INDO AMERICAN JOURNAL OF PHARMACEUTICAL RESEARCH

ANTIMICROBIAL, ANTI-INFLAMMATORY, AND ANTI-PARKINSON'S SCREENING OF AZOMETHINE ANALOGUES THROUGH HSP90 INHIBITION

Dr. M. Ganga Raju*, Divya N

Department of Pharmacology, Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad.

ARTICLE INFO

Article history
Received 15/04/2017
Available online
30/04/2017

Keywords
Hsp90,
MIC,
Antibacterial,
Antifungal,
Anti-Inflammatory,
Anti-Parkinson's Activity,
Antioxidant Activity.

ABSTRACT

Heat-shock protein (Hsp90) a group of molecular chaperones responsible for managing protein folding and quality control in cell environment. Hsp90 requires a series of co-chaperones to assemble into a super-chaperone complex for its function. The current study is piloted to perform antibacterial, antifungal, anti-inflammatory, anti-Parkinson's and antioxidant activities of Hsp90 inhibitors. The compounds S30 and S47 were synthesized and the maximum tolerable dose was found to be 2000 mg/kg, animals were administered with a dose of 100 mg/kg bd. wt and 200 mg/kg bd. wt. From the results dose dependent reduction in symptoms like muscular rigidity, ptosis, tremor, bradykinesia, gait alteration, righting reflex, swim test and locomotor behavior were observed. The histopathological studies of drug treated also showed the restoration. The two test drugs S30 and S47 possess antibacterial, antifungal, anti-inflammatory, anti-Parkinson's and antioxidant activities. Further study is needed to confirm the exact mechanism of action of the test drugs S30 and S47.

Corresponding author
Dr. M. Ganga Raju
Professor and Head,
Department of pharmacology,
Gokaraju Rangaraju College of Pharmacy
mgrpharma@gmail.com

*Please cite this article in press as **Dr. M. Ganga Raju** et al. Antimicrobial, Anti-Inflammatory, And Anti-Parkinson's Screening of Azomethine Analogues Through Hsp90 Inhibition. Indo American Journal of Pharmaceutical Research.2017:7(04).*

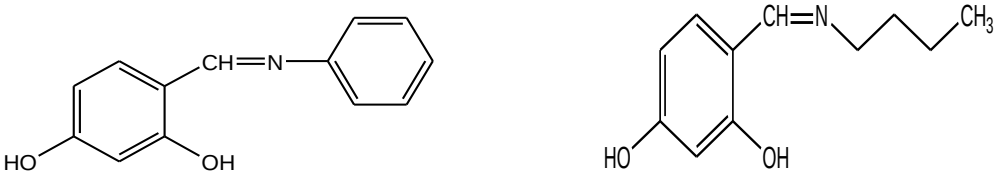
INTRODUCTION

Heat shock proteins (Hsps) are a family of greatly sealed stress proteins which can be persuaded by environmental stress such as heat, hypoxia, DNA damage or UV radiation to regulate cell breakdown and defend prokaryotic and eukaryotic cells from detrimental exogenous stimulus. Heat shock proteins are highly abundant proteins in eukaryotic cells, constituting about 1-2% of total proteins in unstressed cells and increasing to 4-6% of cellular proteins under stress. Hsp90 is an ATP-reliant molecular chaperone which is essential in eukaryotes. It is required for the activation and stabilization of a wide variety of client proteins. Client proteins of Hsp90 play a central pathogenic role in human diseases including cancer, neurodegenerative diseases and viral infections [1].

MATERIALS AND METHODS

Synthesis of compounds

Compounds S30 and S47 were synthesized and characterized as reported previously [2].



S30 **S47**

Figure: 1 Structure of test compounds.

Animals Studies

Healthy adult Sprague Dawley rats of 180-250 gm and Swiss albino mice 20-30 gm were selected for the study. The animals were acquired from Gentox laboratories, Hyderabad. The animals were accommodated according to CPCSEA guidelines (under standard temperature condition). They were given a pellet diet and water ad libitum. The ethical clearance was obtained from Institutional Animal Ethics Committee (IAEC) before the experiment (Reg. no.1175/PO/Ere/S/08/CPCSEA).

Acute toxicity studies

Acute toxicity study was accomplished on compounds S30 and S47 following OECD guidelines (425). The dosage for the pharmacological studies was selected as $1/10^{th}$ of the highest dose (2000 mg/kg) administered.

