To Establish The Involvement Of Central Dopaminergic And Serotonergic Mechanism Of Ginger-Juice On Amphetamine And Lithium Treated Rat

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Abstract: <u>Background & Objective</u>: To establish the involvement of central dopaminergic and serotonergic mechanismof ginger-juice (ZINGIBER OFFICINALE ROSCOE) CNS parameters in rat. <u>Methodology</u>: (A) Albino rats (n=6-12) were administered G.J at two doses (2ml & 4 ml/rat, p.o) as single administration and chronic treatment over period of 30 days. Following this assessment was done. Effect of treatment with G.J acutely and chronically (30days) administered, was assessed. Parameters used during assessment were total acidity, gastric volume & pH and ulcer index 'UI'(acute and chronic). <u>Results & Conclusion</u>: Ginger administered itself did not affect loco motor activity as well as amphetamine-induced enhancement of loco motor activity.Lithium induced head twitches were enhanced after chronic administration indicating enhanced serotonergic system in the central nervous system. [Prasad S NJIRM 2016; 7(1):59-66]

Key Words: Ginger-juice, Amphetamine, Lithium, Locomotor, static activity, analgesic.

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Introduction: A Ginger is one of the most important and oldest spices, consisting of the prepared and sun-dried rhizomes of Zingiberofficinale (Zingiberaceae). It is cultivated in many tropical countries. It is produced all over India from ancient times. It has a good commercial value and is claimed to have many medicinal uses. Because of differences in cultivation pattern, harvesting technique and climatic conditions it's commercial value differs and so also the medicinal actions and uses. It is referred by different names in the languages of different regions and countries.

It is widely consumed almost all over the world however in tropical countries or warm regions like Asia, it is more popular (Katiyar et al., 1996)¹. Because of its typical taste and a pleasant odor it's widely used as flavoring agent in numerous food recipes, beverages, pickles, many popular soft drinks etc (Guenther, 1952)².

From the ancient times it is included in many traditional medicinal systems for treatment of number of diseases. It is widely claimed as a Stomachic, aromatic, carminative, aphrodisiacs, diaphoretic, antiemetic, allergic rhinitis and gastric stimulant and for treating migrane headache. It is also used an antispastic against intestinal colic. Ginger oil is used in mouthwashes and liquors(Evans et al., 1989)³.

Many varieties of ginger are found such as processed, coated or unscraped, unbleached (natural) and bleached ginger having different types of active principles present in the ginger. Many scientists have investigated the ginger oil and found about 50 constituents, mainly aroma, Starch, Volatile oil, Zingiberene, Gingerol, Oleoresin (Gingerin), Zingiberol, Zingerone, Shagaol etc. The acetone extract of ginger contains Zingerberone and ether extract contain Zingerone (Pungent principles).

In view of the available literature, we have tried to screen some actions of ginger-juice; as crude form of ginger. We presume that crude form contains majority of active principles, may be in very low concentrations. Keeping in minded some of its potential therapeutic applications we have carried out animal experiments to investigate the effects of ginger-juice on gastriculceration. We are carrying out animal experiments to investigate the effects of ginger-juice on central nervous system.

Serotonergic System:

Huang et al., (1990)⁴ have found that acetone extract of ginger rhizomes when orally administered, inhibited hypothermia and diarrhoea induced by serotonin. They found that the main active constituent against both disorders was 6-Shogaol, while other anticathartic components were 6-dehydrogingerdione, 8-Gingerol and 10-Gingerol. They isolated anti-5-hydroxytryptamine effect of active principle of galanolactone, diterpenoid from ginger.

Material and Methods: A keeping in view the aims and objectives, experiments were planned to study the effects of ginger in different physiological function.

Preparation of ginger-juice:

The commercially available ginger was obtained from the local market. It was confirmed from the botanist that it was Zingiber officinale. The rhizome of ginger after cleaning and scrapping the superficial skin was cut into small pieces. With the help of mixer-grinder the pieces were made in to paste. The paste was taken on a white clean cloth and the liquid was squeezed out. The juice so obtained was used in the experiments. The stock of juice was kept in a refrigerator for maximum period of 15 days and the required quantity was used for the experiments after removing particulate matter from it.

500gm ginger rhizomes yielded about 250ml juice. 250ml juice was filtered which yielded about 120 -150ml filtrate.

