GENESTEIN, A PHYTOESTROGENS FOR THE TREATMENT OF SCHIZOPHRENIA

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ABSTRACT

The potential therapeutic utility of estrogens in schizophrenia is increasingly being recognised. The goal of the study was to assess the effect of genistein a phytoestrogen in ketamine induced rat model of schizophrenia. Schizophrenia was induced by administering ketamine 50mg/kg i.p. Behavioural models assessed were loco motor activity representing positive symptoms, forced swimming test representing negative symptoms, active avoidance test representing cognitive symptoms. Biochemical parameters like dopamine and acetyl cholinesterase were estimated in rat brain tissues. To assess the possible side effects of genistein on male fertility, andrological parameters of rats such as sperm count, motility, viability and histology of testis were also evaluated. Acute administration of ketamine produced hyperactivity response in loco motor activity test, when administered chronically enhanced the immobility period in animals during the forced swim test and reduced the number of avoidances in active avoidance test. In Genistein, standard (clozapine) and combination of both treated groups we found protective effect of the drugs. Out of three different regimes the combination of clozapine and genistein found to be better in normalizing the levels of various parameters conducted in the present study. So the potentiating effect of the clozapine and genistein drugs can be seen. Genistein a phytoestrogen found to have no adverse effect on andrological parameters in male rats. Based on

the results, genistein was found to be effective in all the symptoms of schizophrenia. Genistein in combination with antipsychotic drug clozapine found to have better protective effect. Genistein, a phytoestrogen has no effect on andrological parameters in male rats. So its use as an adjuvant therapy may be preferred along with standard drug treatment.

INTRODUCTION

Schizophrenia is a chronic, severe, and disabling brain disorder that affects up to 1% of the population and makes it difficult for sufferers to think clearly, make decisions, and interact with people, as well as causing hallucinations, paranoia and many other symptoms. Schizophrenia affects around 24 million people worldwide as of 2011 (World Health Organization. 2011). Like many neurological diseases, the causes of schizophrenia are very complex, so while scientists have a basic idea of how the disease works, much about it is still a mystery.

Symptoms: The symptoms of schizophrenia fall into three broad categories: Positive symptoms: Negative symptoms and cognitive symptoms. The currently available antipsychotics do improve the positive symptoms. They are modestly effective on treating negative and cognition. These residual symptoms especially negative and cognitive symptoms are closely correlated with the degree of disability than the positive symptoms. These drugs were also associated with serious adverse effects. Glutamate is the major excitatory neurotransmitter in the brain, and it is widely distributed throughout our brain. It's involved in fast synaptic transmission, neural plasticity, and higher cognitive functions, such as learning and memory. Glutamate system induces positive and negative symptoms that are not blocked by D2 receptor antagonists. In order to overcome the side effects, identification of new targets, safe and effective medication, naturally occurring antagonists, adjuvant therapies are essential and may helps treatment of Schizophrenia.

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Estrogen and schizophrenia hypothesis:

The ‘estrogen hypothesis’ was derived from epidemiological, clinical and animal studies. Epidemiological studies (Hafner *et al.* 1993) have shown that women with schizophrenia present with first-episode psychosis, on average, about 5 years later than men with schizophrenia. Clinical studies reveal greater differences in the symptoms suffered, with men having more negative symptoms of schizophrenia and women experiencing more affective and paranoid

symptoms (Goldstein, 1988; Goldstein and Tsuang, 1990). Life-cycle studies have also shown that women are more vulnerable for either a first episode of psychosis or relapse of an existing illness at two major periods of hormonal change; firstly during the postpartum period and secondly during the menopause (Seeman, 1986, 1996). There have also been case reports of women whose schizophrenia symptoms were exacerbated at low estrogen phases of the menstrual cycle (Endo et al., 1978). Women are often more responsive to neuroleptic treatment than men. All of these trends seem to indicate that estrogen definitely has a delaying effect on schizophrenia. Estrogen is a generic term that encompasses several different types of similar female hormones. The most potent form of estrogen is generally considered to be estradiol.

