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INVESTIGATION ANTIPYRETIC AND ANALGESIC ACTIVITY OF NICLOSAMIDE

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ABSTRACT
The present pharmacological research work was accomplished to explore the analgesic and antipyretic potential of Niclosamide (50 and 100 mg/kg, p.o.).Acetic acid induced writhing and Eddy's hot-plate model was used to evaluate analgesic effect while antipyretic effect was assessed against brewer`s yeast induced pyrexia in rat. In writhing activity is significantly decreases 27.06% and 49.74% in acetic acid induced analgesia model while latency period of Eddy's hot-plate model is significantly increases 12.17±0.310 and 14.34±0.349 at low and high dose of Niclosamide (50 and 100 mg/kg, p.o.). It also effectively suppress anal temperature at both high and low doses significantly 96.43±0.009 and 96.42±0.003 respectively in Brewer's yeast induced pyrexia. Preliminary experimental outcome is clearly indicates that Niclosamide (50 and 100 mg/kg,p.o.) is having significant analgesic and antipyretic potential.
Keywords-Niclosamide, Pyrexia, Brewer yeast, Accomplished Antipyretic potential.

INTRODUCTION

Shiver of unspecified origin may be caused by numerous illness, along with different dependable region might be septicaemia ,tissue destruction , or some other illness state or other usual characteristic of these state in the intensify formation of cytokines such as interleukin -1 interleukin-6 ,interferon –α and β tumor necrosis factor , the cytokines intensify the synthesis of PGE2 by switch on arachidonic-acid pathway.[1]

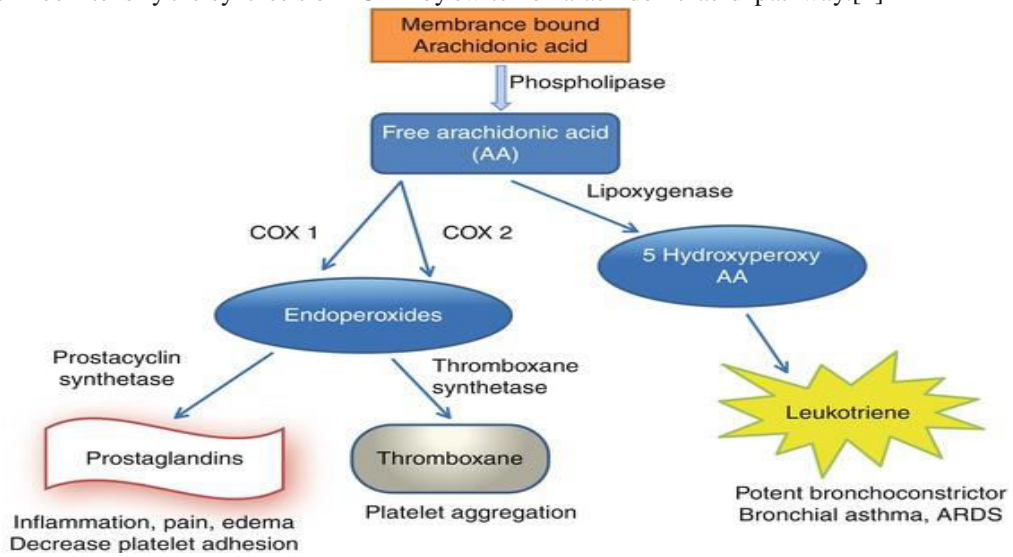

Figure 1. Prostaglandins Synthesis

Enhance of body heat beyond the standard scale 36.5–37.5 °C (97.7–99.5 °F) . Usual body heat is regulated by a central in the hypothalamus who make certain a equilibrium between temperature dropping and temperature making shivers happen when there is disruption of this hypothalamic thermostat descent of fever is being via different procedure. The interplay of external febrifarous (e.g. micro-organisms) or internal febrifarous (e.g. interleukin (IL)-1, IL-6, interferon gamma and ciliaryneurotrophic factor (CNTF) tumour necrosis factor (TNF-α) with the organism vasculosum of the