The liquid portion which was obtained in the course of filtration looked like yellowish hazy opalescent liquid. It was administered orally in acute or chronic experiments. The doses were 4 ml per rat in acute as well as in chronic (for 7 days).

Central Nervous System Functions:

The study was divided with the following aims

- A. Loco motor activity
- B. Lithium-induced head twitches
- *C.* Amphetamine induced locomotor activity

<u>A. Loco motor activity:</u> The loco motor activity of rats was studied using the Benwick activity monitoring equipment (Model-AM1051). Animal movements in principle were detected with help of two sets of infrared beams; Upper and lower; The lower set is used to detect normal movement and the upper set is used to detect if the animal is rearing. The activity detector operated by counting the number of times the beams changed from unbroken to broken (or visa-versa) and incrementing the relevant counters. The activity was split into 6 distinct types. The main distinctions were between mobile, static, fast and slow movement. Here, we have taken only four parameters under the loco motor activity which were as follows:

- 1. Mobile count- Slow
- 3. Rearing count- Slow
- 2. Static count- Slow
- 4. Active time- Total time

1. Study of acute and chronic (seven days') treatment of ginger-juice on loco motor activity in rats:

The rats were divided into following groups, each group consisting of 6-12 rats.

Control group:

Each rats received 4 ml of normal saline orally. After 30 minutes rats were placed in the Benwick activity monitoring equipment (AM1051) for loco motor activity monitoring. After placing inside the animals were given 5 minutes time for acclimatization and 30 minutes reading considered for data analysis.

<u>Test groups:</u>

(a) Effect of acute treatment of ginger-juice:

Each rat of this group was administered ginger-juice 4ml orally. After 30 minutes rats were placed in the activity monitoring and after 5 minutes acclimatization 30 minutes readings were considered for data analysis.

(b) Effect of chronic treatment of ginger-juice:

Each rat of this group was administered orally 4ml of ginger-juice for 7 days orally. After 24 hours of last dose of ginger-juice rats were placed in the activity monitor and data was recorded as described above.

2. Effect of acute and chronic treatment of ginger-juice on amphetamine (1mg/kg) induced changes on loco motor activity.

The rats were divided into following groups, each group consisting of 6-12 rats.

Control group:

Each rat received 4 ml of normal saline orally. After 30 minutes rats were placed in the Benwick activity monitoring equipment (AM1051) for loco motor activity monitoring, after placing inside gives 5 minutes times for acclimatization and 30 minutes readings were considered for data analysis.

Test groups:

(a) Amphetamine treated group:

Rats received amphetamine 1mg/kg i.p and after 15 minutes loco motor activity were recorded.

(b) Ginger-juice plus amphetamine group (acute study):

Each rat received ginger-juice 4 ml orally. After 15 minutes, they received amphetamine 1mg/kg i.p and then after 15 minutes rats were placed in the Benwick activity monitoring equipment for loco motor activity monitoring.

(c) Ginger-juice plus amphetamine group (chronic study):

Each rat of this group was administered ginger-juice 4ml orally for 7 days. After 24 hours of last dose of ginger-

juice rats received amphetamine 1mg/kg i.p, and loco motor activity was recorded as described earlier.

B. Study of acute and chronic treatment of ginger-juice on lithium-induced head twitches in rats.

The rats were divided into following groups, each group consisting of 6-12 rats.

Control group:

Each rat received normal saline 4ml orally. After 30 minutes rats received lithium 150mg/kg, i.p. injection and immediately placed in a transparent glass chamber, and start counting head twitches were counted over period of 40 minutes, this reading considered for data analysis.

Test groups:

(a) Effect of acute treatment of ginger-juice:

Each rat received 4ml of ginger-juice orally. After 30 minutes rats were given lithium 150 mg/kg, i.p. injection and immediately placed in a transparent glass chamber and head twitches were counted as described.

(b) Effect of chronic treatment of ginger-juice:

Each rat received 4ml of ginger-juice orally for 7 days. After 24 hours of last dose of ginger-juice rats were administered lithium 150mg/kg, i.p. and immediately rats were placed in a transparent glass chamber and head twitches were counted.

Results:

CENTRAL NERVOUS SYSTEM FUNCTIONS:

A. Locomotors activity:

1. Effect of acute treatment of ginger-juice (4ml/rat):

1. Mobile count-Slow:

The mean mobile count-'slow' in this vehicle treated group was 26.43 ± 8.84 minutes, while in the gingerjuice treated group it was 26.00 ± 12.06 minutes depicted in table-21. The difference between mean of both groups was statistically not significant. Results reflect that ginger-juice treatment has no effect on mobile count-slow.

