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Antimalarial potential of Nosode 30 and 200 against *Plasmodium berghei* infection in BALB/c mice

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ABSTRACT

Background & objectives: Homeopathy is considered as an emerging area of alternative medicine which could be established for the global health care. One of the greatest objections to this science lies in its inability to explain the mechanism of action of the micro doses based on scientific experiments and proofs. The present study has been undertaken to screen *in vivo* antimalarial activity of Malaria Co Nosode 30 and Nosode 200 against *Plasmodium berghei* infection in BALB/c mice.

Methods: Peter's 4-day test was used to evaluate the *in vivo* schizontocidal effect of Nosode 30 and Nosode 200. One month follow-up study was done to calculate the mean survival time of mice in each group. Biochemical analysis was carried out to assess the liver and kidney function tests using diagnostic kits.

Results: Nosode 30 and 200 exhibited 87.02 and 37.97% chemosuppression on Day 7 and mean survival time (MST) of 18.5 ± 2.16 and 16.5 ± 1.37 days respectively, which were extremely statistically significant when compared to MST of infected control (8.55 ± 0.83 days). The safety of Nosode 30 was also confirmed by the comparable levels of ALP, SGOT, SGPT activities, concentration of bilirubin, urea and creatinine to CQ treated group.

Conclusion: Nosode 30 possesses considerable *in vivo* antiplasmodial activity against *P. berghei* infection as compared to Nosode 200 as evident from the chemosuppression obtained using Peter's 4-day test. Further, studies on the drug can be carried out to establish its antimalarial potential in monotherapy or in combination with other homeopathic drug formulations.

Key words Homeopathy; Malaria Co Nosode; parasitaemia; *Plasmodium berghei*

INTRODUCTION

Antimalarial chemotherapy for decades is cheap, safe and practicable for outpatient use but resistance to cheap efficient antimalarial drugs cause a major problem. Homeopathy is a system of curing diseases with very minute doses of medicine. In homeopathy, crude drug substance is diluted and triturated or succussed to increase its potency by virtue of which, only the medicinal power of the substance is retained and drug related side-effects are eliminated. Hahnemann, father of homeopathy believed that 'Vital force' of a substance was somehow released by the process of 'succussion/potentization' to the 'vehicle' which now behaved as the medicine[1].

Homeopaths also use treatments called Nosodes made from diseased or pathological products such as faecal, urinary or respiratory discharges, blood and tissue. *Malaria officinalis*/Malaria Co Nosode is not a discharge but is called vegetable nosode as it is swamp mire that has been potentised. It was found that the Malaria Nosode is made out of the rotting vegetation from marsh areas where the mosquito breeds. The 'African' homeopaths recommended Malaria Co Nosode 30 on a weekly basis for visitors, starting a week before arrival for recurring parasite which lingers in liver for months. It is one of the most frequently used antimalarials in homeopathy, but there is no provings record. A routine of prophylaxis was given in the form of Abha light complex 30C remedies that contain 5 ingredients, one of them being Malaria Nosode, sold by Ainsworth Pharmacy, UK which contains four malarial (*Plasmodium falciparum*, *P. vivax, P. ovale* and *P. malariae*) and *Anopheles* mosquitoes. The malaria complex was found very effective in preventing malaria in Kenya. All recommended prophylaxis for visitors[2]. Homeopathy is the second largest system in the world, widely used and economical but lacks some evidence based research and data in order to prove its efficacy to the scientific community. *China* another homeopathic antimalarial is also reported to have schizonticidal activity against lethal rodent malaria parasite. The drug enhanced the mean survival time of the mice and also found safe on the morphology of red blood cells of the host as observed in SEM studies[3]. Present research work is an effort to provide most authentic information about antimalarial Nosode 30 and Nosode 200 to their *in vivo* antimalarial activity against *P. berghei* (NK-65) infection in BALB/c mice.