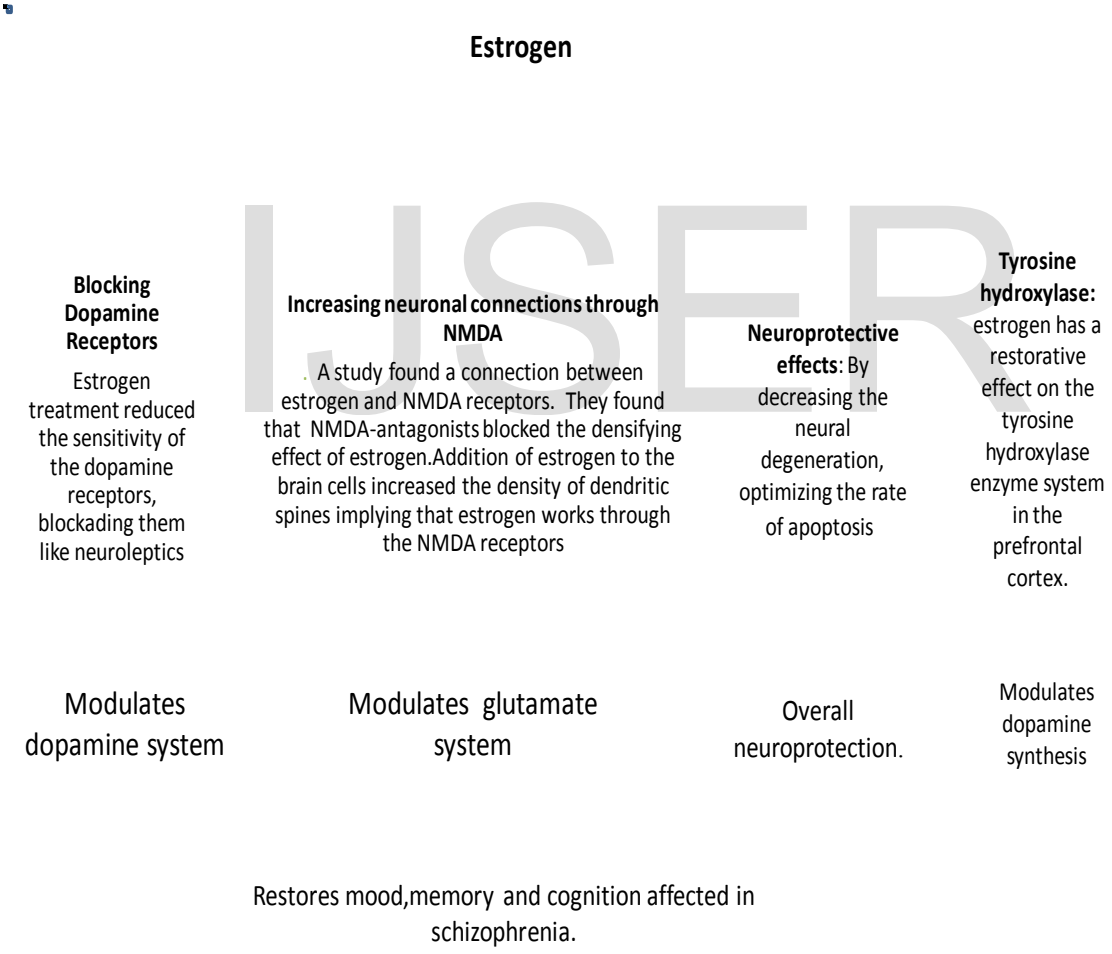

Fig no 1: Mechanisms of estrogens protective effect in schizophrenia.

The hypothesis of the present study was based on role of estrogens in schizophrenia. As discussed above in topic number "Estrogen and schizophrenia hypothesis", the estrogen levels were found to be alarmingly low in patients with schizophrenia. Genistein being a phytoestrogen may be helpful in treating schizophrenia. So the genistein was evaluated for its activity in suitable animal models. The details of the current test drug "Genistein" was elaborated in the next chapter.

MATERIALS AND METHODS:

Animal procurement and maintenance:

Albino wistar rats, (weight 150±20g), males were used for the present study. The animals were procured from Sanzyme limited, Hyderabad, India. They were housed in poly acrylic cages (38cm x 23cm x10cm) with not more than six animals per cage, at an ambient temperature of 25±2°C with 12-h-light/12-h-dark cycle. Rats have free access to standard chow diet and water ad libitum. The maintenance and the handling of animals were performed according to the guidelines and regulations of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi. The research protocols were approved by the Institutional Animal Ethical Committee (IAEC). Approval no: 12/SPIPS/IAEC/12.

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Locomotor Activity (Chatterjee *et al* 2011)

Locomotor activity in rats was measured using the instrument actophotometer. The number of interruptions of the infrared beams along the spatial dimensions of the monitor by the animals was interpreted as horizontal activity counts. In this experiment the rats were divided into 6 groups, each group containing 6 animals (n=6). Prior to the experiment, both the control and the treatment group animals were habituated in the experimental instrument for 15 min and the basal activity scores were noted. Again after 24 h different groups were administered with following drugs, the vehicle (10 ml/kg, oral), clozapine (10mg/kg Oral), Genistein (12.5mg/kg Oral) were administered 60 min prior to the administration of ketamine (50mg/kg I.P). 30 min after ketamine administration each rat was tested for activity scores for 5 min.

Forced swimming test: (Chindo *et al* 2012)

Forced swimming test, is a measure of Despair behaviour. In brief, rat were placed individually in plastic cylinders (approx 45cm height, 21cm diameter) containing 20cm depth of water at 25 °C. After 5min, the animals were removed from water, dried and returned back to their home cages. They

were again placed in the cylinder 24 h later and after the initial 1min acclimatization period, the total duration of immobility was measured for 5min. Rats which were floating motionless were considered to be immobile and the duration of swimming was measured.

Active avoidance test: (DAS *et al* 2003)

Active avoidance test helps to evaluate the associative learning of the animal.

Training for active avoidance test was conducted in Sidman jumping box (Elico, Chennai, India). It was divided in to two equal chambers (27×29×25cm) by Plexiglas partition, with a gate providing access to the adjacent compartment through a 14×17cm space. Prior to avoidance training each rat was habituated to the apparatus for 10 min. At the beginning of each session a rat was placed in the left compartment close to and facing the end wall. In each trial the animal is subjected to a light for 30 s followed by a sound stimulus for 10s. Immediately after the sound stimulus, the rat receives a single low intensity foot shock (0.5 mA; 3 s) through the grid floor. Each animal received a daily session of 15 trials with an inter-trial duration of 15 s for 5 days *i.e.*, a maximum of 75 trials. Transfer time from one compartment to another, number of avoidances (after the stimulus either light alone or both light and sound) and escape (after the foot shock) response are recorded. The criterion for improved cognitive activity was taken as significant increase in the avoidance response on 5th session (retention) as compared to 1st session (training). All the behavioural models were carried out in a semi dark sound proof room in order to overcome external interferences in the experiment.