Experimental design

Antibacterial and antifungal activity

The antimicrobial activity of the test compounds were performed using agar disc diffusion method to reveal the minimal inhibitory concentrations (MIC), i.e., the lowest concentrations of the compounds that inhibit the visible growth of the tested microorganism. Petri plates containing 20 ml of Nutrient agar (NA) media for bacteria and Sabourand's dextrose agar (SDA) for fungi were used. After solidification of the media, ditch was made in the plates with the help of cup-borer (0.85 cm) and then the test compound was inoculated into the well. Ciprofloxacin was used as a standard for bacteria and Fluconazole was used as a positive control for fungi. Plates inoculated with bacteria incubated for 24 h at 37 °C and the fungal culture was incubated for 48 h at 25 °C [3]. The inhibition zone diameters were measured in millimeters. All the tests were performed in triplicate and the average was taken as final reading.

Anti-inflammatory activity

Carrageenan induced paw edema model

The anti-inflammatory activity was evaluated using carrageenan-induced paw edema in the rat model. Sprague Dawley rats are used and acute paw edema was induced by sub-plantar injection of carrageenan suspension into the right hind paw of each rat. The left hind paw was injected with the same volume of 0.1 ml of normal saline. Indomethacin (10 mg/kg bd.wt dose orally) was used as standard. Rats were pre-treated with test drugs (100 mg/kg and 200 mg/kg) and standard drug 1 h prior to carrageenan administration. The paw size was measured in mm using plethysmometer before (0 min) and at 1 h, 2 h, 3 h and 4 h after carrageenan administration. A total of 36 rats were used. The rats were divided into six groups with six animals in each group [4].

Formalin-induced paw edema model

The anti-inflammatory activity was measured using formalin-induced paw edema in the rat model. Female Sprague Dawley rats are used and edema was induced by sub-plantar injection of formalin solution into the right hind paw of each rat. The left hind paw was injected with the same volume of 0.1 ml of normal saline. Indomethacin (10 mg/kg bd. wt. dose orally) was used as standard. Rats were pre-treated with test drugs (100 mg/kg and 200 mg/kg) and standard drug 1 h prior to formalin administration. The paw size was measured in mm using plethysmometer before (0 min) and at 1 h, 2 h, 3 h and 4 h after formalin administration. A total of 36 rats were used. The rats were divided into six groups with six animals in each group [5].
The % inhibition was calculated by the following formula.

$$\% \text{ inhibition in paw edema} = (1 - V_t/V_c) \times 100$$

Where V_t and V_c is the mean increase in paw thickness in treated and control groups respectively.

Anti-Parkinson's activity

Reserpine induced Parkinson's model

Swiss albino male mice 20-30 gm were used. The animals received reserpine of *i.p.* 5 mg/kg bd. wt. of dissolved in 1% glacial acetic acid solution 24 h before the test. The test compounds S30 and S47 at doses of 100 mg/kg and 200 mg/kg and standard drugs administered 30 min prior recording the observations. Bromocriptine 2.5 mg/kg was used as standard drug. The various parameters like muscular rigidity, ptosis, tremor, bradykinesia, catalepsy, righting reflex, actophotometer investigations were measured [6].

Haloperidol induced Parkinson's model

Sprague Dawley rats weighing 180-200 g of either sex were used. The animals received haloperidol 2 mg/kg bd. wt. *i.p* 1 h before the test to induce Parkinson's disease. The animals were dosed for 7 days. On the seventh day, animals were observed for 30 minutes post injection. The test compounds S30 and S47 at doses of 100 mg/kg and 200 mg/kg and standard drugs administered 30 min prior recording the observations. Bromocriptine 2.5 mg/kg bd.wt. used as standard drug. The various parameters like muscular rigidity, ptosis, tremor, bradykinesia, catalepsy, righting reflex, actophotometer test were measured [7].

Muscular rigidity

For the investigation of muscular rigidity, the mice/rat was suspended by its forelimbs on a metal rod of 0.25 cm in diameter located approximately 20 cm above the surface. The time taken by the animal to remain on the rod (maximum 1 min) was recorded. To assess rigidity in a bracing task, the number of steps taken with each forelimb when the mouse is pushed sideways over a distance of 50 cm was recorded [6].

Palpebral ptosis

The irregular bending of the upper lid, caused by partial or total decrement in elevator muscle function was scored in the following way - 0 wide-open eyes, 2 half-open eyes, 4 eyes completely closed, 1 and 3 indicating intermediate values [6].