MATERIAL & METHODS

Mouse and parasite strain

White swiss mice *Mus musculus* of BALB/c strain (weighing 22–26 g and 4-6 wk old) of either sex, obtained from the Central Animal House, Panjab University, Chandigarh were used as experimental model. They were maintained on a standard pellet diet and water *ad libitum*. *P. berghei* (NK- 65) was maintained by i.p. inoculation of 1×10^6 parasitized RBCs from infected to naïve mice. Parasitaemia was checked by preparing Giemsa stained thin blood smears through tail vein incision of infected mice[4]. The treatment of mice was according to the guidelines of committee for the purpose of control and supervision on experiments on animals (Reg. No. 45/1999/CPCSEA), Panjab University, Chandigarh.

Drugs used

The 30 and 200 potency of homeopathic Nosode (Malaria Co Nosode [Nd]) manufactured by M/s. S.B.L. India were used in the present study. These drugs were diluted with distilled water in the ratio 1:2 (v/v) before administration. Chloroquine (LARIAGO Chloroquine phosphate suspension containing 50 mg base) manufactured in India, was given to positive control and chloroquine 20 mg/kg was prepared in distilled water.

Evaluation of antiplasmodial activity of Nosode (Nd 30 and Nd 200)

Six groups having 6 mice each (same sex and age) were used for the present study and the groups were designated as G1 to G6 (Table 1). All groups except G-1 (normal control) were injected with 1×10^6 *P. berghei* parasitized RBCs i.p. on D0 and treated for 4 consecutive days (D0–D3). G1and G2 mice were administered 0.2 ml of distilled water and were kept as normal control and infected control group respectively. Homeopathic drugs Nd 30 and Nd 200 and CQ (20 mg/kg) as positive control were evaluated for their schizontocidal effect using Peter 4-day test.

Blood smears were prepared from tail vein incision on D5 and D7 followed by weekly examination of blood smears up to 1 month follow up period (i.e. Day 14, 21 and 28). Blood films were stained with Giemsa and examined microscopically. The degree of infection was recorded as the percentage parasitaemia. Average chemosuppression for each group and mortality of mice (evaluated as mean survival time period (MST) in each group were determined arithmetically.

Biochemical analysis

The therapeutic efficacy of antimalarial drugs was checked by biochemical analysis (liver and kidney function tests) on Day 7 for G1 and G2 mice and on Day 10 and Day 28 for G3 to G6 mice. The biochemical kits used for assays were manufactured by M/s. Reckon Diagnostic Pvt. Ltd. and M/s. Span Diagnostic Ltd. Blood was collected by tail vein incision of mice from each group. Unhaemolyzed serum was collected and diluted with 0.9% saline.

Statistical analysis

Data have been presented as mean and standard deviation (SD). Statistical evaluation of differences between the experimental groups was determined by the Student's *t*-test with the level of significance of $p < 0.05$.

RESULTS

In vivo antiplasmodial efficacy of Nd 30 and Nd 200

All the mice of infected control (G2) died by Day 9 (Fig. 1a). Mice of G3 negative control also exhibited normal course of infection with 31.62±3.37% infection on Day 7 after which all the mice died within Day 10 (Fig. 1b) showing that vehicle (nascent alcohol) itself has no effect in clearing the rodent malaria parasite (Table 1).

Table 1. Parasitaemia and chemosuppression along with mean survival time period of various experimental groups

Groups (n=6)	Dosage (0.2 ml OD/Day/mouse) i.p. infection of 1×10^6 infected RBC's	Parasitaemia (%) on Day 7	Chemosuppression (Day 7)	Mean survival time (days)
G1	Distilled water (infected D0)	–	–	–
G2	Distilled water (infected D0)	30.44 ± 3.40	–	8.55 ± 0.83
G3	Nascent alcohol (1:2)	31.62 ± 3.37	–	8.83 ± 1.03
G4	CQ (20 mg/kg), infected on D0	0.6 ± 0.3*	96.7	28 ± 0
G5	Nd 30 (D0–D3), infected on D0	3.95 ± 0.9*	87.02	18.5 ± 2.16
G6	Nd 200 (D0-D3), infected on D0	18.88 ± 1.31*	37.97	16.5 ± 1.37

Note: All the groups except G1 were injected with 1×10^6 infected RBC on Day 0; Data are given as mean ± SD. *p*-value in comparison to infected control (G2 group) is shown as *extremely statistically significant ($p < 0.0005$).