2. Static count- Slow:

In the vehicle treated control group the mean static count-slow was 73.13 ± 16.63 minutes, while in gingerjuice treated group it was 57.83 ± 14.13 minutes. The difference between these counts is not significant statistically. The results are shown in table-21. This shows that ginger-juice treatment has no effect on static count-slow.

3. <u>Rearing count-Slow:</u>

The mean rearing count-slow in this vehicle treated group was 9.94 ± 3.66 minutes and in the ginger-juice treated group it was 20.46 ± 8.08 minutes. There is no significant difference between the two groups indicating that there is no effect of ginger-juice treatment on rearing count-slow. The results are shown in table-1.

4. Active time:

The mean active time in this vehicle treated group was 79.49 ± 12.44 minutes and in the ginger-juice treated group it was 68.96 ± 16.43 minutes. It is evident that this difference was not significant statistically. So it indicates that there is no effect of ginger-juice treatment on the active time. The results are shown in table-1.

The above results are shown in the following table-1.

Table 1: Effect Of Active Treatment With Ginger-Juice (4ml/Rat) On Locomotor Activity.

Parameters	Control (n=6)	Ginger (n=6)
	minutes	minutes
Mobile Count- S	26.43± 8.84	26.00 ± 12.06
Static Count-S	73.13 ± 16.63	57.83± 14.13
Rearing Count-S	9.94 ± 3.66	20.46 ± 8.08
Active Time	79.41 ± 12.44	68.96 ± 16.43

Table 1: It shows the effect of acute treatment with ginger-juice on the parameters like mobile count-slow, static count-slow, rearing count-slow and active time of loco motor activity in rat, compared to the vehicle treated control group. The statistical significance vis-à-vis the vehicle treated control is presented as *p<0.05 **P<0.01 ***P<0.001.

The results of the above experiment indicate that ginger-juice-treatment has not produced any effect on any parameter of the loco motor activity.

2. <u>Effect of chronic treatment of ginger-juice (4ml/rat for 7 days):</u>

1. <u>Mobile count-Slow:</u> The mean mobile count-slow in this vehicle treated group was 26.43 ± 8.84 minutes, while in the ginger-juice treated group it was 28.60 ± 4.61 minutes depicted in table-2. The difference between means of both groups was statistically not significant. This proves that ginger-juice treatment has no effect on mobile count-slow.

2. <u>Static count- Slow:</u> In the vehicle treated control group the mean static count-slow was 73.13 ± 16.63 minutes, while in ginger-juice treated group it was 59.55

 \pm 9.49 minutes. These counts are not significant statistically. The results reflect that there is no effect of ginger-juice on static count-slow. The results are shown in table-2.

3. <u>Rearing count-Slow:</u> The mean rearing count-slow in the vehicle treated group was 9.94 ± 3.66 minutes and in the ginger-juice treated group it was 11.38 ± 2.82 minutes. There is no significant difference between the two groups indicating that there is no effect of ginger-juice treatment for 7 days on rearing count-slow. The results are shown in table-2.

4. <u>Active time:</u> The mean active time in the vehicle treated group was 79.49 ± 12.44 minutes and in the ginger-juice treated group it was 53.77 ± 8.99 minutes. It is evident that this difference was not significant significantly. So it indicates that there is no effect of ginger-juice treatment on the active time. The results are shown in table-2.

Table 2: Value Of Different Parameters In Vehicle Control Group And Ginger-Juice Treated Group (Acute & Chronic).

Parameters	(Ctrl. Vehicle) n=6	Ginger-juice 4ml/rat (acute) n=6	Ginger-juice 4ml/rat (7days) n=6		
1.Mobile Count- S	26.43±8.84	26.00±12.06	28.60±4.61		
2.StaticCount-S	73.13±16.63	57.83 ± 14.13	59.55±9.49		
3.Rearing Count-S	9.94 ± 3.66	20.46 ± 8.08	11.38±2.82		
4.Active Time	79.41±12.44	68.96±16.43	53.77 ± 8.99		

Table 2: It shows the effect of acute and chronic (4ml/rat for 7 days) treatment with ginger-juice on the parameters like mobile count-slow, static count-slow, rearing count-slow and active time of loco motor activity in rat compared to the vehicle treated control group. The statistical significance vis-à-vis the vehicle treated control is presented as *p<0.05 **P<0.01 ***P<0.001.

