard. Phytochemical analysis is very important in the evaluation of active biological component of plant. The phytoconstituents

Table 6. Result of L.D.M showing the number of entries and time spent in both arms

S. N.	Groups/ Dose	Time spent in open arm(Mean ±SEM)	No. of entries in open arm (Mean±SEM)
1.	Disease control group/Normal saline	3.3392 ± 0.140	5.96± 0.354
2	Test Sample treated group/520mg/kg	6.588 ± 0.069**	13.12 ±0.120**
3	Test Sample and Standard drug treated group	4.0256± 0.300**	5.48 ± 0.338*
4	Standard drug treated group (1mg/kg)	6.386 ± 0.065**	11.48± 0.3720**

P< 0.0001 considered Extremely Significant; * P> 0.05 - Non Significant; **P<0.01 Compared to control

quantified in the present study exhibit great deal of medicinal importance specially Alkaloid part plays major role in anxiolytic activity. Quantitative estimation of ashwagandha churna, T.L.C. spot of sample was very nearest to standard sample spot and HPLC analysis of herbal formulation sample value almost similar to standard sample value.

Acute oral toxicity study of the AC (50mg/kg, 300mg/kg and 2000 mg/kg orally) revealed that there was no toxicity of any nature, all animals survive during observation period.

The study was undertaken in view of the therapeutic use of AC in Ayurveda system of medicines for treatment of various diseases like nature stress increasing body strength, treatment of body weakness anxiety, increase memory, brain power, cordial disorders, antioxidant, cancer etc. The anxiolytic activity effects of Ayurvedic formulation were investigated in present study and also investigated interaction study of allopathic drug.

The EPM is the prime apparatus that is responsible for finding the anxiolytic potential of a drug. The Treatment AC (520 mg/kg) revealed anxiolytic activity, since the number of open arm entries and time spent in open arm parameters was most delegate guide for anxiolytic activity (Kokate, 2006). When used in combination AC (520 mg/kg) with Diazepam (1 mg/kg) had shown a non significant anxiolytic activity and decrease in open arm entry and time spent in open arm.

Light dark box is the prime apparatus for screening of anxiolytics. It is carved in such a way to observe trend of rodents to analyze a novel environment when confronted with aversive properties of brightly illuminated area. Anxiolytic tend to increase the time spent in light area and increase the number of entry in light area. The treatment AC (520 mg/kg) revealed anxiolytic activity, since the time spent in light, area increase and increases the numbers of entry in light area parameter was most delegate guide Po anxiolytic activity. When used in combination AC (520 mg/kg) with diazepam (1 mg/kg) had shown a non significant anxiolytic activity and decrease time spent in light area and decrease No. of entry in light area. In our study, we demonstrated significant antianxiety activity when used diazepam but used combination AC and diazepam non-significant anxiolytic activity shown(Shri, 2008).

Conclusion

Results obtained in the present study suggest that the herbal formulation shows significant antianxiety activity when AC and Diazepam were used separately. While in case of combination the results obtained were not significant this may be due to some interaction among AC and Diazepam. However further studies are required at molecular level to evaluate its exact mechanism of action and specific interaction among the use of combination of herbal formulation and Diazepam.

Conflicts of interest: Not declared.

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