

**SOME PHARMACOLOGICAL ACTIONS OF 'NIMBIDIN'-
A BITTER PRINCIPLE OF AZADIRACHTA INDICA-
A JUSS (NEEM)**

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ABSTRACT: *Nimbidin the major bitter principle from oil of seeds of Azadirachta indica A. Juss was investigated for various pharmacological actions in a number animal models. On central nervous system it exhibited moderate sedative effect it did not show any significant cardiovascular effects in experimental animals. Autonomic pharmacological studies (in-vitro) revealed pronounced anticholinergic antihistaminic (H1-receptor), anti 5 HT and antinicotinic activities. But in-vivo tests did not show any anticholinergic or anti-histaminic activity. Nimbidin possessed moderate diuretic activity and was found to be devoid of local anaesthetic and antiandrogenic effects in rodents.*

Introduction

Nimbidin, the major bitter principle from the oil of neem seed (*Azadirachta indica* seeds) kernels has been shown to possess a number of medicinal properties. Recently we have reported that it possessed significant antigastric ulcer², antiarthritic and anti-inflammatory³, analgesic and antipyretic⁴ as well as hypoglycaemic effects⁵ in various experimental models. In the present study, efforts were made to explore its other potential pharmacological actions in selected experimental animal models.

Materials and Methods

Nimbidin used in the study has been isolated from commercially available neem oil by the method of Siddiqui and Mitra.⁶ Nimbidin was used as colloidal solution of alcohol in water (10% v/v) in concentration of 10-20

mg/ml. Various pharmacological actions were tested by the following methods.

Effects on Central Nervous System (CNS)

On gross behavioural effects: Gross behavioural, neurologic, autonomic and toxic effects were observed as described by Irwin⁷, at 15, 30, 60, 120 min, and thereafter at 6 hourly intervals upto 72 hours in orally administered group and 24 hours in i.p. group. Nimbidin was given in graded doses of 10-2000 mg/kg (p.o.) and 10-1000 mg (i.p) to both albino rats and mice. Control groups received only the vehicle, 10% alcohol.

Potentiation of pentobarbitone hypnosis:

Pentobarbitone sodium (50mg/kg i.p) was injected to groups of 6 male mice (20-25g) in each, 30 min after oral administration of nimbidin in doses of 20, 40 and 80 mg.kg,

and the vehicle control group received 10% alcohol orally. The period between the loss and return of righting reflex was taken as the sleeping time and this was calculated for each group and compared.

Anticonvulsant activity:

Chemoshock seizure were induced in 18h fasted mice (20-25g) by a single s.c injection of metrazol (90 mg/kg) and nimbidin (20 and 40 mg/kg) was given orally 30 min prior to the convulsant. Percentage protection afforded was observed and the anticonvulsant activity assessed in terms of absence of clonic convulsions.

In rats (100-125g) anticonvulsant electroshock seizure pattern test (MES), according to the method of Swinyard et al¹⁸; Nimbidin (20,40 and 80 mg/kg p.p) was given one hour prior to the challenge of electroshock. Prevention of tonic extension of hind limbs was taken as the evidence of anticonvulsant activity. Phenobarbitone sodium 50 and 100mg/kg was the reference drug.

Effects on cardiovascular system (CVS)

Effect on arterial Blood Pressure (B.P.):

In mongrel dogs of either sex (10-12 kg) anaesthetized with pentobarbital sodium (35 mg/kg i.v.), nimbidin was administered i.v. for its effect on blood pressure and its ability to antagonize Various agonists influencing cardiovascular function. Carotid (right) blood pressure (B.P.), ECG (lead II) and respiration was monitored through the Encardio Rite Polygraph. Blood pressure responses were obtained with adrenaline, acetylcholine, histamine and isoprenaline (2

ug.kg i.v., each), carotid occlusion (CO) for 45 sec. and stimulation of peripheral and central cut end of the vagus (5V-10/sec. for 2 sec.) before and after test drug. Nimbidin was injected i.v. through the cannulated femoral vein in dose levels of 100 ug to 80 mg/kg as colloidal solution in 10% alcohol. Normal 'response bracket' was taken before and after the test drug (i.v) as described by Smith⁹. Hypotensive activity of nimbidin when observed was also studied after atropinization (Atropine SO₄ 1.5 mg/kg i.v.) and bilateral vagotomy. Effect of nimbidin on nicotine induced (10-20 µg/kg i.v). hypertensive effect was also studied on dog B.P.

