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Research Article

A PHARMACOLOGICAL STUDY ON THE EVALUATION OF RASAYANA DRUGS FOR PHYSIOLOGICAL CHANGES IN EXPERIMENTAL MODEL.

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ABSTRACT:

Rasayana drugs are well known for their therapeutic efficacy in human being for its action on physical and mental health. Any new formulation should not be tried directly on children instead it should be studied in experimental model to observe any side effect and to avoid biasness. So, a pharmacological study was conducted to know the efficacy of Rasayana Yogas containing Mandukaparni, Yastimadhu, Guduchi, Shankhapushpi, Aswagandha and Satavari in granules form on mice and rat comprising of three groups for its Anabolic Immunomodulation and Adaptogenic effect. Result was encouraging for wt gain ,cell mediated immunity with percentage changes for Humoral immunity and Adaptogenic action in treated groups compared to control group details of which has been mentioned in main paper.

KEY WORDS: Rasayana, immunity, animal study, Immunomodulation.

INTRODUCTION:

Growth and development are two undifferentiated complex characters of childhood period from the conception till maturity. Both are interrelated but not exchangeable. Between these two characters, Growth is implying to physical changes which can be measured easily and influence development in early childhood period. So,efforts are being taken by physicians, pharmacologists and scientist to maintain the Growth through intervention by holistic approach. Rasayana drugs like Aswagandha, Satavari ,Yastimadhu and Guduchi are well known and were extensively used by ancient vaidyas to improve general health of an individual and the children at large.

Before considering any formulation, safety for an individual or in children especially, it is worth trying in experimental model like mice and rats which may do well are obligatory. The main disadvantage of experimental study is the effect of any drug may not be the same in all species including human. The differences lie in physiology and body constitution. Hence it is necessary to use appropriate model.

Acharya charaka had mentioned that a disciple should always make efforts for the upliftment of knowledge with adoption of suitable methods to acquire proper knowledge. Ayurveda depicts few reference of animal experimentation by using animals in order to prevent from toxicity related unwanted hazards on royal family .It is clear that comparison of humans with animals with respect to their physiological and pathological aspects were existing during ancient periods.

Taking the concept of physiological changesfrom the Ayurvedic point of view in present study Brumhana, Balya as well as rasayana effect of the drug has been emphasized which has been studied pharmacologically by using Anabolic, immunomodulater and adaptogenic test models.

The present formulation Rasayana Yoga containing Aswagandha, Satavari, Yastimadhu, guduchi, sankhapuspi and mandukaparni {Cha Chi} in granules form was tried for its effect on experimental models having Brumhana, balya and rasayana properties. No previous study has been conducted on this particular formulation experimentally. Keeping this point in mind the present study was planned with following aims and objectives.

AIMS AND OBJECTIVES:

1) To evaluate the efficacy of Rasayana yoga on animals for anabolic, immunomodulation and adaptogenic activity.

2) To find out the role of trial drug in experimental models.

MATERIALS AND METHODS:

Animals-healthy Charles foster albino rats of either sex weighing between 140gm -240 gm and albino mice of either sex weighing between 20g-40gm were used for experimentation. The animals were obtained from Animal house attached to IPGT&RA,, GAU, Jamnagar after protocol was approved by Institutions ethics committee .Animals were kept in polypropylene cage with top stainless steel grill. They were maintained on amrit brand pellets obtained from chakan mills pune. Both food and water was given adlibitum. All the animals were kept in the same environment. Six animals of either sex were allotted in each group based on random number generation. The animals were marked with saturated picric acid for proper identification.

Husbandry Conditions-

Animals were kept in polypropylene cage with top stainless steel grill. Paddy husk was used as bedding material for animals. The animals were exposed to light and dark cycle with relative humidity of 50-70% and ambient temperature maintained at around 22± 03degree c. All animals were kept in same environmental conditions.

Preparation for test formulations for administration:

For all tests combined form of Rasayana yoga containing Mandukaparni, Yastimadhu, guduchi, Shankhapuspi, Aswagandha and satavari in granules form was prepared in the pharmacy of Gujarat Ayurveda university, Jamnagar. All the herbal drugs were made into decoction before the formation of granules. The ratio of sugar to the drugs in the granules was 2:1.

Dose: Dose of the drug was calculated by extrapolating the human therapeutic dose to rat on the basis of body surface area ratio (conversion factor 0.018 for rat and 0.0026 for mice) using the table of Paget and Barnes(1964).