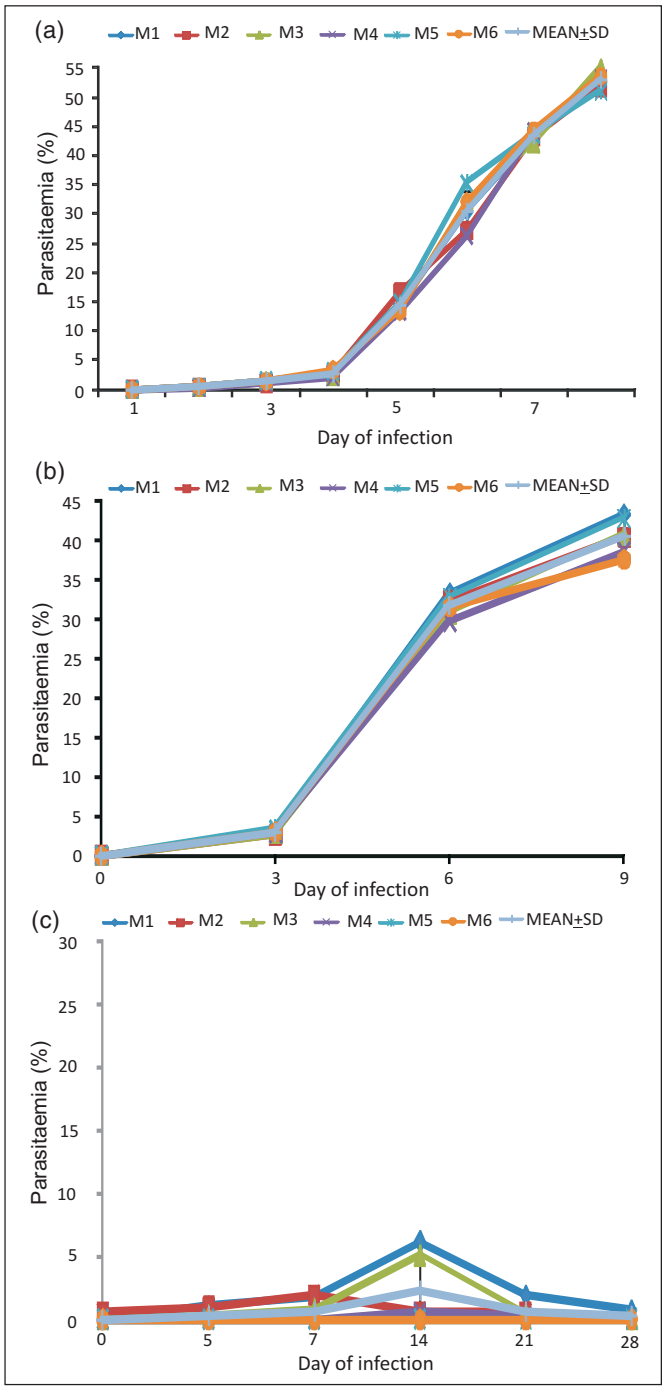

Fig. 1: Graph showing course of infection in BALB/c mice (G2 group) infected with 1×10^6 *P .berghei* parasitized red blood cells : (a) treated with nascent alcohol (G3 group); (b) chloroquine (G4 group); and (c) Data expressed as Mean±SD of six mice. Parasitaemia (%) of G2 group = 30.44±3.40, G3 group = 31.62 ± 3.37 on Day 7; and G4 = 0.6±0.3, 2.3±1 and 0.24±0.2 on Days 7, 14 and 28 respectively.

In group G4 (CQ 20 mg/kg), the parasitaemia was 0.34±0.1% on Day 5 which continuously increased up to 2.3±1% by Day 14 after which the parasitaemia declined to 0.24 ± 0.2% on Day 28 (Fig. 1c). CQ exhibited 96.7% chemosuppression and MST of 28 days which was maximum among the treated groups.

The infection observed in G5 group (Nd 30) was 1.02±0.13% on Day 5 which increased to 3.95±0.9% (p <0.0005) by Day 7 with 87.02% chemosuppression. The parasitaemia further increased to 14.69±0.73% and only one mouse survived till Day 21 (Fig. 2a) showing MST of 18.5±2.16 days. In G6 group (Nd 200), parasitaemia on Day 5 was 1.47±0.43% which increased to 18.18±1.31% by Day 7, followed by decrease in parasitaemia to 16.09±2.41% on Day 14 (Fig. 2b). In this group also, only one mouse survived till Day 21. Average chemosuppression was 37.97% and mean survival time was 16.5±1.37 (p <0.0005) days which was lowest among the treated groups but significantly higher than the MST of infected control.