The results of the above experiments indicate that ginger-juice-treatment has not produced any effect on any parameter of the loco motor activity.

3. Effect of acute and chronic (seven days) treatment of ginger juice on amphetamine (1mg/kg i.p) induced changes on loco motor activity.

A. Amphetamine treated group.

1. Mobile count-Slow:

The mean mobile count-slow in the vehicle treated control group was 26.43 ± 8.84 minutes, while in the

amphetamine treated group was 135.44 ± 26.46 minutes. The difference between these counts is significant statistically (P<0.001) as compared to the vehicle treated control group. It is evident that amphetamine has effects on mobile count-slow. The results are depicted in table-3.

2. Static count- Slow:

The mean static count-slow in vehicle treated control group was 73.13 ± 16.63 minutes, while in the amphetamine treated group it was 160.10 ± 4.70 minutes. The difference between these counts is significant statistically (P<0.001). The results are shown in table-3.

3. Rearing count-Slow:

The mean rearing count-slow in the vehicle treated control group was 9.94 ± 3.66 minutes and in amphetamine treated group it was 80.58 ± 35.67 minutes. There is significant (P<0.001) difference between the two groups. The results are expressed in table-3.

4. Active time:

The mean active time in the vehicle treated control group was 79.49 ± 12.44 minutes, while in the amphetamine treated group it was 215.58 ± 18.82 minutes. It is evident that this difference was significant statistically (P<0.001). The results are depicted in table-3.

The above results prove that amphetamine (1mg/kg, i.p.) administration significantly increased the counts of all parameters of loco motor activity in rats.

B. Effect of acute administration of ginger juice on amphetamine (1mg/kg i.p) induced changes in loco motor activity in rats.

1. Mobile count-Slow:

The mean mobile count-slow in the amphetamine treated group was 135.44 ± 26.46 minutes, while in the ginger-juice treated group it was 141.24 ± 25.23 minutes. It is evident that this difference was not significant statistically as compared to the amphetamine treated group. So it indicates that ginger-juice has no effect on amphetamine-induced mobile count-slow. The results are shown in table-3.

2. Static count- Slow:

In the amphetamine treated group the mean static count-slow was 160.10 ± 4.70 minutes, while in the

NJIRM 2015; Vol. 7(1) Jan – Feb

ginger-juice treated group it was 165.16 ± 5.84 minutes. The difference between counts is not significant statistically as compared to the amphetamine treated group. The results are depicted in table-3:

3. Rearing count-Slow:

The mean rearing count-slow in the amphetamine treated group was 80.58 ± 35.67 minutes and in gingerjuice treated group it was 79.43 ± 38.86 minutes. The differences between counts are not significant statistically. The results indicate that there is no effect of ginger-juice treatment on amphetamine induced rearing counts-slow. The results are shown in table-3.

4. Active time:

The mean active time in the amphetamine treated group was 215.58 ± 18.82 minutes, while in ginger-juice treated group it was 239.93 ± 20.16 minutes. The difference between counts of both groups was not significant statistically. The results are depicted in table-3.

The results of the above experiment indicate that acute ginger-juice-treatment has not produced any effect on any parameters of the amphetamine (1mg/kg i.p) induced loco motor activity in rats.

C. Effect of chronic administration of ginger-juice (4ml/rat for 7 days) on amphetamine (1mg/kg i.p) induced changes in loco motor activity in rats.

1. <u>Mobile count-Slow</u>: The mean mobile count-slow in the amphetamine treated group was 135.44 ±26.46 minutes, while in the pre-treated ginger-juice group it was 121.69 ±18.67 minutes. The results are depicted in table-3. The difference of mean between both groups was not significant statistically. This proves that gingerjuice treatment has no effect on the amphetamineinduced mobile count-slow of loco motor activity in rats.

2. Static count- Slow:

In the amphetamine treated group the mean static count-slow was 160.10 ± 4.70 minutes, while in pretreated ginger-juice group it was 155.71 ± 5.86 minutes. These counts are not statistically significant as compared to the amphetamine treated group. The results reflect that there is no effect of ginger-juice on amphetamine-induced static count-slow of loco motor activity in rats. The results are shown in table-3.