Trial drug-The adult dose of trial drug was 10 gm.It was considered for the study.It was triturated with distilled water in a specific proportion to administer the drug in the forms of drops.

Route of drug administration- oral route with help of a no.3 gastric catheter sleeved on to a syringe for rats & incase of mice p.42 plastic tube was used for oral feeding.

Time of drug administration-Between 8.30 am-9.30 am

Instruments Used-

Weighing scale, micro liter plate,serological water bath, syringe, catheter, surgical instruments, centrifuge machine, refrigerator and swimming jars.

Chemicals Used-

Normal saline, Alsever's solution and formaldehyde and cyclophosphamide .All the chemicals were procured from the standard and reputed firms.

Experimental models

1.Anabolic study- Effect on body wt. of albino rats.

2.Immunomodulator study- For humoral and Cell mediated immunity.

3.Adaptogenic activity- Swimming endurance induced changes in rectal temperature.

Procedure for Anabolic study-

For the anabolic effect of test drug R. Yoga 18 albino rats of both sexes were taken in the body wt of 60-80 g and divided in three groups. Soluble form of test drug was administered with the help of rubber catheter in low and high dose for 30 days.The wt. of the rats were noted before, during and after the drug administration. The change in body wt of both female &male were noted along with anthropometric parameters (Mid Arm circumference, Mid leg circumference, chest circumference, snoot to Neck circumference, neck to root of tail, root of tail to end of tail.

EFFECT OF TEST DRUG ON BODY WEIGHT OF ALBINO RATS

Table 1

Group	Dose Mg kg-	Initial Wt. Mean±SEM	After 10 days	% changes	20 days	% changes	30 days	% changes
Contol Gr.	-	71±4.58	114±3.46	60.56↑	143±01	102.85↑	173.33±3.33	146.25↑
R.Yoga(3)	500	82±6.11	131.33±3.42*	61.04↑	164±6.66*	100.00±	186.67±3.33*	129.73↑
High dose(2)	1000	74±3.06	124±9.89	68.24↑	180±10*	141.96↑	195±15	162.5↑

(Gr: Group; R. Yoga: Rasayan Yoga;)

The data depicted in the table 1 shows effect of test drug on body wt. was significant after 10 and 20 days in low dose Gr. Highly statistically significant effect was found in high dose gr. After 30 days of treatment. The body wt. was marginally low in control group. Effect of test drug on anthropometric measurement in male rats was statistically significant.

The data related to the effect of drug on the body wt. of female albino rats was higher in trial groups compared to control group. However changes in anthropometric measurement of the female rat were insignificant statistically.

Experiment 2:

For this experiment mice of either sexing the body wt. ranges between 20-30 g were selected and divided in three groups. Mice in Gr. A were trial drug R. yoga in high dose, In Gr.-B the drug R. yoga were administered the in low dose. In control Gr. C tap water was given. The drug was administered for 10 consecutive days. On 3rd day, SRBC was injected intraperitonially as sensitizing agent. in the dose of 10ml/kg. The SRBC was collected from a slaughter house in a sterilizing bottle and prepared for experimentation .on 10th day of drug administration the blood was collected from the mice and was used for both sensitization and to determine the antibody titer after serum separation and two fold dilution by normal solution kept in a micro litre plate . 0.1ml of thrice saline washed 2% SRBC was added to each wall of the tray. The tray was covered over night to observe antibody titer next day.

Effect of test drug on Cell Mediated immunity-

The test drug was evaluated to assess its effect on cell mediated immunity by noting its effect on immunological inflammation produced by pedal injection of a suspension of SRBC. Sterile thrice washed SRBC was injected 0.2 ml /100g body wt. on the first day of drug administration. The drug was administered for six days. Injecting 0.05ml of similar suspension in the right hind paw on the sixth day of sensitization induced immunological edema which was measured by volume displacement method before sensitization, 24 and 48 hrs after the second injection of SRBC into hind paw. Percentage increase in paw volume after SRBC in comparison to initial value was noted.

OBSERVATIONS AND RESULTS:

EFFECT OF R. YOGA ON ANTIBODY FORMATION:

As shown in the table 2 the percentage the test drug didn't affect the antibody formation against SRBC significantly. The % decrease in antibody formation was 6.30 in low dose group whereas percentage increase was 14.41 in high dose treated group.