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lamina terminalis connection to the produce pyrexia.[2] Cryogens inclusive of anti-inflammatory cytokines (IL-10) hormones (e.g.α melanocyte stimulative hormone corticotrophin and corticotrophin discharge hormone) and numerous neuroendocrine gland product ,cytochrome P-450 between another they exert their anti-pyrexia impact through the preventing agglutination of febrifarous cytokines (glucocortcoids, IL-1 finder adversary . [3]

Paregorics are the medicaments that comfort in anguish in absentia loss of sentience comfort of anguish is necessary mediated following make into 2 grouped. Narcotics and Nor-narcotics .Narcotics whereas get rest from soreness even they making depression of the C.N.S those are of naturally containing opium alkaloid: e.g. morphine, codeine, nor-narcotics those medicament do not make valuable depression of C.N.S. whereas getting rest e.g. Salicylates and respective compounds. NAISDs are principally dominant in opposite to rest associative with swelling distemper or tissue harm since they lack making of prostaglandins (pain, fever, tissue injury). NASIDs exert their function by prohibit biosynthesis of prostaglandine and prohibit the cyclooxygenase enzyme COX. COX prohibit are an indiscernible most part of analgesic regimens. COX enzymes exist having sub class, COX-1 and COX-2. COX-1 isoform is pressing and made prostanoids requisite for physiological rising inclusive protection of gastric mucosa. COX-2 is infusible and making prostanoids which intermediary swelling procedure as in today's competitive world people are always in race to surge ahead of each other.[4] In doing so we compromise with our health and suffer with pain. Headache, back pain etc. Many of these pains are commonly due to our lifestyle and occupational hazards. In order to relieve ourselves from the pain, we rely on pain killer. Worldwide scientific research has been focused on inflammation because of its repercussion in practically all human and animal diseases. [5]

Niclosamide is an FDA assumptive thalminitics medicament .which is highly effective medicament in opposition to cestodes contaminate human being - taeniasaginataT.solium,Diphyllobothriumlatumandhymenolepis *nana,* together with pin worm (*enterobius)* to treat the tapeworm contamination in men and variant animal the medicaments seems to work by intercept oxidative phosphorylation in the mitochondria and tampering with anaerobic procreate of ATP by the tapeworm traumatic due to niclosamide, these day niclosamide is being used by human being as a oral antihelminthic medicaments to treat the parasitic contamination. [6]

According to existing research have irradiate that niclosamide can have broad ranging medical supplication for the remedy of illness different than those account by parasites. These illness and indication could include carcinoma .microorganism and viral germ contamination , metabolic ill health like that Type II diabetes mellitus, NASH and NAFLD, artery constriction, endometriosis, neuropathic discomfort, particular rheumatism, sclerodermatous graft-versus-host disease, and intrinsic sclerosis. Amongst the prime procedure associative with the medicament activity of niclosamide are approaching of oxidative phosphorylation, and modulation of Wnt/β-catenin, mTORC1, STAT3, NF-κB and Notch signaling paths.[7]

Niclosamide significantly inhibited HUVEC proliferation, migration and cord-like structure formation. Niclosamide also suppresses VEGF-induced angiogenesis in vivo. Niclosamide attenuated IKK-mediated activation of NF-jB pathway in TNFa-induced endothelial cells. Niclosamide also suppresses VEGF-induced endothelial VEGFR2 activation and downstream P-AKT, P-mTOR and P-p70S6K Vascular endothelial cells play an important role in the regulation of vascular tone, vascular permeability, angiogenesis and vascular inflammatory response Angiogenesis is the process of forming new blood vessels from an reproduction, pregnancy and wound healing, but it is also involved in pathologic processes such as inflammation, tumour growth and metastasis, several studies reported the inhibitory effects of niclosamide on multiple intracellular signaling pathways.[8]