Myocardiography:

Experiment were carried out in selected dogs (10-20 kg body weight) for studying the effect of nimbidin (1, 5, 10 and 20 mg/kg) on the rate and amplitude of auricular (left) and ventricular (right) contractions.¹⁰

Isolated rabbit heart preparations:- were put up and the effect of nimbidin on the heart rate and amplitude of contraction in-vitro was recorded.¹¹

Continuous heart perfusion of frog (in-situ):

Contractions of the perfused frog heart was recorded on a smoked paper attached to the slow moving drum using starling heart lever. Nimbidin was injected into the perfusing fluid in varying doses (10/ug to 10mg) in a constant volume. Change in the rate, tone and force of contractions were recorded.¹²

Chromodacryorrhea test

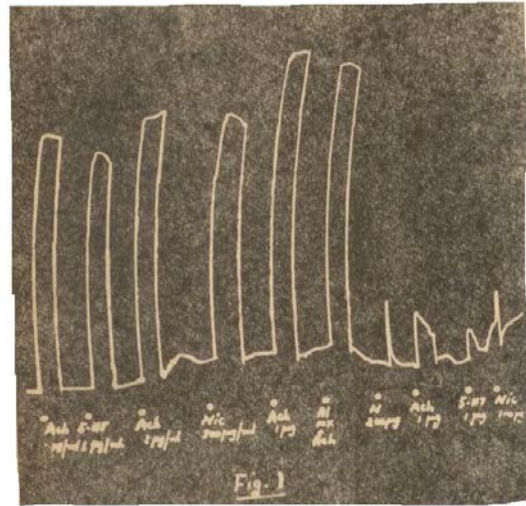
Effect of nimbidin (20 and 40 mg/kg) and atropine sulfate (3mg/kg) were studied for their effect on methacholine – induced chromodacryorrhea in Holtzman male rats.¹³ Treatments were assigned to groups of 6 rats and test drugs or the vehicle were injected i.p.; 30 min. later, the animals were checked for mydriasis and then given methacholine (10 mg/kg i.p.). The presence or absence of chromodacryorrhea was recorded 10 min. After the methacholine administration on ‘Yes’ or ‘No’ basis.¹⁴

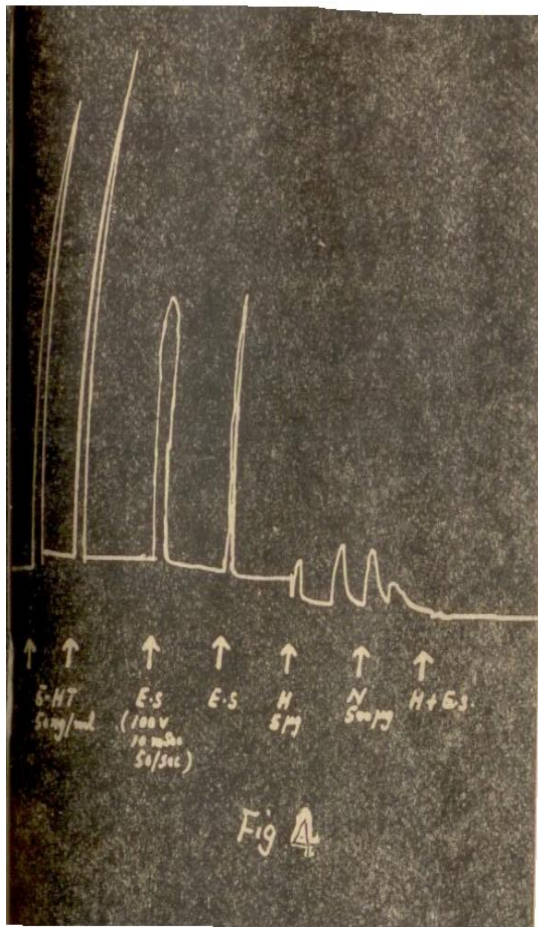
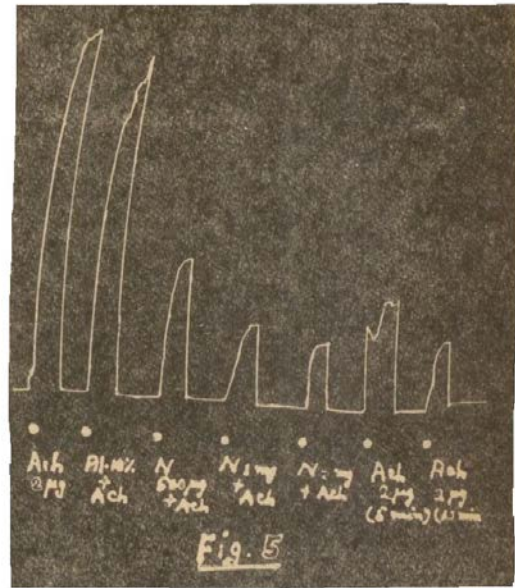
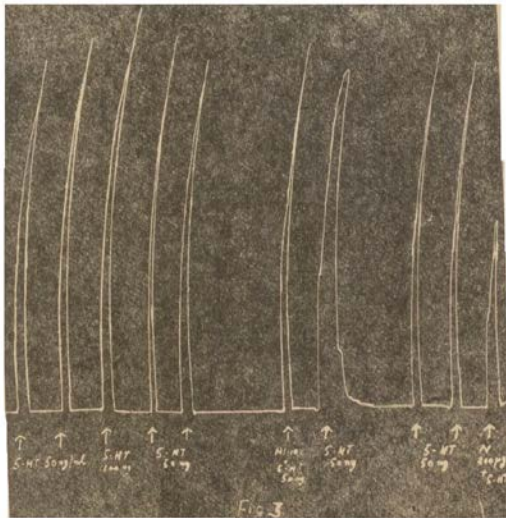
Gastrointestinal Motility