Estimation of Dopamine: Dopamine in the brain homogenates was measured using a method adopted from Schlumpf *et al.,* 1974. On the day of estimation rats were sacrificed by cervical dislocation, whole brain was dissected out and separated. Weighed quantity 1.4 g of the brain tissue in 14ml of HCl- butanol was used for the homogenization. The sample was then centrifuged for 1 min at 2000 r pm. An aliquot of supernatant phase was removed and added to the centrifuge tubes containing 5ml of n- heptane and 625µl of 0.1 M HCl. After vigorous shaking for ten mins the tubes were centrifuged under the same conditions to separate aqueous and organic phases. Upper organic phase was discarded and the aqueous 1.5 ml was used for the estimation of dopamine.

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Estimation of Acetyl cholinesterase (AchE):

Acetyl cholinesterase enzyme activity was estimated by Ellman method. The rats were decapitated; brains are removed quickly and placed in saline. The brain tissues are weighed and homogenized in 0.1M phosphate buffer (pH 8). 0.4ml aliquot of the homogenate is added to a cuvette containing 2.6ml phosphate buffer (0.1M, pH 8) and 100µl of DTNB. The contents of the cuvette are mixed thoroughly by bubbling air and absorbance is measured at 412nm in a spectrophotometer. When absorbance reaches a stable value, it is recorded as basal reading. 20µl of substrate i.e., acetylthiocholine is added and change in absorbance is recorded. Change in absorbance per minute is thus determined.

Sperm collection and evaluation:

This study is aimed to evaluate the effect of genistein on male fertility by evaluating some andrological parameters of rats such as sperm count, motility, viability and morphology which are some of the indices that determine the ability of male to produce viable spermatozoa.

Immediately after killing, the epididymis was removed and trimmed of fat. Spermatozoa were obtained and prepared by method (kato *et al* 2002). Briefly caudal epididymis was minced in saline solution and incubated at 37^0C for 30 min to allow dispersion of spermatozoa.

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Sperm count:

The caudal sperm count test was performed according to (d'souza 2004). The spermatozoa count was obtained by counting the number of sperm cells in four WBC chambers using a neubauer's slide.

Sperm viability:

Microcopic examinations of seminal smears staind with eosin were carried out to determine the % of sperm viability. Ratio of alive/ dead.

Histopathology of testis:

Testis of treated rats were taken and fixed in 10% neutral formalin solution. the fixed specimen were then trimmed, washed and dehydrated in ascending grads of alcohol. Specimens were cleared in xylene, embedded in paraffin, sectioned at 4-6 microns thickness and stained.

EXPERIMENTAL DESIGN:

The rats were divided into five groups, each group consisting of six animals. The rats were used for studying the protective effect of genistein against ketamine induced psychosis.

Grouping of animals

GROUP-I	Normal control
GROUP-II	Positive control- treated with ketamine
GROUP-III	Clozapine + ketamine
GROUP-IV	Genistein + ketamine
GROUP-V	Clozapine + Genistein + ketamine

Drug treatment protocol

- Ketamine- 50mg/kg; intra peritoneal (i.p).
- Clozapine- 10mg/kg; per oral (p.o)
- Genistein- 12.5mg/kg; p.o

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Preparation of drug solutions:

Clozapine dissolved in DMSO and genistein dissolved in sesame oil were administered (p.o) to the rats according to the treatment protocol.

Groups III, IV, V were pre-treated with clozapine and genistein prior to the administration of ketamine. All groups of rats were assessed for the behavioural activities like locomotor activity, forced swimming, active avoidance test according to the given procedures and the observations are recorded. Finally the rats were sacrificed and the brain homogenates are used for the estimation of dopamine and Ach. Testis were collected from this cauda epididymis was isolated and used for the estimation of sperm count, viability and motility.

Statistical analysis:

Statistical analysis of all the obtained results was performed by one way ANOVA using graph pad prism software version 5.0 followed by Bonferroni's multiple comparison test. All the results were expressed as mean±SEM. A probability of $p<0.05$ was considered as significant.

The experiments were conducted according to the above procedures and the observations are recorded. The results are drawn from observations and these results are discussed in the next chapters.

RESULTS

Locomotor activity:

Animals were pre-treated with either Clozapine (10mg/kg) or Genistein (12.5mg/kg) or both through oral route 1hr prior to the ketamine administration. The locomotor activity was measured at 30 min after ketamine administration by using actophotometer. Each rat was tested for activity scores for 5 min.

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Table no 1: Effect of genistein on ketamine induced hyperlocomotor activity

Treatment Groups (n=6)	Horizontal activity counts Mean ± SEM
Normal control	100±12
Positive control - ketamine 50mg/kg	450±30#
Standard - clozapine 10mg/kg	150±4.4*
genistein - 12.5mg/kg	260±16*
clozapine(10mg/kg)+genistein(12.5mg/kg)	91±5.2*

*p<0.05 as compared to positive control; #p<0.05 as compared to normal control.