EFFECT OF R.YOGA ON CELL MEDIATED IMMUNITY:

The data pertaining to the effect of the test drug on cell mediated immunity after 24hrs and 48hrs have been shown in the table. The drug induced immunological paw edema measured after 24 and 48 hrs in comparison to control group. Statistically significant effect was found in high dose group whereas percentage change was observed in low dose group which was insignificant statistically

Table 2

GROUP	Dose Gkg-1	Haemagglutination Titre log 2 Mean ± SEM	Percentage of changes
Control(6)	-	6.66 ±0.58	
R. Yoga(4)	1.3	6.24 ±0.63	6.30↓
R. Yoga(4)	2.6	7.62±0.57	14.41↑

Table 3

Group	Dose g kg—1	Mean ± SEM Percentage increase in paw volume			
		After 24hhrs	%	After 48 hrs	%
Control(5)	-	51.59±13.70		168.06±31.41	
R. Yoga(4)	1.3	86.16±18.86	67%	238.97±44.13	44.19↑
R. Yoga(3)	2.6	71.07±28.65	37%	254.76*±12.04	50.99↑

ADAPTOGENIC ACTIVITY

Procedure:

The probable adaptogenic activity in the test drug was assessed by noting the effect of pre treatment on temperature test drugs on swimming induced changes in rectal temperature of rats. The experiment was employed to assess the drug effect on swimming endurance induced changes in rectal temperature of rats after modification by Bhargava and Singh.

12 albino rats of both sexes in the body weight range between 170-210 g were taken and randomly divided into two groups after recording of body wt. Six rats were taken in control group and given distilled water till the experiment whereas the six rats were in the treated group administered rasayana yoga for 8 days once daily .The rats of the group were subjected to acute swimming stress I a specially designed jars .The rats were subjected to immersion in water for 40 minutes , one hr after drug administration. The observation made was compared with the control.

EFFECT OF TEST DRUG ON STRESS INDUCED HYPOTHERMIA IN RATS:

Table 4

Group	Dose mg/ kg-1	Hypothermia (0C) Mean ± SEM	Percentage of changes
Control (6)	-	4.1±0.66	
R. yoga	1000	4.3±0.63	4.87

The data presented in above table 4 shows statistically non-significant effect on rectal temperature in the treated group in comparison to the control group. The percentage increase in temperature was 4.87 in treated group.

DISCUSSION:

Anabolic study:

From the observation made with respect to effect of drug on body wt. it was evident that the test drug possesses anabolic effect. It was non-significant for female rats. The increased body wt. especially in male rats indicates positive effect on nitrogen balance in the body.It may be mediated through androgenic activity. Aswagandha and satavari in the formulation are well known for their action on male

reproductive system working as Vrusya for sukravardhana property. It needs to ascertain the effect on clinical study in both sexes as it has been universally accepted for the action of rasayana irrespective of sexes.

Immunomodulation activity**:**

Immunomodulation activity in the test drug was assessed for both antibody and cell mediated immunity effects. The drug didn't affect antibody formation against SRBc significantly. However, moderate statistically non-significant increase was observed at higher dose level.

Data obtained from immunological paw edema test shows that the test drug has moderate stimulation effect at lower dose level and significant stimulation at higher dose level. It is a well-known fact that CMI is mediated through lymphocytes. It is possible that the active principles present in the test formulation may be acting at the induction stage, through stimulation of cytotoxic cells, through stimulation and release of cytokines and activation of macrophages to enhance the CMI.

Adaptogenic activity-

The probable adaptogenic activity in the test drug was assessed by noting the effect of pre treatment of test drugs in swimming induced changes in rectal temperature. The observed magnitude of change with test drug did not reach statistically significant level. However, apparent changes is an indicator of future study with modification of dose as the drug is rasayana in nature.

SUMMARY AND CONCLUSION:

The experimental study was undertaken to avoid the biasness of clinical study in future and lay a strong foundation work to to prove the validity of the study. Brumhana or anabolic effect of the drug was studied by anthropometric measurement before and after the drug administration .The percentage change so observed was statistically calculated. Immunomodulation activity was observed by the established model for vyadhikshamatwa i.e. for humoral immunity and cell mediated immunity. Adaptogenic activity in the test drug was assessed by noting the effect of pre-treatment of test drug in swimming induced changes in rectal temperature

CONCLUSION:

The principle of Ayurveda with the support of modern parameter is efficacious for the assessment of physical and physiological changes. Rasayana drugs are well known for their action on physical and mental health. In the present study combination of medhya rasayana with balya and Brumhana drug proved to be useful in enhancing Vyadhikshamatva, Brumhana and Rasayana effect through Anabolic, immunomodulatory and Adaptogenic study in the experimental model.

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