MATERIALS AND METHODS

Experimental Rodents

Wistar albino rodents of either sex weighing between 150-200g were used for this study. They were procured in the AIBPS animal house Kanpur recognized by the Institutional Animal Ethics Committee (IAEC). Polypropylene limits were used to house (3 for each pen) theanimal at a temperature of 28 ±50C and 12 h light /dull cycle. Hindustan Lever chow pellets were used to feed the animal and water not basic. The animals were kept fasting medium –term going before the examination and this study was approved by IAEC for animal studies (1122/PO/Re/S/2007/CPCSEA) include all framework used in the research.

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Drugs and Chemicals

Niclosamidetablets, Batch No.CTE202028 were obtained from Juvenor Healthcare Private Limited and Diclofenac injection (Abaris Healthcare Private Limited Mumbai, Batch No.UO40072) and Paracetomol injection (Batch No. –TAB 9D11) was purchased Tablets (india) Limited.

STUDY DESIGN

IN VIVO ANALGESIC ANDANTIPYRETIC ACTIVITY

Writhing test

Six albino (n = 6) mice (Musmusculas) of either sex weighing between 30 to 40 gram was used in each group

- **Control Group** - Carboxyl methyl cellulose 1% Suspension (1 ml/100 g body weight);
- **Standard Group** - diclofenac sodium 10 mg/kg suspended in CMC;
- **Niclosamide** Group - 50mg/kg orally.
- **Niclosamide**Group- 100 mg/kg orally.

1% CMC (1 ml/100 g b.w.) was orally given to control group (Group I) while Standard group (Group II) treated orally with Diclofenac (10 mg/kg) suspended in 1% Carboxymethyl cellulose. Niclosamide 1 and Niclosamide 2 (Group III and Group IV respectively) received 50 mg and 100 mg/kg drug orally .After Thirty minutes 0.1 ml/10 g of 1% aceticacid solution (i.p.) injected to each group. The number of writhes was counted for 15 min after Five minutes after acetic acid injection. The percentage inhibition of writhing was calculated according to the following formula,

%Inhibition= ((AWC -AWT)/AWC) ×100.

Where,

AWC =Average number of writhing activity in control group,

AWT =Average writhes in Treated group (Purnima A. et al., 2010).

HOT PLATE METHOD

The paws of mice and rats are very sensitive to heat at temperatures which are not damaging the skin. The responses are jumping, withdrawal of the paws and licking of the paws. The time until these responses occur is prolonged after administration of centrally acting analgesics, whereas peripheral analgesics of the acetylsalicylic acid or phenyl-acetic acid type do not generally affect these responses. [9]

Procedure

Adult Albino Mice (n = 6) of body weight ranging from 30 to 40 gram of both sexes were randomized into four groups of six each.

- **Control Group -** Carboxyl methyl cellulose 1% Suspension (1 ml/100 g body weight)
- **Standard Group** - Diclofenac sodium 10 mg/kg suspended in CMC;
- **Niclosamide**I-Group - 50 mg/kg orally.
- **Niclosamide** II-Group- 100 mg/kg orally

Eddy's Hot plate Temperature was maintained at 55°C to 56°C throughout the study. The animals were individually placed on the hot plate. The time either lacking or jumping occurs was recorded. Repeated reading was taken before and after 30 mint and 39 1 h, 2 h and 3 h following administration of the drugs to the respective groups. The cutoff time for mice was 15 sec to avoid any injury.[10]

Statistical observation

The measurable investigation was finished using Graphpad 5.0 programming. Information was recorded as mean ± S.E.M. The measurable outcome of variety between bunches was controlled by examination of change (ANOVA) worked by Dunnett's test. Contrasts of $P<0.05$ were inspected statically huge.