Effect of genistein on ketamine induced hyperlocomotor activity in rats. Each point is Mean ± S.E.M. Number of rats used per treatment for group are 6. One way ANOVA followed by Boenferoni's multiple comparison test revealed significant difference between control and various treatment groups.

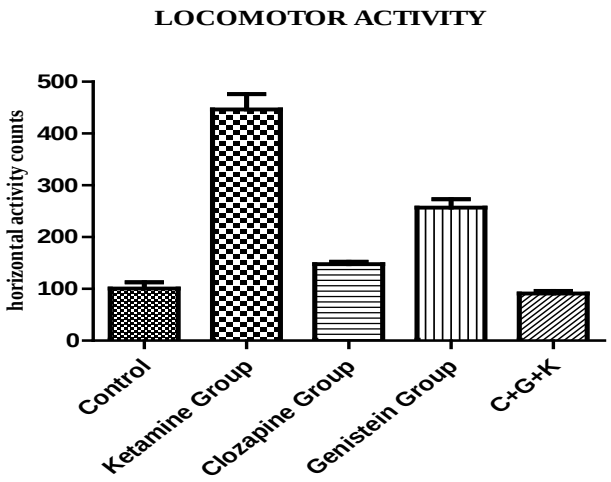

Fig 5: Locomotor activity counts in different animal groups

Ketamine significantly increased the locomotor activity. In Genistein, cloazipine and combination of both treated groups we found protective effect of the drugs. Out of 3 different regimes the combination of clozapine and genistein found to be better in maintaining the locomotion almost at normal levels. So the potentiation effect of the above drugs can be seen.

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Forced swimming test:

This is chronic study for about 10 da ys. Animals were pre-treated with either Clozapine (10mg/kg) or Genistein (12.5mg/kg) or both through oral route 1hr prior to the ketamine administration for 8 days, then on 9th day rats were placed individually in plastic cylinders containing water. After 5min, the animals were removed from water, dried and returned back to the cages. They were again placed in the cylinder 24 h later and after the initial 1min acclimatization period, the total duration of immobility was measured for 5min.

Table 2: Effect of genistein on ketamine induced immobility in forced swimming test:

Treatment Groups (n=6)	Immobility duration (secs)
Normal control	87±8.3
Positive control - Ketamine 50mg/kg	170±8.5$^{\#}$
Standard - Clozapine 10mg/kg	94±6.9*

Genistein 12.5mg/kg	130±7.6*
Clozapine10mg/kg+Genistein12.5mg/kg	43±11*

*p<0.05 as compared to positive control; #p<0.01 as compared to normal control

Effect of genistein on ketamine induced immobility in rats. Each point is Mean ± S.E.M. Number of rats used per treatment for group are 6. One way ANOVA followed by Boenferoni's multiple comparison test revealed significant difference between control and various treatment groups.

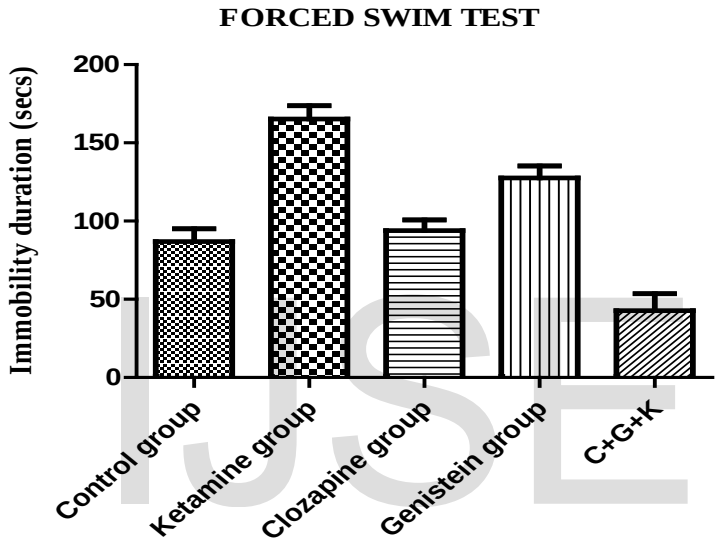

Fig 6: Immobility duration in different animal groups

Ketamine significantly increased the immobility duration. In Genistein, cloazipine and combination of both treated groups we found protective effect of the drugs. Out of 3 different regimes the combination of clozapine and genistein found to have better effect. The potentiation effect of the above drugs can be seen.

Active avoidance test:

It's also a chronic study for period of 10 days, for successful induction memory impairment by ketamine. Rats were pretreated with drugs for 10 days. From the 5th day each animal received a daily session of 15 trials with an inter-trial duration of 15 s for 5 days *i.e.*, a maximum of 75 trials in shuttle box. The number of avoidances was recorded.