Tremor

Tremor also defined as shaking, the phasic tremor of the whole body was evaluated visually in mice utilizing the rating scale - 0 no tremor, 1 occasional isolated twitch, 2 moderate tremor associated with short periods of calmness, 3 pronounced continuous tremor [6].

Akinesia/bradykinesia

It is the diminished capability to start movements or akinesia test, the mouse was held by the tail so that animal is standing by his forelimbs and moving on his own. The number of steps taken with both forelimbs was recorded for 30 s [6].

Catalepsy

It is measured by placing the animal forepaws on a horizontal wooden bar (for mice 0.7 cm diameter & 4 cm above the table top, for rats 2-5 cm diameter & 6 cm above the top) the time until the animal removed forepaws from the bar was recorded, with a maximum cut off time of 3 min [6].

Gait alteration

The variation of the march was assessed only once by recording footprints (can be done by marking mice's paws with ink). Uninterrupted walking over a sheet of paper allows analysis of gait patterns and pathways [6].

Righting reflex

The righting reflex was assessed by rotating the mouse onto its back five times. Normal mice immediately turn themselves over, to right themselves onto all four feet. Righting reflex was scored as follows – 0 -no impairment, 1- on side one to two times, 2- on side three to four times, 3- on side five times, 4 - on back one to two times, 5 - on back three to four times, 6 - on back five times, 7 - sluggish when placed on back and 8 - righting response absent when on back and tail pinched [6].

Actophotometer

Each animal placed in the actophotometer and the readings of interrupting the light electrodes were recorded for 3min [8].

Swim test

Each mouse was introduced individually into a pool (45 cm long; 22 cm wide diameter and 20 cm high) filled with 10 cm deep water (21-23°C). Mice were allowed to swim maximum time 5 minutes. The time was recorded until the mice float on water without any movement [6].

Histopathological studies

Brain of normal control, haloperidol control, test S30 (200 mg/kg) and test S47 (200 mg/kg) treated groups and standard bromocriptine were stored in containers for 12 h in 10% formalin solution and subjected to histopathological studies. Observed microscopically for histopathological changes i.e., normal, damaged and recovered brain was studied and compared. The results were shown in Figure 2.

***In vitro* Antioxidant studies**

Antioxidant studies such as reducing power assay and hydrogen peroxide scavenging assay were evaluated [9].

Statistical analysis

All the values were expressed as mean ±SEM. The data were statistically analyzed by one way ANOVA followed by Dunnett's t-test.

RESULTS

Acute toxicity studies

The test drugs S30 and S47 were showed safe upto a dose of 2000 mg/kg, bd.wt. with no signs of mortality, hence the dose of 100 mg/kg and 200 mg/kg were considered for the study.

Antimicrobial activity

a. Antibacterial activity

Minimum inhibitory concentration of S30 found to be *Bacillus pumilus* 50 µg/mL whereas remaining all strains minimum inhibitory concentration is 100 µg/mL. For S47 the minimum inhibitory concentration was found to be 500 µg/mL in all the strains.

b. Antifungal activity

Minimum inhibitory concentration of S30 in fungi is 100 µg/mL and S47 is 500 µg/mL. The standard drugs ciprofloxacin and fluconazole has shown MIC at concentration 10 µg/mL.

Table: 1 Antibacterial activity and Antifungal of test drugs S30 and S47.

Microorganisms	S30		S47		Ciprofloxacin		Fluconazole	
	MIC (µg/ml)	Zone of inhibition (mm)	MIC (µg/ml)	Zone of inhibition (mm)	MIC (µg/ml)	Zone of inhibition (mm)	MIC (µg/ml)	Zone of inhibition (mm)
Bacillus subtilis	100	14.0±0.57	500	17.0±0.57	10	22.3±0.54	—	
Bacillus pumilis	50	11.3±0.33	500	16.3±0.33	10	22.5±0.34	—	
Escherichia coli	100	12.0±0.00	500	17.0±0.57	10	22.6±1.20	—	
Pseudomonas aureginosa	100	12.6±0.66	500	17.6±0.88	10	23.0±0.30	10	23.0±0.66
Candida albicans	100	14.5±0.33	100	500 18.0±0.57	—		10	24.0±0.33
Aspergillus fumigates	16.5±0.50			500 20.3±0.33	—			

Anti-inflammatory activity

a. Carrageenan induced paw edema models

Percentage reduction in carrageenan induced paw edema of the test drug S30 at a dose of 100 mg/kg bd.wt and 200 mg/kg bd.wt were found to be 19.58% and 52.22% respectively. The percentage reduction in paw edema of the test drug S47 at a dose of 100 mg/kg bd.wt and 200 mg/kg bd.wt were 10.80% and 44.22%. The standard drug indomethacin 10 mg/kg bd.wt has shown 65.27% reduction in paw edema. All the values were tabulated in table 2. The test drugs S30 and S47 have shown significant reduction in paw edema when compared to control and standard group.