BREWER'S YEAST INSTIGATED PYREXIA IN WISTAR RAT

Principle

The subcutaneous implantation of Brewer's yeast blend is known to move fever in Wistar rodents. A decline in temperature can be ended by the association of blends in with antipyretic activity.

Technique

Twenty Wistar rodents (150-200 gm) of either sex were arbitrarily partitioned into four gatherings.

- Control Group - 1% Tween 80% in normal saline solution orally
- Standard Group - Paracetamol 100 mg /kg
- Test Group – I Niclosamide 50 Milligram/ kilogram orally
- Test Group – II Niclosamide 100Milligram/ kilogram orally

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15% W/v suspension of Brewer's liquefaction in refined water at a portion of 10 ml/kg body weight was infused in the back below the nape of Wistar rodents. Before experimentation, the rectal degree of heat of all animal groups was recorded using a clinical digital thermometer.After 18hr Brewer's yeast injection increase the rectal degree of heat was noted and only creatures showing an influence in the degree of heat of at least 0.6^0 C or (1^0F) were chosen for the study. Control group creatures have given 1% Tween 80% in typical saline arrangement orally and Standard gathering creatures have given Paracetamol drug 100 Milligram/ kilogram, Test bunch I creatures given Niclosamide 50 Milligram/ kilogram orally and Test gathering – II given Niclosamide 100 Milligram/ kilogram orally. After the treatment, the level of warmth of the considerable number of rodents in each gathering was noted at 0 hours, 1 hours, 2 hours, 3 hours, and 4 hours .[11]

Statistical observation

The statistical investigation was finished using Graphpad 5.0 programming. Information was noted as mean ± S.E.M. The measurable outcome of variety between bunches was finished up by the examination of various two way (ANOVA) followed by means of Bonferroniposttests. Contrasts of $P<0.001$ were analyzing statically demonstrative

RESULTS AND DISCUSSION

The study was started to study analgesic and anti-pyretic effect of Niclosamide at 50 and 100 mg/kg dose. The result of different study is given below:

Result of Acetic Acid induced Writhing Activity

In acetic acid induced writhing method Six albino (n = 6) mice was taken in each group. The study was divided in four group. Control Group, Standard Group and Test Group-I niclosamide 50mg/kg orally and Test Group-II niclosamide 100mg/kg. Animal group treated with niclosamideclearly shows significant increase in reaction time at 100 mg/kg (as given in table1).

**Result of Eddy`s Hot Plate Method** In eddy`s Hot plate method Six albino (n = 6) mice was taken in each group. The study was divided in four group. Control Group, Standard Group and Test Group-I Niclosamide 50 mg/kg orally and Niclosamide group II-100mg/kg. Animal group treated with Niclosamide clearly shows significant increase in reaction time at 100 mg/kg (as given in table 2).

Result of Brewer`s induced Pyrexia

The impact of Niclosamide on the rectal degree of heat in rodents is depicted in (Table3). The subcutaneous infusion of brewer's yeast suspension uniquely expanded the rectal degree of heat after 18h of the organization. prevention with Niclosamide 50mg or 100 mg/kg diminished the rectal temperature of the rat in portion subordinate kind. It was discovered that the Niclosamide 50 Milligram/ kilogram created significant bringing down the internal heat level at 4 hours following its organization (96.42 ± 0.003). This impact was maximal at a portion of 100 Milligram/ kilogram at which created significant bringing down the internal heat level ($P<0.01$) as long as 4 hours after its organization (93.40 ± 0.015). The antipyretic activity began as ahead of schedule and 1 h and the impact were kept up for 4h, after its organization. Standard medication paracetamol 100mg/kg and test tranquilize (Niclosamide) were essentially lessened the yeast-raised rectal temperature, at second, third, and fourth hour contrasted with the benchmark group.