Table3: Effect of genistein on active avoidance paradigm against ketamine induced cognitive impairment:

Treatment Groups (n=6)	No of avoidances
Normal control	11±0.49
Positive control - Ketamine 50mg/kg	5.0±0.37#
Standard - Clozapine 10mg/kg	7.5±0.43*
Genistein 12.5mg/kg	7.6±0.67*
Clozapine10mg/kg+Genistein12.5mg/kg	12±0.67*

*p<0.05 as compared to positive control; #p<0.01 as compared to normal control

Effect of genistein on ketamine induced cognitive impairment in rats. Each point is Mean ± S.E.M. Number of rats used per treatment for group are 6. One way ANOVA followed by Boenferoni's multiple comparison test revealed significant difference between control and various treatment groups.

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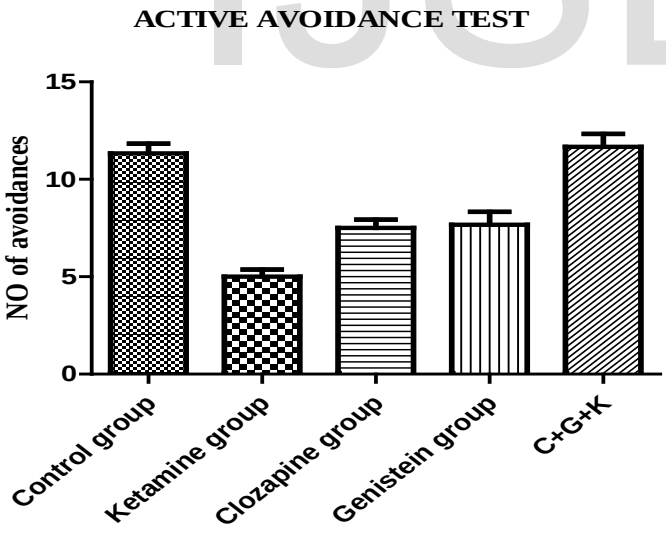

Fig 7: Number of avoidances in different animal groups

Ketamine significantly decreased the number of avoidances. In Genistein, cloazipine and combination of both treated groups we found protective effect of the drugs. Out of 3 different

regimes the combination of clozapine and genistein found to be better in increasing the number of avoidances almost at normal levels. The potentiation effect of the above drugs can be seen.

Estimation of Dopamine:

Dopamine levels in rat brain were estimated by using photoflourimeter. The test and the standard serial dilutions of dopamine were recorded against blank at excitation and emission wavelength 340- 580.

Table4: Effect of genistein on ketamine induced alteration of dopamine levels in rat brain:

Treatment Groups (n=6)	Concentration of dopamine(ng/mg)
Normal control	39±0.52
Positive control - Ketamine 50mg/kg	58±2.1$^{\#}$
Standard - Clozapine 10mg/kg	41±0.52*
Genistein 12.5mg/kg	51±0.52*
Clozapine10mg/kg+Genistein12.5mg/kg	36±1.0*

*p<0.05 as compared to positive control; #p<0.01 as compared to normal control

Effect of genistein on ketamine induced alteration of dopamine levels in rat brains. Each point is Mean ± S.E.M. Number of rats used per treatment for group are 6. One way ANOVA followed by Boenferoni's multiple comparison test revealed significant difference between control and various treatment groups.

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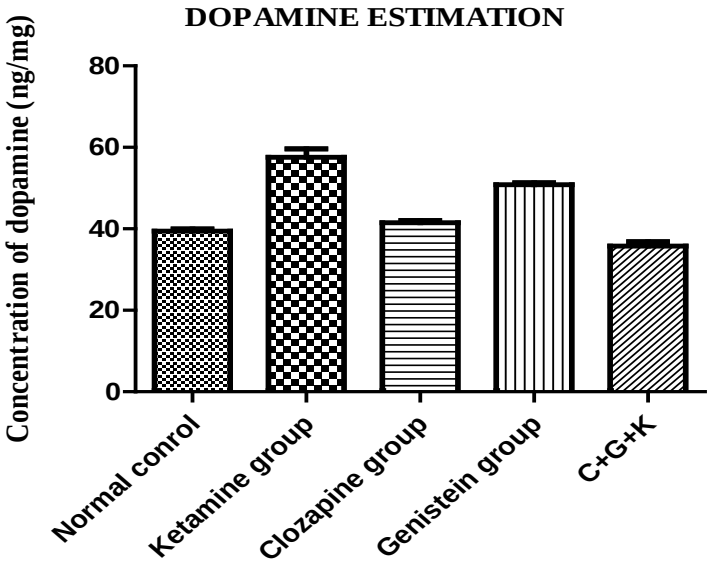

Fig 8: Dopamine levels in different animal groups

Ketamine significantly increased the dopamine levels in rat brains. In Genistein, cloazipine and combination of both treated groups we found protective effect of the drugs. Out of 3 different regimes the combination of clozapine and genistein found to be better in decreasing the dopamine almost to normal levels. The potentiation effect of the above drugs can be seen.

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Estimation of Acetyl cholinesterase:

Table5: Effect of genistein on Acetylcholine esterase activity in ketamine treated rats:

Treatment Groups (n=6)	Concentration of AchE μ moles/ml/min/mg protein
Normal control	118±1.76
Positive control - Ketamine 50mg/kg	480±12.5$^{\#}$
Standard - Clozapine 10mg/kg	312±8.36*
Genistein 12.5mg/kg	302±10.3*
Clozapine10mg/kg+Genistein12.5mg/kg	119±3.76*

*p<0.05 as compared to positive control; #p<0.01 as compared to normal control

Effect of genistein on ketamine induced alteration of dopamine levels in rat brains. Each point is Mean ± S.E.M. Number of rats used per treatment for group are 6. One way ANOVA

followed by Boenferoni's multiple comparison test revealed significant difference between control and various treatment groups.