Table: 2 Carrageenan induced anti-inflammatory activity of test drugs S30 and S47 in Sprague Dawley rats.

	Change in paw edema (mL)				
GROUPS	1h	2h	3h	4 h	% reduction in Paw edema at 3h
Carrageenan control	3.50±0.25	5.41±0.20	7.66±0.24	6.75±0.25	---
S30 (100 mg/kg)	3.33±0.05	4.83±0.21	6.91±0.20	6.33±0.16	19.58 %[a*]
S30 (200 mg/kg)	2.75±0.17	4.16±0.21	3.66±0.16	5.66±0.16	52.22%[a*]
S47 (100 mg/kg)	3.33±0.05	4.41±0.20	6.83±0.21	6.88±0.08	10.80%[a *]
S47 (200 mg/kg)	3.00±0.129	4.25±0.17	4.25±0.21	5.75±0.25	44.22%[a*]
Indomethacin (10 mg/kg)	2.41±0.20	3.41±0.20	4.33±0.16	3.58±0.20	65.27%[a*]

Values are expressed as mean±SEM, (n=6). All the groups were compared with control induced group and standard group. Significant values are expressed as control (a= p<0.01) standard (*=p<0.01)

b. Formalin induced paw edema model

The percentage reduction in formalin induced paw edema of the test drug S30 at a dose of 100 mg/kg bd.wt and 200 mg/kg bd.wt were found to be 19.00% and 38.08% respectively. The percentage reduction in paw edema of the test drug S47 at a dose of 100 mg/kg bd.wt and 200 mg/kg bd.wt was 12.76% and 25.71%. The standard drug indomethacin 10 mg/kg has shown 64.28% reduction in paw edema. All the values were tabulated in table 3. The test drugs S30 and S47 have shown significant reduction in paw edema when compared to control and standard group.

Table: 3 Formalin induced anti-inflammatory activity of test drugs S30 and S47 in Sprague Dawley rats.

	Change in paw edema (mL)				
GROUPS	1h	2h	3h	4 h	% reduction in paw edema at 3h
Formalin control	4.50±0.02	6.51±0.02	10.5±0.02	7.52±0.25	---
S30 (100 mg/kg)	5.52±0.02	7.13±0.04	8.51±0.02	7.00±0.08	19.00% [a*]
S30 (200mg/kg)	5.16±0.01	5.91±0.02	6.58±0.02	7.10±0.02	38.08% [a*]
S47 (100mg/kg)	5.33±0.02	7.08±0.00	9.16 ±0.03	8.50 ±0.02	12.76%[a*]
S47 (200 mg/kg)	4.66±0.02	6.16±0.04	7.80±0.03	7.61±0.04	25.71%[a*]
Indomethacin (10 mg/kg)	2.04±0.00	3.52±0.02	3.75±0.02	3.32±1.20	64.28%[a*]

Values are expressed as mean±SEM, (n=6). All the groups were compared with control induced group and standard group. Significant values are expressed as control (a= p<0.01) standard (*=p<0.01)

Anti-Parkinson's activity

a. Reserpine induced Parkinson's model

The various parameters in reserpine induced Parkinson's model like bradykinesia, ptosis, tremor, righting reflex muscular rigidity, catalepsy, locomotor behavior, actophotometer and swim test were measured. The values were reported in the Table 4 and 5. The reduction in symptoms of the test drug S30 and S47 at a dose of 100 mg/kg bd.wt, 200 mg/kg bd.wt was increased in dose dependent manner. The standard drug Bromocriptine has shown significant reduction in all the above parameters. All the results were tabulated in table 4 and 5.The test drugs S30 and S47 have shown significant reduction in all parameters when compared to control group, reserpine control and standard group.