Effect on Nimbidin on intestinal transport was determined by using the method of Macht and Barba-Gosa with modifications.¹⁵ Nimbidin (20 and 40mg/kg) or carrier vehicle (10% alcohol) or atropine (25 mg/kg) was administered orally to the respective groups. One hour later, the rats received a charcoal meal (2 ml/100g body weight) consisting of 10% charcoal and 10% gum acacia suspended in 0.5% CMC. Ten minutes later all animals were sacrificed and the distance traversed by the charcoal meal was expressed as percent of the total length of the small intestine and the results were compared statistically.

Antihistaminic test (in-vivo)

Nimbidin was evaluated for its ability to protect against the lethal toxicity of histamine aerosol in guinea pigs^{10, 16} in various dose levels (20, 40 and 80 mg/kg.)





LEGENDS TO ILLUSTRATIONS

Fig. 1: Showing the anticholinergic and antinicotinic (ganglionic blocking) activities of nimbodin on guinea pig ileum.

Ach – Acetylcholine (1 µg) 5-HT – hydroxytryptamine (1 µg)

Nic – Nicotine (100 µg) N-Nimbodin (200 µg) (con- µg/ml bath fluid)

Fig.2: Showing antihistaminic effect of Nimbodin on g. pig ileum.

H-Histamine (3 µg) Al-Alcohol (10%), N-Nimbodin (1 mg)

Fig.3: Anti-5-HT effect of nimbodin on rat uterus (in-vitro)

5-HT-5-Hydroxytryptamine (50mg) Al-Alcohol. N-Nimbodin (200 µg)

Fig.4: Effect of nimbodin on Histamine.

H₂ – receptors in rat uterus.

5-HT-5-Hydroxytryptamine (50ng) E.S-
electrical stimulation (100 v, 10 m. Sec.
50/sec, 10 sec)

H- Histamine, N-Nimbidin.

Fig.5. Effect of nimbidin on skeletal muscle
of frog (rectus Abdominis) Ach-
acetylcholine (2 µg)

Al-alcohol-10% N-Nimbidin (1&2 mg/ml)

And the animals observed for respiratory
distress. They were considered unprotected
when they fell on sides with asphyxia
convulsions.

Autonomic Pharmacology-Isolated Tissue studies

Guinea pig ileum: Anticholinergic and
antihistaminic activity.

Longitudinal contraction of 2cm length of
guinea pig ileum to alternate doses of
acetylcholine and histamine (1-3 µg/ml) was
recorded on smoked paper by isotonic
frontal writing levers (load 1g). The tissue
was suspended in Krebs solution at 37°C
and bubbled with fresh air, A contact time of
30s in each 3 min dose cycle was used for
each agonist. Dose response curves were
recorded both before and after contact of
nimbidin (100 to 500 µg/ml). Nicotine or 5-
HT act at different receptors of the
intramural nerve plexus of Auerbach,
nicotine responses being hexamethonium
sensitive and 5 – HT responses
hexamethonium resistant. Acetylcholine
was the control agonist.¹⁷

Rat ileum-Anti 5-HT activity

Longitudinal contractions of 2 cm lengths of
terminal rat ileum to alternate doses of
acetylcholine and 5 – HT were recorded (1
µg/ml). The tissue was suspended in Krebs
solution at 37°C. The agonist contact time
in each 4 min dose cycle was 30s, the tissue
being washed three times by over flow
between doses. Dose response curves were
made before and after exposure of the tissue
to nimbidin (100-500 µg/ml) for one
minute.¹⁷

Rat Uterus-Histamine H₂-receptor blockade
and anti 5-HT effects.