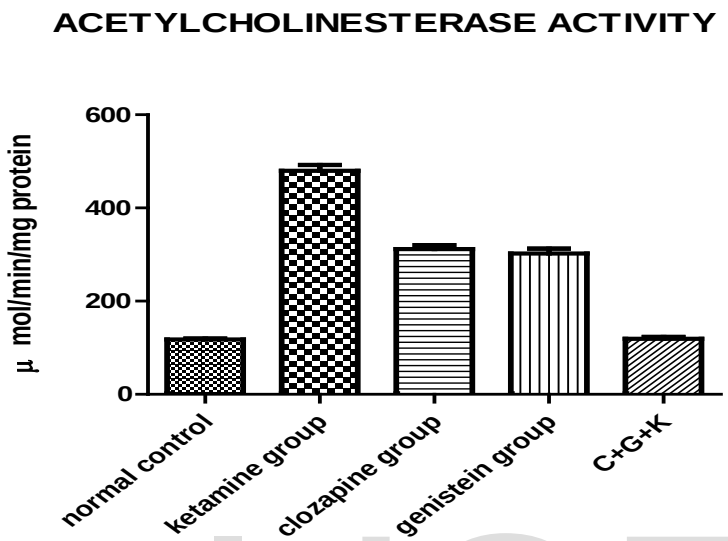

Fig 9: AchE levels in different animal groups

Ketamine significantly increased the AchE levels in rat brains. In Genistein, cloazipine and combination of both treated groups we found protective effect of the drugs. Out of 3 different regimes the combination of clozapine and genistein found to be better in decreasing the AchE levels almost to normal levels. The potentiation effect of the above drugs can be seen.

Sperm count and evaluation:

Table6: Effect of genistein on sperm count, motility, viability for evaluating its effect on male reproductive organs:

Groups	Normal control	Genistein	Clozapine +genistein
Sperm count (10^6 cells)	63.33±2.6	62±2.06	64±1.07
Motility	79±5.70	72±1.76	76±1.45
Viability	66.33±0.69	64±2.10	70±4.02

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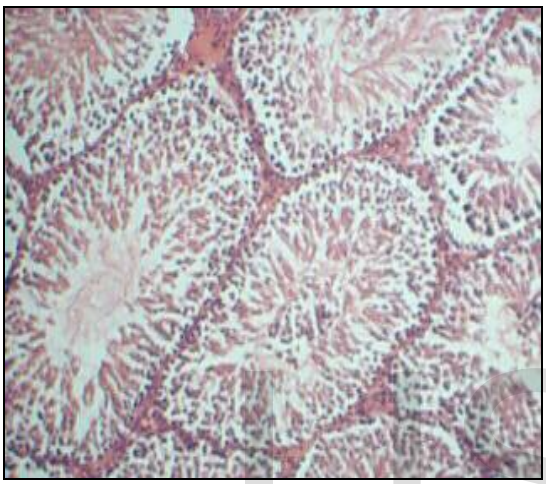
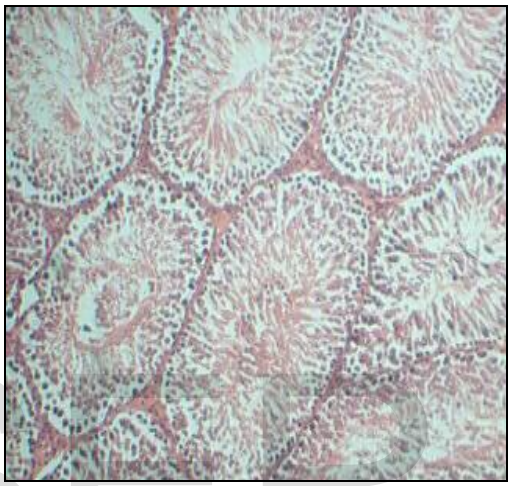
No significant difference observed in sperm count, motility, viability in different treatment groups.

Histology of testis:

Testis of normal treatment group: **Genistein group:**





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Genistein and clozapine treated group:

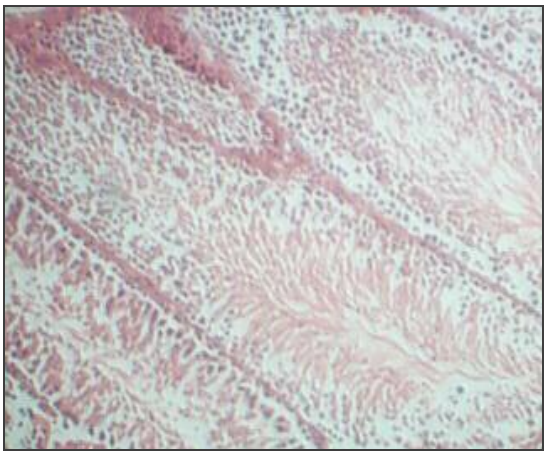

Fig 10: Photographs representing the histology of testis of different groups.

Genistein isoflavone, a major constituent of soybeans doesn't produce any adverse effect on male reproductive organs. There is no significant difference observed in sperm count, motility, and viability when compared with normal control group.