Biochemical assays

The alkaline phosphatase (ALP) activity of enzyme in sera of normal mice was recorded to be 7.33±0.98 KA. It increased significantly in the infected mice 35.27±4.69

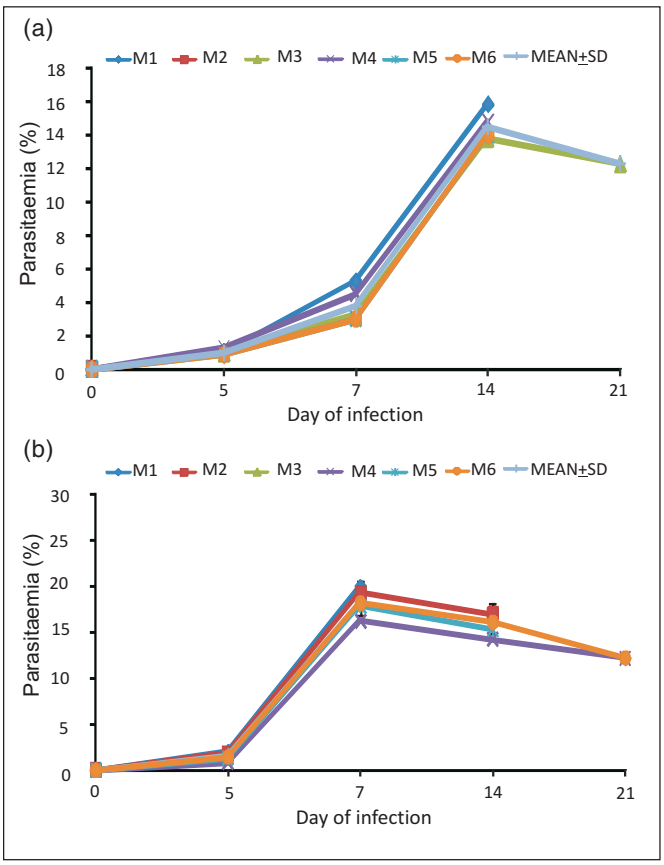

Fig. 2: Graph showing course of infection in BALB/c mice treated with Nosode 30 (G5 group): (a) Nosode 200 (G6 group); and (b) Data expressed as Mean±SD of six mice. Parasitaemia (%) of G5 group = 3.95±0.9, 14.69; G6 group = 18.18±1.31, 16.09±2.41 on Days 7 and 14, respectively.

Table 2. Alterations in ALP activity, bilirubin concentration, SGOT activity, SGPT activity, concentration of urea and creatinine in various experimental groups on Day 7 and Day 10

Group	ALP (KA units)	Bilirubin (mg/dl)	SGOT activity (U/l)	SGPT activity (U/l)	Urea (KA units)	Creatinine (mg/dl)
G1 (D 7)	7.33 ± 0.98	0.65 ± 0.27	13.68 ± 0.55	29 ± 0.2	32.0 ± 1.05	1.04 ± 0.18
G2 (D 7)	35.27 ± 4.69	2.31 ± 0.31	76.46 ± 0.82	133.19 ± 3.09	62.6 ± 2.25	4.57 ± 0.18
G3 (D 7)	34.34 ± 5.41	1.87 ± 0.12	80 ± 12.24	131.05 ± 6.12	74.05 ± 10.47	4.49 ± 0.08
G4 (D 10)	12.3 ± 1.3***	1.78 ± 0.2**	44 ± 5.02***	60 ± 3.8***	59.3 ± 3.3$^{\#}$	5.5 ± 1.02$^{\#}$
(D 28)	6.6 ± 2.5***	1.5 ± 0.11***	23 ± 4.3***	58 ± 6.2***	52 ± 6.2**	4.1 ± 0.3*
G5 (D 10)	12 ± 4.45***	1.10 ± 0.21***	47.05 ± 4.06***	87.23 ± 8.82***	73.33 ± 5.22***	2.04 ± 0.30***
G6 (D 10)	28.84 ± 3.21*	2.29 ± 0.23$^{\#}$	56.46 ± 9.54***	128.63 ± 1.2*	106.66 ± 9.81***	2.34 ± 0.16***

Note: Data are given as mean±SD; *p*-value in comparison to infected control (G2) is shown as ***extremely statistically significant ($p<0.0005$), **very statistically significant ($p<0.005$), *statistically significant ($p<0.05$), $^{\#}$not statistically significant ($p>0.05$) .