Table 4: Reserpine induced Parkinson's activity of test drugs S30 and S47 in Swiss albino mice

GROUPS	Bradykinesia	Ptosis	Tremor	Righting reflex
Control	39.5±1.50	---	---	---
Reserpine control	1.50±0.50[aA]	3.50±0.50[aA]	3.00±0.00[aA]	7.50±0.50[aA]
S30 (100 mg/kg)	3.00±1.00[a*A]	2.50±0.50[a**B]	2.00±0.00[a*B]	1.50±0.50[a.**A]
S30 (200 mg/kg)	9.50±0.50[a**A]	2.50±0.50[a**B]	1.5±0.00[a**B]	1.50±0.50[b**A]
S47 (100 mg/kg)	3.50±0.51[a*B]	4.00±0.00[a*A]	3.00±0.0[a*A]	4.50±0.50[a**B]
S47 (200 mg/kg)	6.50±0.50[a*A]	3.00±0.17[a**A]	2.00±0.00[a*A]	2.50±0.50[a*A]
Bromocriptine (2.5 mg/kg)	18.5±0.20[a**]	1.50±0.50[a**]	1.00±0.00[a**]	1.50±0.50[a**]

Values are expressed as mean±SEM, (n=6). All the groups were compared with normal, reserpine induced group and standard group. Significant values are expressed as control (a= p<0.01) (b= p<0.05) reserpine control (**= p<0.01) (*= p<0.05) standard (A=p<0.01) (B= p<0.05).

Table 5: Reserpine induced Parkinson's activity of test drugs S30 and S47 in Swiss albino mice.

GROUPS	Muscular rigidity	Catalepsy	Actophotometer	Righting reflex
Control	89.0±1.0	2.00±0.0	230±10.0	260±1.00
Reserpine control	6.00 ± 0.5^{aA}	165.0 ± 5.00^{aA}	8.00 ± 1.00^{aA}	15.0 ± 5.00^{aA}
S30 (100 mg/kg)	$37.5\pm2.5^{a**B}$	$125\pm5.00^{a**A}$	$11.5\pm0.50^{a*A}$	$49.0\pm9.00^{a*A}$
S30 (200 mg/kg)	$56.5\pm0.1^{a**B}$	$118\pm2.00^{a**A}$	$15.5\pm0.50^{a**A}$	$94.5\pm5.5^{a**A}$
S47 (100 mg/kg)	$22.5\pm7.5^{a**B}$	$156\pm4.00^{a*A}$	$12.0\pm1.0^{b*A}$	$39.0\pm9.0^{a**A}$
S47 (200 mg/kg)	$40.0\pm0.5^{a**A}$	$116\pm1.50^{a**A}$	$13.0\pm0.50^{a*A}$	$69.0\pm1.0^{a*B}$
Bromocriptine (2.5 mg/kg)	$58.5\pm3.5^{a**}$	$65.0\pm5.0^{a**}$	$26.0\pm1.00^{a**}$	$125\pm5.0^{b**}$

Values are expressed as mean±SEM, (n=6). All the groups were compared with normal, reserpine induced group and standard group. Significant values are expressed as control (a= p<0.01) (b= p<0.05) reserpine control (**= p<0.01) (*= p<0.05) standard (A=p<0.01) (B= p<0.05).

b. Haloperidol induced Parkinson's model

The various parameters in haloperidol induced Parkinson's model like bradykinesia, ptosis, tremor, righting reflex, muscular rigidity, catalepsy, actophotometer test were measured. The values were reported in the Table 6 and 7. The reduction in symptoms of the test drug S30 and S47 at a dose of 100 mg/kg bd.wt, 200 mg/kg bd.wt was increased in a dose dependant manner. The standard drug Bromocriptine has shown significant reduction in all the above parameters. All the results were tabulated in table 6 and 7. The two test drugs S30 and S47 have shown significant reduction in all parameters when compared to control group, haloperidol control and standard group.

Table 6: Haloperidol induced Parkinson's activity of test drugs S30 and S47 in Sprague Dawley rats.

GROUPS	Bradykinesia	Ptosis	Tremor	Righting reflex
Control	50.5±1.50	---	---	---
Haloperidol control	12.0 ± 0.50^{bA}	3.50 ± 0.50^{aA}	3.00 ± 0.00^{aB}	7.00 ± 0.50^{aA}
S30 (100 mg/kg)	$3.50\pm0.50^{a**B}$	$2.50\pm0.50^{a*A}$	$2.00\pm0.00^{a**A}$	$1.50\pm0.50^{a**A}$
S30 (200 mg/kg)	$11.0\pm1.00^{a**A}$	$2.00\pm0.50^{a**B}$	$1.00\pm0.0^{a**B}$	$1.50\pm0.50^{a**A}$
S47 (100 mg/kg)	$3.00\pm1.00^{a*A}$	$3.00\pm0.00^{a**A}$	$2.50\pm0.00^{a*A}$	$4.50\pm0.50^{a*A}$
S47 (200 mg/kg)	$8.50\pm0.50^{a**A}$	$3.00\pm0.00^{b*A}$	$2.50\pm0.00^{a**B}$	$4.00\pm0.00^{a**A}$
Bromocriptine (2.5 mg/kg)	$16.50\pm1.50^{a*}$	$1.50\pm0.50^{a**}$	$1.00\pm0.00^{a**}$	$2.00\pm0.00^{b**}$