The intraperitoneal injection of acetic acid (1%) caused strong nociceptive response in the control group, with (91.18± 2.78) abdominal contortions. At high and low doses (ETS) it showed more number of writhing's (66.51±6.76 and 32.51 ±3.53, respectively) as compared to standard (42.68±3.49). Treated animals with Niclosamide (50,100 mg/kg) inhibited writhing's caused by acetic acid by 27.05 and 49.73%, respectively. Diclofenac sodium (10mg/kg), the standard for this experiment, reduced contortions by 52.90 %.

Table-1Effect of Niclosamide on Acetic Acid Induced Writhing Activity in Mice

Treatment Dose mg/kg	**No. of writhing per 15 minute**	**Percentage of inhibition**
Control	91.18 ± 2.78	-------
Diclofenac 10mg/kg	42.68 ± 3.49	52.90
Niclosamide(50 mg/kg)	66.51 ± 6.76	27.06
Niclosamide(100 mg/kg)	32.51 ± 3.53	49.74

Hot plate result showed significant reduction of pain at 120 min following Niclosamide medication (50 and 100 mg/kg) as compared to control. The animals pre-treated with Niclosamide showed a dose dependent increase in latency of response in the hot-plate method. Increase in mean reaction time by diclofenac in the standard group was significantly higher (15.08±0.266 s) at 2 h than both low and high doses, which showed the mean reaction time at 12.17±0.310 s and 14.34±0.348, respectively when compared to control at 4.29±0.254s

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Table2: Analgesic effect of Niclosamidewith Eddy`s Hot plate Test in Mice

Treatment Dose mg /kg	Reaction time in second in different hours				
	0Mint	30 Mint	1hour	2 hour	3 hour
Control	4.49 ± 0.149	4.54±0.248	4.66±0.254	4.29±0.254	5.56±0.231**
Diclofenac(10mg/kg)	5.42±0.287	12.70±0.306**	14.39±0.361**	15.08±0.266**	14.56±0.285***
Niclosamide (50mg/kgorallly)	5.44±0.121	9.13±0.307**	10.25±0.338***	12.17±0.310***	11.517±0.344***
Niclosamide(100mg/kg orally)	6.12±0.234	11.22±0.232**	13.94±0.234***	14.34±0.349****	13.49±0.174***

All values were described as mean± SEM. All the data were statistically analyzed by one-way ANOVA followed by Dunnett's multiple comparison test and values P<0.05 were studied to the significant. *** P<0.0001 when compared to the control group.

Table: 3Antipyretic effect of Niclosamideon Brewer's yeast induced pyrexia in Rat

Treatment 0 hour	Dose(mg/kg) (after18hr)	Basel temp.(^{0}F)	Rectal temperature (^{0}F) 1 hour	2 hour	3 hour	4hour
Control	Dose	95.57 ± 1.222	101.27 ± 0.414	101.12±0.005	101.08± 0.052	101.19±0.009	101.08 ± 0.007
Paracetamol	100mg/kg	95.42± 0.005	100.17±0.006	98.24±0.006***	97.21± 0.008***	96.50± 0.009***	93.49±0.402***
Niclosamide	50mg/kg	96.13 ± 0.006	101.09 ± 0.005	99.02.13 ± 0.004***	97.52 ± 0.017***	96.72± 0.008***	96.43± 0.009***
Niclosamide	100mg/kg	96.21± 0.409	101.47 ± 0.011	98.43 ± 0.005***	96.48 ± 0.406***	96.47± 0.005***	96.42 ± 0.003***

Each value represents the mean ± SD, n=6 in each group, obtained data were analyzed by using two way ANOVA followed by Bonferroniposttests to compare the values, where statistical significance was ***P<0.001 when compared to control group