Rat uterus preparation was used to detect a
histamine H₂ receptor blocking potential of
the test drug as per the method of Patnaik et
al.¹⁸

In another set of experiments using isolated
rat uterine horn in an organ bath containing
De-Jalon solution, effect of nimbidin in
antagonizing the 5-hydroxy-tryptamine (5-
HT) induced contractions was studied.
Estrogen primed rat uterus was taken for the
study using graded doses of 5-HT
(10,15,20,25,50 and 100 mg), contractions
of uterus were elicited at room temperature
and thereafter test drug (10 µg-1mg/ml) was
added 1 min before 5-HT and effect of the
rug on 5-HT induced contractile response
was observed.

Effect on skeletal muscle

To detect whether nimbidin is a peripherally
acting muscle relaxant or not, isolated frog's
rectus abdominis muscle preparation was set
up as described by Burn.¹²

Diuretic Activity

Diuretic activity of nimbidin was investigated in Holtzman male rats (175-200g) using the method of Lipschitz et al with modifications.¹⁹ The test drug in 20 and 40 mg/kg as solution in 10% alcohol was given orally, and hydrochlorothiazide 2.5 mg/kg was taken as the standard diuretic for comparison. Control rats received 10% alcohol. The total volume of urine excreted in a 5h period was measured. Urinary excretion, diuretic activity and ionic excretion (Na⁺, K⁺) in urine were calculated for all groups and compared.

Effect on Fertility

In females: Preliminary antifertility effect on nimbidin (20 and 40 mg/Kg p.o) was evaluated in Holtzman female rats of proven fertility, by administering from day one (D1) of pregnancy to day seven (D7), On D10 they were laparatomised and uterine horns examined for the number and size of implants.²⁰

In males: To detect the effect of test drug on the spermatogenesis and male sex organs, the drug was daily fed in dose levels of 25, 50 and 100mg/kg in groups of fertile male albino rats (125-150g) of Holtzman strain, for a period of 6 weeks. Control group was also maintained with the vehicle, 10% alcohol for the above period. At the end of medication they were sacrificed, male sex organs were collected, weighed, histological and biochemical investigations (cholesterol, total proteins, fructose and phosphatases) were carried out for each group.

Antiandrogenic activity

In castrated young male rats (60-90g) testosterone-phenyl propionate 1 mg/kg was given s.c., alone in control group and simultaneously with nimbidin (20 and 40 mg/kg orally, for a period of 7 days beginning with one day after castration. On the 8th day all rats were killed, seminal vesicles, prostate and levator ani were dissected out and wet weight noted. Inhibition of androgen induced weight gain would indicate antiandrogenic activity.²¹

Local Anaesthetic activity

Surface anaesthesia was tested on the rabbit cornea by instilling the test drug as 10% alcohol solution into the eye. Local anaesthesia was regarded complete when the animal failed to blink in response to all the 6 bristle touches to cornea.²²

Infiltration anaesthesia – was tested in male guinea pigs using the intradermal skin wheal test method of Bulbring and Wajda.²³ Test drug in different concentrations in 0.25 ml volume was injected intradermally in shaved backs of guinea pigs in four areas. Anaesthesia was regarded complete when the animal failed to react to all the 6 pin pricks on the site of injection.

Results

Effects on Central Nervous system

On gross behavioural effects: At lower doses (upto 100mg/kg) no appreciable gross effects were noticed both in rats and mice. But from 250 mg/kg onwards nimbidin produced a dose dependent mild CNS depressant activity in rodents 15 min after i.p. injection and 60 min after oral dosing

Potentiation of pentobarbitone hypnosis

Nimbidin in 40 and 80 mg/kg dose levels potentiated the sleeping time effect was not statistically significant.

Anticonvulsant activity

In metazol-induced seizures in mice and supramaximal electroshock seizure test in rats nimbidin failed to protect animals against the clonic and tonic convulsions respectively, whereas, phenobarbitone (50 and 100 mg/kg) exhibited 60% and 80% protection.