Values are expressed as mean±SEM, (n=6). All the groups were compared with normal, haloperidol induced group and standard group. Significant values are expressed as control (a= p<0.01) (b= p<0.05) haloperidol control (**= p<0.01) (*= p<0.05) standard (A=p<0.01) (B= p<0.05).

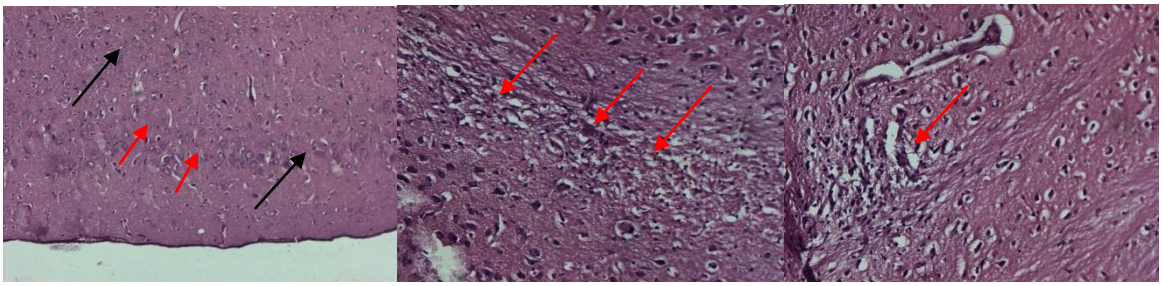
Table 7: Haloperidol induced Parkinson's activity of test drugs S30 and S47 in Sprague Dawley rats.

GROUPS	Muscular rigidity	Catalepsy	Actophotometer
Control	60.0±0.0	2.00±0.00	269±0.54
Haloperidol control	3.50 ± 0.5^{aA}	165 ± 5.00^{bA}	25.0 ± 5.00^{aA}
S30 (100 mg/kg)	$12.5\pm2.05^{a**A}$	$125\pm5.00^{b**A}$	$33.0\pm1.00^{a**B}$
S30 (200 mg/kg)	$21.0\pm1.0^{a**B}$	$118\pm2.00^{a**B}$	$46.5\pm4.50^{a**A}$
S47 (100 mg/kg)	$19.0\pm1.00^{a*A}$	$156\pm4.00^{a*A}$	$29.6\pm0.50^{a*A}$
S47 (200 mg/kg)	$26.5\pm1.5^{a**A}$	$116\pm1.50^{a**A}$	$39.5\pm0.50^{a**A}$
Bromocriptine (2.5 mg/kg)	$31.0\pm0.50^{a**}$	$65.0\pm5.00^{a**}$	$57.5\pm0.20^{a**}$

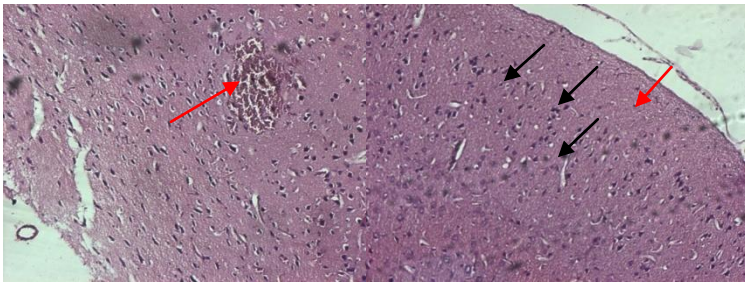
Values are expressed as mean±SEM, (n=6). All the groups were compared with normal, haloperidol induced group and standard group. Significant values are expressed as control (a= p<0.01) (b= p<0.05) haloperidol control (**= p<0.01) (*= p<0.05) standard (A=p<0.01) (B= p<0.05).