3. Rearing count-Slow:

The mean rearing count-slow in this amphetamine treated group was 80.58 ± 35.67 minutes and in the pretreated ginger-juice group it was 74.21 ± 12.63 minutes. This shows that there is no significant difference between the two groups indicating that there is no effect of ginger-juice treatment for 7 days on amphetamine induced rearing count-slow of loco motor activity in rats. The results are shown in table-3.

4. Active time:

The mean active time in this amphetamine treated group was 215.58 ± 18.82 minutes and in the pretreated ginger-juice group it was 198.71 ± 11.21 minutes. It is evident that this difference was not significant statistically. So it indicates that there is no effect of ginger-juice treatment on amphetamine induced active time of loco motor activity. The results are shown in table-3.

Table 3: Value Of Different Parameters In Vehicle Control, Ginger-Juice (Acute & Chronic) And Amphetamine Treated Group.

Parameters	(Control -	Amphetamine	Pretreated G. juice	Pretreated G. juice
	vehicle)	(1mg/kgI.P)	acute	chronic-
	n=6	n=6	amphetamine.	amphetamine
			(1mg/kg I.P)	1mg/kg I.P
			n=6	,(n=6)
1.Mobile Count- S	26.43 ± 8.84	135.44 ± 26.46***	141.24 ± 25.23	121.69 ± 18.67
2.Static Count-S	73.13 ±	160.10± 4.70***	165.16 ± 5.84	155.71 ± 5.86
3.Rearing Count-S	9.94 ± 3.66	80.58 ± 35.67***	79.43 ± 38.86	74.21 ±12.63
4.Active Time	79.41± 12.44	215.58 ± 18.82***	239.93 ± 20.16	198.71 ± 11.21

Table 3: It shows the effect of amphetamine (1 mg/Kg I.P) on various parameters like mobile count-slow, static count-slow, rearing count-slow and active time in pre-treated acute and chronic (for 7 days) with ginger-juice

(4 ml/rat) on rats. The statistical significance vis-à-vis the vehicle treated control and amphetamine treated is presented as p<0.05 **P<0.01 ***P<0.001.

The above data indicates that amphetamine (1mg/kg) increases various counts of loco motor activity, however, the activity induced by amphetamine is not altered by acute or chronic treatment with ginger-juice.

B. Lithium induced head twitches in rats:

1. Count of head twitches after administration of lithium is in different groups of rats is as under.

(a) Lithium treated control group:

The mean head twitches in this group were 13.16 ± 3.59 counts. The results are shown in table-4 and figure-1.

(b) Effect of acute treatment of ginger-juice on lithium induced head twitches:

The mean head twitches in the lithium treated control group was 13.16 ± 3.59 counts, while in ginger-juice treated group it was 12.16 ± 0.48 counts expressed in table-4 and figure-1. The differences of mean between two groups are not significant statistically as compared to the lithium treated control group. This reflects that acute administration of ginger-juice has no effect on lithium induced head twitches.

(c) Effect of chronic treatment of ginger-juice (4ml/rat for 7 days) on lithium induced head twitches:

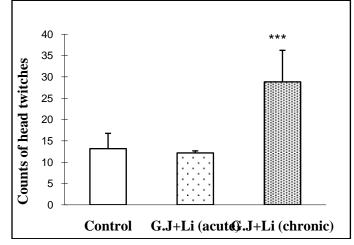
In the lithium treated control group the mean head twitches was 13.16 ± 3.59 counts, while in ginger-juice treated group it was 25.83 ± 7.40 counts. The differences of mean between two groups are significant statistically (P<0.001). It is evident that 7 days treatment of ginger-juice increased the head twitches. The results are expressed in table-4 and figure-1.

 Table 4: Effect Of Lithium And Ginger-Juice Treated

 Group (Acute And Chronic).

Parameters	Head	
	twitching	
	(counts)	
Control (Li 150mg/kg) (n=6)	13.16 ± 3.59	
(G. juice + Li 150mg/kg), acute (n=6)	12.16 ± 0.48	
(G. juice + Li 150mg/kg),chronic (n=6)	28.83 ±	
	7.40***	

Table 4: The effect of lithium (150 mg/Kg) on parameter like head twitches in pre-treated with ginger-juice (4 ml/rat) 30 minutes before the administration of lithium (150mg/kg I.P) on rats. The statistical significance vis-àvis the vehicle treated control is presented as *p<0.05 **P<0.01 ***P<0.001. Figure 1: Head twitches in acute and chronic treated ginger-juice + Lithium(Li) - 150mg/kg).