Histopathology of testis of individual genistein treated and combination of genistein & clozapine treated groups doesn't showed any significant difference when compared with control group. All groups showed normal histological arrangements of blood vessels, Leydig cells and a seminiferous tubule with lumen.

The results observed here are interpreted and discussed in the next chapter.

DISCUSSION

Schizophrenia is a chronic debilitating psychiatric disorder affecting as many as 1% of the population worldwide (Fauci 2008).

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The clinical features of the heterogeneous disorder schizophrenia usually appear in late adolescence or early adulthood, and can be divided into positive, negative and cognitive symptoms. Delusions (paranoia, false beliefs), hallucinations (visual and auditory), and thought disturbances are classified as positive symptoms, whereas negative features include depression, withdrawal from social contacts, incapability to feel pleasure (anhedonia) and flattening of emotional responses. Cognitive defects involve deficits in attention and memory. (Rajiv Tendon *et al* 2009)

The residual symptoms especially negative and cognitive symptoms are closely correlated with the degree of disability than the positive symptoms. Atypical antipsychotic medication provides hope in the management of negative symptoms, but the success remains limited. (Buckley *et al.* 2007)

The dopaminergic hypothesis came about from the observation that drugs that antagonized dopamine were found to be effective in the treatment of schizophrenia. Dopamine system has been proposed to account for the positive and negative symptoms of schizophrenia

that emerge during adolescence. Dopamine antagonists are effective only in treating positive symptoms associated in schizophrenia.

The estrogen hypothesis suggests that estrogen provides protection from the development of Schizophrenia and decreased the severity of negative symptoms (Seeman 1982; Seeman& Lang 1990). Preclinical data supports the involvement of estrogen in the regulation of several neurotransmitter systems (Dopamine, Serotonin, Noradrenalin and Glutamate) (McEwen 2002; Cyr *et al.* 2002). Beside direct influence on neurotransmission, estrogen may play a role in Schizophrenia by susceptibility gene regulation (Olsen *et al.* 2008). It involves estrogen effects variety processes during the brain development includes neuronal differentiation, survival and excitability (Boulware & Mermelstein 2005; Garcia-Segura *et al.* 2001). Many studies have indicated that estrogen replacement therapy has a beneficial effect on cognition function.

The use of estrogen as adjuvant treatment appears promising, but its use in long-term treatment has the disadvantage of the potential negative effect estrogen can have on breast and uterine tissue. (Chua *et al* 2005; Chlebowski *et al* 2009) Estrogen non-selectively acts on ER-alpha receptors are associated with the traditional female sexual effects and are found in the uterus, testis and adrenal gland.

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Soy isoflavones, which are referred to as phytoestrogens and include genistein, can bind to estrogen receptors (ERs) and affect estrogen-mediated processes (Molteni *et al* 1995). Pan *et al* 2000) Genistein is a relatively selective estrogen receptor β agonist (Tzagarakis-Foster; Lomri & Leitman 2001). ER-beta is present in more diverse parts of the body, including the brain.

The goal of the study was to assess the efficacy of genistein using ketamine-induced working model in rat with respect to some selected behavioural phenotypes that correlate with certain sections of symptoms observed in schizophrenia. The present study was also aimed at evaluation of the neurochemical changes in the brain tissues which are implicated in the pathophysiology of schizophrenia. To assess the possible side effects of genistein on male fertility, andrological parameters of rats such as sperm count, motility, viability and morphology were also evaluated.

Ketamine was a proven inducing agent of schizophrenia by blockade of the NMDA receptor channel complex by non-competitive antagonism induces symptoms commonly seen in schizophrenia (Krystal *et al.* 1994). Sub-anaesthetic doses of NMDA receptor antagonists, such as phencyclidine (PCP), MK-801 and ketamine, were reported to induce a w ider spectrum of behavioural responses that encompass positive, negative, and cognitive schizophrenia-like symptoms in healthy human volunteers (Javitt and Zukin, 1991) and rodents (Chatterjee *et al.* 2011). In the present study the behavioural models such as locomotor activity, forced swimming test, active avoidance test were conducted and Acetylcholinesterase enzyme and dopamine levels in brain tissue were estimated.

Locomotor activity is a measure of the positive symptoms in schizophrenia. Positive symptoms include aggressive and stereotypy behaviour. Results in the present study indicate that acute treatment of ketamine (50 mg/kg, i.p.) produced hyper locomotor response. The hyperlocomotory activity observed here are believed to be the result of dopamine agonistic action induced by ketamine. The locomotor activity was almost normal when genistein was given along with clozapine in ketamine received rats and it's an indication of protection from the occurrence of positive symptoms in schizophrenia. But genistein alone was not effective on par with combination of genistein and clozapine. We found this phenomenon was in agreement with the published literature. The past literature suggests, A 28- day clinical study has found that addition of 100 mcg of transdermal estradiol provided better clinical improvement of psychotic symptoms of hallucinations, thought disorder and delusion in women patients (Kulkarni *et al* 2001).

Forced swimming test is a suitable animal model that reflects negative symptoms of schizophrenia. Depression is one of the major negative symptoms of schizophrenia. Forced swim-induced immobility in rodents is an acceptable animal model of schizophrenia that reflects a state of despair in the rat and the reduction in the immobility time serves as the index of antidepressant activity.