KA which was five times the normal value. Similar activity was found in G3 group (34.34±5.41 KA). A significant decrease in ALP activity was observed in G4 group by the Day 28 (6.6±2.5 KA) as compared to Day 10 (12.3±1.3 KA). In G6 group, ALP activity was maximum, i.e. 28.84±3.21 KA followed by G5 group (12±4.45 KA) on Day 10. Serum bilirubin level was also found to be increased in G2 group (2.31±0.31mg/dl) and G3 group (1.87±0.12 mg/dl) as compared to G1 group (0.65±0.27 mg/dl). The concentration of bilirubin was maximum in G6 group (2.29±0.23 mg/dl) followed by G4 and G5 groups. In G4 group, the bilirubin concentration decreased from 1.78±0.2 mg/dl on Day 10 to 1.5±0.11 mg/dl on Day 28 (Table 2).

Serum glutamate oxaloacetate transaminase (SGOT) activity was found to be 13.68±0.55 U/l in G1 group while in G2 and G3 groups it was increased more than six times, i.e. 76.46 ± 0.82 U/l and 80±12.24 U/l respectively. In G4 group, SGOT activity was 44±5.02 U/l on Day 10 and 23±4.3 U/l on Day 28. SGOT activity was observed maximum in G6 group (56.46±9.54 U/l), followed by G5 group (47.05±4.06 U/l). Serum glutamate pyruvate transaminase (SGPT) activity was also found five times more in G2 (133.19±3.09 U/l) and G3 (131.05±6.12 U/l) than G1 (29.0±0.20 U/l). SGPT activity was found maximum in G6 group (128.63±1.2 U/l), followed by G5 group (87.23±8.82 U/l), G4 group (60±3.8 U/l on Day 10 and 58±6.2 U/l on Day 28).

In G1 group the level of serum urea was determined to be 32.0±1.05 mg/dl which increased two fold to 62.6± 2.25 mg/dl in G2 group than normal value. In positive control, there was decrease in concentration of urea on Day 10 from 59.3±3.3 mg/dl to 52±6.2 mg/dl on Day 28. Serum urea was found maximum in G6 group (106.66± 9.81 mg/dl) followed by G5 group (73.33±5.22 mg/dl). Increased creatinine concentration is an indicative of renal diseases which was evident in G2 group (4.57±0.18 mg/dl) and G3 group (4.49 ± 0.08 mg/dl) as compared to normal mice (1.04 ± 0.18 mg/dl). Concentration of creatinine in G4 group was 5.5 ± 1.02 mg/dl on Day 10 which decreased to 4.1 ± 0.3 mg/dl on Day 28. Its concentration was 2.34 ± 0.16 mg/dl in G6 group and 2.04 ± 0.30 mg/dl in G5 group.

DISCUSSION

The antimalarial chemotherapy is the keystone of malaria control efforts. But the major problem to the use of antimalarial drugs is resistance developed by the parasite[5]. Homeopathy offers more affordable and safer approach to disease management. In the present study, antiplasmodial efficacy of homeopathic drugs Nd 30 and Nd 200 has been evaluated *in vivo*.

Lethality of *P. berghei* to mice has been reported by many workers[6]. *P. berghei* preferentially invades reticulocytes[7–9] and its infection is associated with mortality rates which are confirmed in the present study too. When vehicle (nascent alcohol) was administered to BALB/c mice the parasitaemia on Day 7 was comparable to infected control group, indicating that vehicle does not provide any protection and have no placebo effect. The homeopathic medicines have been shown effective than placebo controls in 60 (43.5%) findings of 138 randomized controlled trials till 2008. In drug treated groups, the chemosuppression of Nd 30 was 87.02% on Day 7 which was higher than Nd 200 treated group. The mean survival time period was also more in Nd 30 (18.5±2.16 days).