Head twitches induced by lithium administered were not antagonized by acute treatment of ginger-juice.

Chronic treatment of ginger-juice induced the head twitches significantly. The above data indicates that lithium in dose of 150mg/kg induced head twitches. Acute treatment with ginger-juice did not affect this activity. However, chronic treatment increased the number of head twitches induced by lithium significantly.

Discussion: This was as a multifaceted study, of which only screen the actions, on certain central nervous system parameters, and to establish the involvement of central dopaminergic and serotonergic mechanismwere selected for the current analysis. Other parameters are either already published¹³⁻²¹ or in the process of preparation/submission.

Observations on amphetamine-induced locomotor activity may give some clue about effects of ginger-juice treatment on central dopaminergic action. Amphetamine in the dose (1mg/kg i.p) administered to the rats exhibited enhanced locomotor activity this may be considered by virtue of stimulation of central dopamine receptors (Shigenabu et al., 1994)⁵. Ginger-juice on acute as well as chronic treatment failed to modify the amphetamine-induced locomotor activity. This indicates that there is neither prodopamenergic nor antidopaminergic effect on part of ginger.

Further, lack of antidopaminergic effect may also possibly rule out the possibility of acute dystonia as is the case with anti-dopaminergic antiemetics.

The role of dopaminergic mechanisms in the regulation of stress responses has been studied in experimental animals. The complex dopaminergic mechanisms are involved in the regulation of visceral, endocrinological and immune responses during stress (Puri et al., 1994)⁶. As ginger-juice is not affecting the central dopaminergic system.Any speculation that ginger-juice treatment may modify stress or endocrinal and immune responses may not be sustained.

Lithium is reported to cause release of serotonin in central nervous system (Wielosz et al., 1979)⁷. Head twitches induced by administration of lithium are considered as model to exhibit this kind of effect (Wielosz et al., 1979)⁷.

In the present study, it is observed that ginger-juice administration over a period of 7 days increased lithiuminduced twitches indicating enhanced serotonergic transmission in CNS.

In acute treatment with ginger-juice head twitches are not antagonized by it. Therefore it is evident that acute treatment with ginger-juice has no interaction with 5-HT system but chronic treatment enhanced the 5-HT mediated response. The possibility of increased sensitivity of 5-HT response is worth considering.

Administration of ginger-juice acutely, however did not increase the lithium induced head twitches. This indicates lack of serotonergic or serotonin modulating effect on the part of Z. officinale when administered as a single dose. Huang et al., (1990)⁴ reported effect of acetone extract of Zingiber officinale on serotonin system. They had observed inhibition of serotonininduced hypothermia and diarrhoea, and 6-shagoal was considered as active principle responsible for these effects.

Ginger-juice administered in the present study was in the Crude form. Possibility remains that active ingredient separated in acetone extract may be present in a very small quantity in crude form of the ginger-juice used in the present study. Possibly that may not be enough to exhibit serotonin antagonist effect in the present study. Even if we presume enough quantity of 6-Shagoal present in crude form, antiserotonin effect might have not manifested in the present study because of possibility of presence of other components. Why chronic treatment caused enhanced serotonergic responses? The question cannot be answered easily. The remote speculation that can be made, which of course does not have any evidence to support, is that the unmanifested antagonist effect of "crude" form of Z. officinale on chronic administration might be responsible for up- regulation of 5-HT receptor. As it is obvious, that chronic treatment with antagonists results into up regulation of the receptors enhancing agonistic response (Tripathi, 1999)⁸.

Observations in the present study, Ginger-juice on acute as well as chronic treatment failed to modify the amphetamine-induced locomotor activity. Lithium induced head twitches were enhanced after chronic administration indicating enhanced serotonergic system in the central nervous system.

Conclusion: Ginger administered itself did not affect loco motor activity as well as amphetamine-induced enhancement of loco motor activity.

Lithium induced head twitches were enhanced after chronic administration indicating enhanced serotonergic system in the central nervous system.

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Conflict of interest: None

Funding: None

Cite this Article as: Prasad S, Shah C, Desai A, Vajpeyee S, Bhavsar V. To Establish The Involvement Of Ginger-Juice On Amphetamine And Lithium Treated Rat. Natl J Integr Res Med 2016; 7(1): 59-66