Effects on cardiovascular system

Nimbidin upto 8 mg/kg (i.v) failed to produce any effect on B.P., E.C.G and respiration in anaesthetized dogs, but from 10 mg/kg onwards it produced a transient fall in B.P which was not blocked by atropine or antihistaminics pretreatment. In higher doses it showed an increase in the rate and amplitude of respiration. Test drug also showed a dose-dependent blockade of the hypertensive effect of large dose of nicotine (20 µg/kg)

It had no effect on the rate and amplitude of both auricular and ventricular contractions upto 20 mg/kg in intact dog heart as well as in isolated rabbit heart.

On frog heart (in-situ) nimbidin showed bradycardia from 2 mg onward and this effect was neither cholinergic nor sympatholytic.

Chromodacryorrhea: Nimbidin did not block the methacoline induced chromodacryorrhea in rats.

Gastrointestinal motility: Nimbidin failed to influence intestinal transit of charcoal meal, whereas atrophine-sulphate decreased the translocation of intestinal contents.

Antihistaminic test (Invivo): it did not protect g. pigs against histamine aerosol-induced convulsions.

Autonomic Pharmacology

There was 50 and 70% blockade of acetylcholine-induced contractions with 100 and 200 µg/ml of nimbidin respectively (Fig.1). It produced 40% inhibition of histamine-induced (3 µg/ml) spasms of g. pig ileum in a concentration of 1 mg/ml bath (Fig2). But this blockade was found to be of weak, transient and noncompetitive.

Nimbidin at concentrations of 200 and 500 µg/ml produced 50 and 90% blockade of the spasmogenic effect of nicotine (100 µg/ml) in g. pig ileum indicating ganglion blocking activity of test drug (Fig.1).

Nimbidin (200 µg/ml) exhibited 50% and 80% antagonism of 5-HT induced spasms in rat ileum and rat uterus (Fig.3) respectively.

In electrically stimulated rat uterus nimbidin up to 500 µg/ml bath fluid failed to exhibit any histamine H₂ – receptor blocking activity (Fig.4)

On frog skeletal muscle preparation, nimbidin from 100 µg/ml onwards showed antagonism of Ach (2µg/ml) induced spasms and with 2 mg/ml dose there was 60% inhibition, which was also found to be reversible (Fig .5).

Diuretic activity

Nimbidin showed only moderate diuretic effect (48% urinary excretion at 40mg/kg) as compared to that hydrochlorothiazide (94%) in rats.

Effect on fertility

Nimbidin failed to exhibit any significant effect on implantation in proven fertile female and supermetogenesis in male rats. It was also found to be devoid of any antiandrogenic potential.

Local anaesthetic activity

In both test patterns nimbidin did not exhibit any local anaesthetic effect in rabbits as well as g. pigs.

Discussion

Present investigation on nimbidin, the crude bitter principle from neem seeds clearly demonstrated that this compound does not possess any undesired pharmacological actions. It was observed to have only moderate CNS depressant effect as it potentiated the pentobarbitone sleeping time in mice at 40 and 80 mg/kg dose levels. But it had no anticonvulsant activity in rodents. Nimbidin did not show any significant cardiovascular effects, but at higher dose levels (10 and 20 mg/kg i.v) it exhibited partial antinicotinic action in anaesthetized dogs predictive of its ganglion blocking effect.

In autonomic pharmacological studies (in-vitro) nimbidin significantly inhibited the spasmogenic action of acetylcholine, histamine, nicotine on isolated smooth

muscles of the G.I tract of g.pigs. it also exhibited anti-5-HT effect on smooth muscle preparations of rat. However the drug failed to exhibit any effect on the normal motility of the gastro-intestinal tract in rodents (in-vivo). Similarly this in-vitro anticholinergic and antihistaminic effects could not be demonstrated in in-vivo tests like chromodacryorrhea in rats and histamine aerosol test in g. pigs. On the contrary, nimbidin exhibited antinicotinic activity in both in-Vitro and in-vivo test patterns suggestive of the ganglion blocking effect. But it was devoid of H₂-receptor blocking action in-vitro.

Nimbidin showed moderate diuretic activity without influencing the ionic concentration. It was devoid of any local anaesthetic and antifertility effects in rodents. The pronounced anticholinergic, anti-5-HT and antinicotinic effect exhibited by this crude compound without any significant CNS or CVS effects warrant further detailed evaluation before it is being exploited in therapy.

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