Histopathology

In normal brain cerebral hemisphere appeared normal and meninges surrounding the brain appeared normal. In haloperidol control brain mild to moderate demyelination and necrosis noticed in the cerebral hemisphere of brain. In S30 treated brain mild foci of necrosis and inflammation noticed in the cerebral hemisphere. In S47 treated brain foci of hemorrhage or hematoma formation noticed in the cerebral hemisphere of brain. The standard bromocriptine treated brain. Cerebral hemisphere appeared normal-black and meninges surrounding the cerebral hemisphere appeared normal.



Normal **Haloperidol control** **S30**



S47 **Bromocriptine**

Figure: 2 Histopathology of haloperidol induced parkinson's model.

Reducing power assay

In vitro antioxidant activity was performed using reducing power assay. The IC_{50} concentrations of S30 and S47 are 42 µg/mL and 49 µg/mL the standard ascorbic acid IC_{50} was found to be 31 µg/mL. From the results it is clear that the test drugs S30 and S47 showed significant antioxidant activity.

H_2O_2 Scavenging assay

In vitro antioxidant activity was performed using hydrogen peroxide scavenging assay. The IC_{50} concentrations of S30 and S47 are 16 µg/mL and 22 µg/mL the standard ascorbic acid IC_{50} was found to be 10 µg/mL. From the results it is clear that the test drugs S30 and S47 showed significant antioxidant activity.

DISCUSSION

Acute toxicity studies of test drugs S30 and S47 at the dose of 2000 mg/kg showed no toxic symptoms or death in any of the animal's upto one week and till the end of the study. Thus the drug was considered to be safe.

In bacterial cells Hsp90 expressed as HtpG (High temperature protein G), a homologue of Hsp90. Under stress conditions, bacterial Hsp90 seems to be involved in supporting *de novo* protein folding [10]. As the test drugs (S30 and S47) are Hsp90 inhibitors, the antibacterial activity of these test drugs might be due to inhibition of high temperature protein G (HtpG).

Fungi uses fungal Hsp90-calcineurin pathway in cell wall synthesis, ion homeostasis and other functions. Calcineurin function is governed by Hsp90 as it is one client protein of Hsp90. The antifungal activity of test drugs might be due to inhibition of fungal Hsp90 [11].

The two test drugs S30 and S47 100 mg/kg and 200 mg/kg showed ($p<0.01$) respectively. Histamine, vascular endothelial growth factor, acetyl choline, oestrogen and fluid shear stress activates a mechanism in Hsp90. Hsp90 associates with endothelial nitric oxide synthase (eNOS) and is rapidly forms complex with eNOS by agonist that stimulate the production of nitric oxide. So Hsp90 inhibitors impede the endothelial nitric oxide synthase that decreases the nitric oxide production [12]. Administration of two test drugs S30 and S47 to carrageenan induced rats and formalin induced rats have shown a dose dependent inhibition of the paw thickness and this might be due to blocking the eNOS through Hsp90 inhibition.

The two test drug S30 and S47 100 mg/kg and 200 mg/kg showed ($p<0.05$) and ($p<0.01$) respectively. Hsp90 plays a role in maintaining the functional stability of neuronal proteins. Neurodegenerative disorders associated with protein aggregation, the rationale has been that inhibition of Hsp90 activates heat shock factor-1 (HSF-1) to induce production of Hsp70 and Hsp40, as well as of other chaperones, which in turn, promote disaggregation and protein degradation [13]. Administration of two test drugs S30 and S47 to reserpine induced and haloperidol induced Parkinson's disease models have shown a dose dependent reduction in symptoms and this might be due to inhibition of Hsp90 which induces the Hsp70 and that leads to protein degradation.

Additionally, histopathological examinations were also done to support the biochemical investigations. The histopathology studies of brain confirmed the anti-Parkinson's activity of synthesized compounds S30 and S47. The brain of haloperidol treated group showed marked demyelination and necrosis in the cerebral hemisphere indicating the induction of Parkinson's disease. The compounds treated groups showed significant variation from that of haloperidol induced group. The brain of S30 treated group showed mild foci of necrosis and inflammation without significant damage to meninges. The brain of S47 treated group showed hematoma formation in the cerebral hemisphere with considerable damage to meninges. The activity of S30 was found to be significant than S47 when compared to standard bromocriptine in which both cerebral hemisphere and meninges were normal.

The two test compounds showed significant IC_{50} values in both models of antioxidant activity. The antioxidant activity might be due to presence of hydroxyl groups in the compounds.