In our study chronic administration of ketamine for 10 days induced enhancement of the immobility duration of rats in forced swimming test. The reduction in immobility duration was almost normal when genistein was given along with clozapine in ketamine received rats and it's an indication of protection from the occurrence of negative symptoms in schizophrenia. But

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genistein alone was not effective on par with combination of genistein and clozapine. This phenomenon was in agreement with the previous published literature, and it suggests that, raloxiphene, a selective estrogen receptor modulator, when given along with antipsychotic treatment in postmenopausal women with schizophrenia who are exhibiting negative symptoms significantly reduced both positive and negative symptoms. (Judith Usall *et al* 2010). The enhancement of immobility after chronic administration of phencyclidine, ketamine has been used previously as a model for the negative symptoms of psychosis, such as flattening of affect and avolition (Noda *et al* 1995; Chatterjee *et al* 2011).

Cognitive impairments such as deficits in attention, executive function, working (short-term) memory, and long-term memory, are core symptoms in patients with schizophrenia. Among these, learning and memory impairments are known to be particularly severe, and they are suggested to be major determinants of the amount of disability patients with schizophrenia. The active avoidance test has been used to evaluate the effects of antipsychotic drugs on learning and memory function. Genistein improved the ketamine induced cognitive symptoms in schizophrenic rats in our study. The increase in the number of avoidances was normal when genistein was given along with clozapine in ketamine received rats and it's an indication of protection from the occurrence of cognitive symptoms in schizophrenia. Both genistein and clozapine equally increased the number of avoidances. This phenomenon was in agreement with the previous published literature, and it suggests that Genistein improves memory of Ab-injected rats in (M. Bagheri*et al*. 2011) study. Acute and chronic ketamine administration, differentially and site specifically, modulated the levels of acetylcholine, dopamine, serotonin and noradrenaline. (Chatterjee *et al.* 2012)

Acetylcholine plays a critical synaptic role in the initial stages of memory formation (Hasselmo, 2006). Ketamine blocks nicotinic cholinergic receptors (Scheller *et al*, 1996) and there by suppresses glutamate release. Blockade of these receptors, induces increased ACh release activating cholinesterase enzyme which, in turn, interferes with hippocampal memory formation. Genistein individually reduced the Ache levels and in combination with clozapine produced potentiating effect.

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Increased locomotor activity was found in locomotor activity test. It has been reported that dopaminergic pathways are critical for the control locomotor activities. Ketamine increased the dopamine levels (Chatterjee *et al* 2012). Dopamine levels were almost normal when genistein was given along with clozapine in ketamine received rats and it's an indication of protective effect. But genistein alone was not effective on par with combination of genistein and clozapine.

This phenomenon was in agreement with the previous literature and it suggests Animal models have found that estrogen can inhibit the actions of dopamine (Hafner *et al*. 1991). Various animal studies found that estrogen treatment has been shown to reduce the dopamine concentration in the striatum and modulate sensitivity as well as the number of dopamine receptors (Foreman and Porter, 1980; Koller *et al.* 1980; Gordon *et al*. 1980; Dupond *et al.* 1981; Bedard *et al.* 1984). The effect of genistein on male fertility is assessed by evaluating some andrological parameters of rats such as sperm count, motility, viability and histology of testis.

Genistein isoflavone, a major constituent of soybeans has no adverse effect on male reproductive organs. There is no significant difference observed in sperm count, motility, and viability in genistein and combination of genistein and clozapine groups when compared with normal control group. Histopathology of testis of individual genistein treated and combination of genistein & clozapine treated groups doesn't showed any difference when compared with control group. All groups showed normal histological arrangements of blood vessels, leydig cells and a seminiferous tubule with lumen.

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This phenomenon was in agreement with the previous literature and it suggests as, A 2010 meta-analysis of fifteen placebo-controlled studies said that "neither soy foods nor isoflavone supplements alter measures of bio available testosterone concentrations in men"(Hamilton-Reeves *et al*. 2010). Furthermore, isoflavone supplementation has no effect on sperm concentration, count or motility, and leads to no observable changes in testicular or ejaculate volume (Dabrowski, Waldemar 2004, Mitchell *et al* 2001). This makes sense, as estradiol, the endogenous estrogen agonist, and a metabolite of testosterone, inhibits sperm cell apoptosis.

CONCLUSION:

- Schizophrenia is a chronic and disabling mental illness affecting millions of people worldwide. The symptoms of schizophrenia are classified into positive, negative and

cognitive symptoms. New receptor targets and drugs have being evaluated for addressing the multifaceted syndrome of schizophrenia. "Estrogen hypothesis" of schizophrenia posits that estrogen has a protective effect in women who are susceptible to presenting with this illness. In the present study genistein being a phytoestrogen is evaluated for its effect in schizophrenic rats. The results suggest that treatment with genistein is effective in all the symptoms of schizophrenia. Genistein was found to modulate the levels of dopamine and acetyl cholinesterase enzyme. Genistein in combination with antipsychotic drug clozapine found to have better protective effect. Genistein, a phytoestrogen doesn't produce any adverse effect on andrological parameters in male rats. Its use as an adjuvant therapy can be preferred along with standard drug treatment.

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