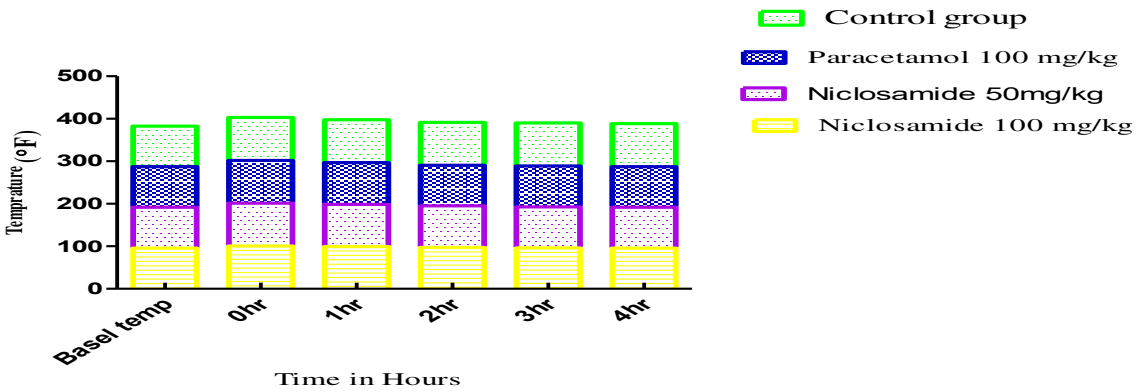

Figure-2 Column Graph Displaying the Effect of Niclosamide Drug 50mg/kg and 100mg/kg Against Brewer's Yeast Instigated Pyrexia

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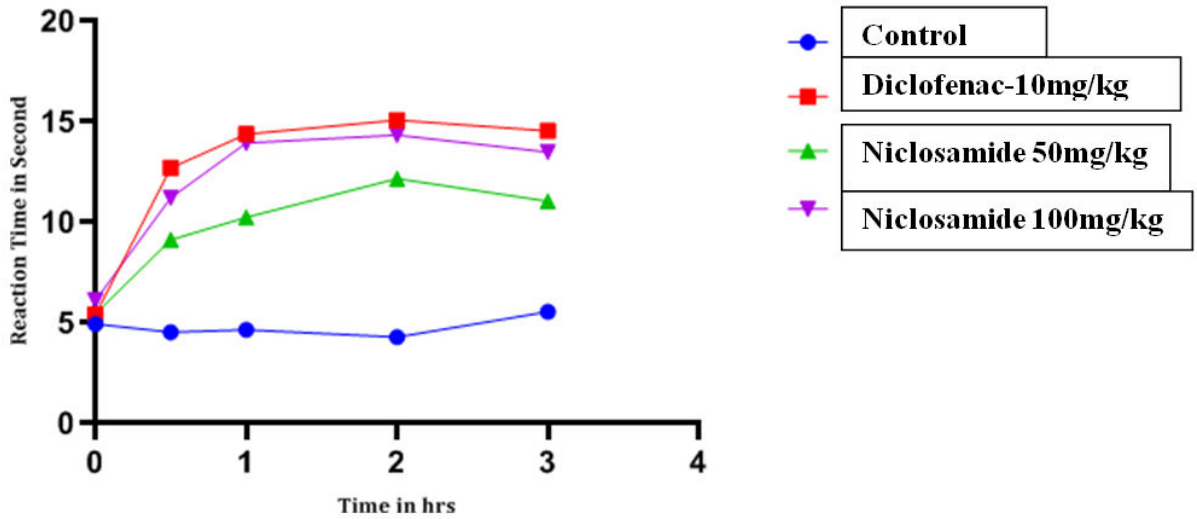

Figure-3 Line Graph Showing Effect of Niclosamide 50 mg/kg, 100mg/kg against Eddy`s Hot Plate induced nociception

CONCLUSION

Pyrexia and pain is considered as a secondary impact of infection, tissue damage, inflammation, graft rejection, malignancy or other diseased state. It is the body's natural defence to create an environment where infectious agent or damaged tissue can't survive. Most of the NSAIDS drugs inhibit COX-2 expression to reduce the elevated body temperature by inhibiting PGE2 biosynthesis. Moreover, these synthetic agents irreversibly inhibit COX-2 with high selectivity but are toxic to the hepatic cells, glomeruli, cortex of brain and heart muscles, whereas natural COX-2 inhibitors have lower selectivity with fewer side effects. Acetic acid induced writhing and Eddy's hot-plate model was used to evaluate analgesic effect while antipyretic effect was assessed against brewer`s yeast induced pyrexia in rat.