CONCLUSION

It was concluded that the test drugs S30 and S47 possess antibacterial, antifungal, anti-inflammatory, anti-Parkinson's and antioxidant activities. When the two test drug are compared to each other S30 has shown better anti-Parkinson's, anti-inflammatory and antioxidant activities over S47. Further studies are required to elucidate the exact mechanism of action.

REFERENCES

1. Kevin C. Kregel, Invited Review: Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. Journal of Appl Physiol. 2002; 92: 2177-2186.
2. Sayan Dutta Gupta, Revathi B, Gisela Mazaira, Mario D Galigniana, Subrahmanyam CVS, Gowrishanker NL, Raghavendra NM. 2,4dihydroxy benzaldehyde derived schiff bases as small molecule Hsp90 inhibitors: Rational identification of a new anticancer laed, bioorganic chemistry. 2015; 97-105.
3. Ana Zovko, Maja Vaukner Gabric, Kristina Sepcic, Franc Pohleven, Domen Jaklic, Nina Gunde-Cimerman, Zhibao Lu, RuAngelie Edrada-Ebel, Wael E. Houssen, Ines Mancini, Andrea Defant, Marcel Jaspars, Tom Turk. Antifungal and antibacterial activity of 3-alkylpyridinium polymeric analogs of marine toxins. International Biodeterioration & Biodegradation. 2012; 68: 71-77.
4. Udaybhan Singh P, Parveen Kumar, Manish M. Wanjari, Thenmozhi S, Balakrishnan BR. Analgesic and anti-inflammatory activity of root bark of *Grewia asiatica* Linn. in rodents. Ancient Science of Life. 2013; 32(3): 150-155.
5. Singh GB, Surjeet Singh, Bani S, Gupta BD and Banerjee SK. Anti-inflammatory activity of oleanolic acid in rats and mice. Journal of Pharmacy and Pharmacology. 1992; 44(5): 456–458.
6. Jose Luis Calderon,Rodrigo Bolanos. behavioral Analysis of the Reserpine Induced Motor Changes in Parkinsonian Mouse Model. Rivista Neuropsicologia, Neurosiquiatria Y Neurociencias. 2011; (11): 49-61.
7. Manikandaselvi S, Mahalakshmi R, Thinagarbabu R, Angumeenal AR, Neuroprotective activity of s-Allylcysteine on Haloperidol induced Parkinson's disease in albino mice. International Journal of PharmaTech research. 2012; 4(2): 669-675.
8. Tadaiesky MT, Andreatini R, Vital, M. A. B. F. Different effects of 7- nitroindazole in reserpine-induced hypolocomotion in two strains of mice. European Journal of Pharmacology. 2006; 535: 199-207.
9. Ali Ebrahimzadeh MD, Syed Mohammad Nabavi, Syed Fazel Nabavi, Fatemeh Bahramian and Ahmad Reza Bekhradnia. Antioxidant and free radical scavenging activity of *H. officinalis* .*V. angustifolius*, *V. odorata*, *B. hyrcana* and *C. speciosum.* Pak. J. Pharm. Science. 2010; 23 (1): 29-34.
10. Hitoshi Nakamoto, Kensaku Fujita, Aguru Ohtaki, Satoru Watanabe, Shoichi Narumi, Takahiro Maruyama, Emi Suenaga, Tomoko S. Misono, Penmetcha K.R. Kumar, Pierre Goloubinoff and Hirofumi Yoshikawa. Physical Interaction between

Bacterial Heat Shock Protein 90 (Hsp90) and Hsp70 Chaperones Mediates Their Cooperative Action to Refold Denatured Proteins. Journal of biological chemistry. 2014; 1-23.

11. Leah E. Cowen. The evolution of fungal drug resistance: modulating the trajectory from genotype to phenotype. Nature Reviews Microbiology Journal. 2008: 187-198.
12. Jessica L. Lowry, Viktor Brovkovych, Yongkang Zhang, and Randal A. Skidgel. Endothelial Nitric-oxide Synthase Activation Generates an Inducible Nitric-oxide Synthase-like Output of Nitric Oxide in Inflamed Endothelium. The Journal of biological chemistry. 2012; 288(6): 4174–4193.
13. Wenjie Luo, Wellin Sun, Tony Taldone, Anna Rodina and Gabriela chiosis. Heat shock protein 90 in neurodegenerative disease. Molecular neurodegeneration 2010; 5(24): 45-47.

5487847845**1170431**