This observation is in accordance with the earlier studies where 30 potency of homeopathic drugs *China, Chelidonium* and *Artemisia vulgaris* were found to be very effective against rodent malaria parasite exhibiting maximum chemosuppression and enhancement of mean sur-

vival time of mice[10–13]. According to the Hahnemann, any disease may remain unaffected by the lowest potency, whereas, higher potency probably is too powerful which may cause adverse effect, so use of correctly selected medium potency may prove more effective[14]. It has been reported that *Cinchona officinalis* (*China*) can be taken as prophylactic drug in a 30 C dose weekly prior to entering a malarial zone[15]. All these findings establish that the 30 potency of homeopathic drug formulations is most effective against the malaria parasite as compared to lower/higher potencies which is observed in the present study too.

In positive control (CQ 20 mg/kg) on Day 7, chemosuppression was 96.7% and mean survival time was 28 days which was again in accordance with the earlier studies in which mice inoculated with 1×10^5, 1×10^6, 1×10^7 pRBC and then treated with chloroquine (20 mg/kg) showed a marked effect with the mean survival more than 28 days[16] .

The functioning of liver and kidney was also checked by measuring the levels of serum alkaline phosphatase activity, concentration of bilirubin, SGOT activity, SGPT activity, urea and creatinine concentration. The increased serum ALP activity among patients indicate that the liver stage of malaria infection accompanied by perturbation of the host hepatocytes membrane leading to leakage of this enzyme out of liver cells[17]. In the present study also, the activity of serum alkaline phosphatase in G2 (35.27± 4.69 KA) and G3 (34.34±5.41 KA) groups was increased twice in comparison to normal mice (7.33±0.98 KA). In positive control, it was 12.3±1.3 KA on Day 10 which decreased to 6.6±2.5 KA on Day 28. Studies have shown that chloroquine affects a wide range of biochemical processes including inhibition of key metabolic enzymes such as alcohol dehydrogenase, succinate dehydrogenase and glucose-6-phosphate dehydrogenase[18,19]. ALP activity was maximum in G6 (28.84±3.21 KA) group, followed by G5 group (12±4.45 KA) almost comparable amount of ALP activity to normal mice prompting the safety of homeopathic formulation for liver of host.

Serum bilirubin concentration (≥3 mg/dl) is a common feature attributed in part to liver damage and haemolysis of parasitized and non-parasitized cells[20]. In infected control, bilirubin concentration was observed to rise four times as compared to normal. While in chloroquine treated group bilirubin concentration was 1.78±0.2 mg/dl. The serum bilirubin levels were observed to increase in all treated groups indicating jaundice like symptoms.

The ratio of AST to ALT is useful in differentiating between causes of liver damage[21,22]. SGOT activity increased six times in infected control and vehicle treated group than the normal control. In G4 group, SGOT activity was decreased by Day 28 which was comparable to normal value. The results of the present study revealed a significant increase of serum transaminases. The elevation during acute infection results in necrosis of hepatic cells. These enzymes are released into the circulation with consequent rise in the serum levels[23–25]. SGPT activity was observed to rise in all treated groups. In chloroquine treated (G4) group also, considerable increase in SGPT activity was recorded.

Serum urea and creatinine concentration are used for the assessment of renal sufficiency[26]. There was significant increase in serum urea and creatinine in infected control as compared to normal. Higher levels of serum urea and creatinine are indications of deficiency in renal function[27–29]. All the treated groups recorded increase in serum urea levels. Chloroquine administration is also reported to impair kidney function, resulting inappropriate Na^+ and Cl^- retention[30]. In the present study also, the creatinine levels were also considerably elevated in CQ treated group indicating impairment of kidney function. In treated groups, the increase in creatinine concentration was less as compared to *P. berghei* infected mice.

CONCLUSION

These findings suggest that chemosuppression observed in Nd 30 treated group is considerable. Further studies are needed to establish Malaria Co Nosode 30 (Nd 30) as potent antimalarial in monotherapy or in combination therapy with other homeopathic formulations.

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