In writhing activity is significantly decreases 27.06% and 49.74% in acetic acid induced analgesia model while latency period of Eddy's hot-plate model is significantly increases 12.17±0.310 and 14.34±0.349 at low and high dose ofniclosamide (50 and 100 mg/kg, p.o.).

It also effectively suppress anal temperature at both high and low doses significantly 96.43± 0.009 and 96.42± 0.003 respectively in Brewer's yeast induced pyrexia. Preliminary experimental outcome is clearly indicates that niclosamide (50 and 100 mg/kg, p.o.). is having significant analgesic and antipyretic potential

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Author contribution

All author participated Equally.

Conflict of interest None

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Reference

1. Pise,HN and Jadhav SS, Evaluation of analgesic and antipyretic activity of Nigella sativa: an experimental study. National Journal of Physiology, Pharmacy and Pharmacology, 2016; 6(4): 291-295.

2. Tajuddin NB, Mohamed HMI and Arumugam VA, Antipyretic activity of Azimatetracantha in experimental animals, International Journal of Current Biomedical and Pharmaceutical Research, 2011; 1(2): 41-44.

3. Akapa TC, Kehinde AO, Beatrice OO and Olajide OJ, Antipyretic activity of Abutilon mauritianum (Jacq.) roots in Wistar rats, Int J Pharm Sci Res., 2014; 5(2): 42-45.

4. Pavani AN, Somashekara SC, Jagannath N, Govindadas D and Shravani P, Antipyretic activity of Piper nigrum in Wistar albino rats, Int J Pharm Biomed Res., 2013; 4(3): 167-169.

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 04, 2021

5. Vasundra DPA and Divya PS, Antipyretic activity of ethanol and aqueous extract of root of Asparagus racemosus in yeast induced pyrexia, Asian Journal of Pharmaceutical and clinical research, 2013; 6(3): 190-193.

6. Pan JX, Ding K and Wang CY: Niclosamide, an old antihelminthic agent, demonstrates antitumor activity by blocking multiple signaling pathways of cancer stem cells. Chin J Cancer. 31:178–184. 2012.

7. Satoh K, Zhang L, Zhang Y, Chelluri R, Boufraqech M, Nilubol N, Patel D, Shen M and Kebebew E: Identification of niclosamide as a novel anticancer agent for adrenocortical carcinoma. Clin Cancer Res. 22:3458–3466. 2016.

8. Ye T, Xiong Y, Yan Y, Xia Y, Song X, Liu L, Li D, Wang N, Zhang L, Zhu Y, et al: The anthelmintic drug niclosamide induces apoptosis, impairs metastasis and reduces immunosuppressive cells in breast cancer model.

9. Wang C, Zhou X, Xu H, Shi X, Zhao J, Yang M, Zhang L, Jin X, Hu Y, Li X, et al: Niclosamide inhibits cell growth and enhances drug sensitivity of hepatocellular carcinoma cells via STAT3 signaling pathway. J Cancer. 9:4150–4155. 2018.

10. Subedi NK, Rahman SM and Akbar MA, Analgesic and antipyretic activities of methanol extract and its fraction from the root of Schoenoplectusgrossus, Evidence-Based Complementary and Alternative Medicine, 2016: 1-8.

11. DubeyAnubhav, Tiwari M, Singh Yatendra, Kumar N, Srivastava K. Investigation of anti-Pyretic activity of vinpocetine in wistar rat, International Journal of Pharmaceutical Research 2020;12(2):1901-1906.