

# Effects of quinine sulfate- an antimalarial drug on palatal growth in developing mice embryo: A histopathological study



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## ABSTRACT

**Background:** Malaria is one of the most common infectious and fatal diseases worldwide. Quinine sulfate is one of the antimalarial drug used to treat chloroquine resistant malaria and malaria falciparum. **Aims and Objectives:** The present study was designed to assess its developmental defects on mice palate. **Materials and Methods:** Thirty adult pregnant female Swiss albino mice were used in the present work. They were divided into five groups of six each. One control group (c) with 6 pregnant mice received normal saline orally from day 6 to 15 of gestation. Four treated groups of 6 pregnant mice each were formed. Treated groups T1, T2, T3 and T4 were administered Quinine sulfate orally with 50, 100, 200, 400 mg/kg bw/day from day 6 to 15 of gestation respectively. **Results:** No palatal abnormalities were observed histologically in the control and T1 treated group. However, varying degrees of clefting of the palate, from failure of elevation of the palatal shelves to failure of fusion in the midline were observed in the Quinine sulfate treated groups (T2, T3 & T4). **Conclusion:** Quinine sulfate induced defects in the developing palate in the present study by disturbing the process of palatogenesis, which is characterized by incomplete growth, elevation or fusion of the palatal shelves.

**Key words:** Quinine; Teratogenesis; Clefting; Mesenchyma

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## INTRODUCTION

Many great discoveries have been made by chance but some have been the result of human perseverance and ingenuity. A sterling example is the discovery of quinine in Peru and is now produced in Java. Quinine has gone through centuries without losing its medical efficacy that efficacy allowed the exploration and colonization of Africa and played a key role in the ability to conduct overseas military campaigns.<sup>1</sup> Quinine is the oldest drug used to treat malaria and it remains widely used. It is especially valuable in the treatment of severe or drug resistant *P. falciparum* infections. It is considered to be safe during pregnancy although the evidence is limited and it is known to cause hypoglycaemia.<sup>2</sup> Quinine was first used in the 17<sup>th</sup> century as a prophylactic against malaria.

Quinine remains the prototype blood schizonticide for the suppressive treatment and cure of chloroquine-resistant and multidrug-resistant falciparum malaria.<sup>3</sup> At the beginning of the 1970s, researchers became interested in the antiviral properties of antimalarial drugs. Today, quinine is still used to treat chloroquine and multidrug resistant *Plasmodium falciparum*, as well as severe cerebral infestation.<sup>4-6</sup> Recently quinine is used in the treatment of autoimmune disorders such as Lupus and rheumatoid arthritis.<sup>7</sup> QS is also widely used to treat patients with autoimmune dermatologic and rheumatologic diseases, and additional therapy for patients with HIV infection. These patients, who are often immuno-compromised, may receive a secondary advantage from these antimalarials, which may provide some protection against staphylococci and *E. agglomerans*.<sup>8</sup> The efficacy of the quinine will

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continue to play a significance role in the management of multidrug-resistant malaria.<sup>9</sup>

Although the efficacy of quinine is immense in the treatment of chloroquine-resistant and multidrug-resistant falciparum malaria, there are also a few reports on its teratogenic effects in pregnancy. However, there are not enough evidence to demonstrate the teratogenic and toxic effect of quinine sulfate (QS) on the craniofacial abnormalities in the humans and experimental animals. Present study was carried out to determine the effect of QS on the palatal growth of mice embryos; in control and various treated groups in detail.

## MATERIALS AND METHODS

**Chemical:** Quinine sulfate was obtained from “LobaChemiePvt Ltd” (Mumbai, India). The chemical was of analytical grade.

**Animals:** A total of 30 sexually mature Swiss albino female mice (*musdomesticusdomesticus*), each of 8 to 10 weeks old weighing approximately 20-35 g were housed in the institutional animal house. The mice were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. The animals were maintained under standard laboratory conditions of temperature (23-28°C), humidity (30-70%), and a 12-h light dark cycle. All animals were allowed free access to filter-purified tap water ad libitum and fed on a commercial diet (Hindustan Lever, Mumbai, India). All the studies conducted were approved by the Institutional Animal Ethical Committee (No. IAEC/KMC/06/2006\_2007), Kasturba Medical College, Mangalore, according to prescribed guide-lines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Female mice during their pro-estrous phase of estrous cycle were caged overnight with the males of the same stock (Female: Male=3:1). The vaginal smear was examined next morning at 8.00 a.m. presence of spermatozoa in the smear was taken as day ‘zero’ of pregnancy. A total of 30 pregnant mice divided into 5 groups of 6 each were studied.

**Acute Toxicity Studies:** The five-day LD50 (Lethal Dose) for QS was found to be 800 mg/kg bw. The dose selected for assessment of teratogenic potential of QS was 1/2, 1/4, 1/8 and 1/16 of the LD50 i.e. 400 mg/kg bw, 200 mg/kg bw, 100 mg/kg bw and 50 mg/kg bw respectively.

**Experimental Groups:** Thirty adult pregnant female Swiss albino mice (*musdomesticusdomesticus*) were used in the present work. They were divided into five groups

of six each. One control group (c) with 6 pregnant mice received normal saline orally from day 6 to 15 of gestation. Four treated groups of 6 pregnant mice each were formed. Treated groups T1, T2, T3 and T4 were administered QS orally (oral gavage) with 50, 100, 200, 400 mg/kg bw/day from day 6 to 15 of gestation respectively (Table I). At the end of the experiment period; pregnant mice were sacrificed with overdose of ether anesthesia on day 18 of pregnancy (i.e. two days prior to full term). The uterine horns were exteriorized after opening the abdomen by midline incision. The sacs were inspected for sites of resorption and viable fetuses. The fetuses were removed from the uterus and were dried by wiping on a blotting paper, then weighted in Essae digital weighing balance. Bouin’s solution was used for fixation (96 hr, and then washed in 70% alcohol).<sup>10</sup>

**Evaluation of Embryo Teratogenicity:** The uterine horns were cut along the anti-mesometrial (greater) curvature and macroscopically examined. Finally each fetus was separated from its amniotic sac and placenta, which was individually weighed and grossly inspected. The fetuses were euthanized with intraperitoneal injection of pentobarbital.<sup>11</sup> Following external observation, the heads of control and QS treated fetuses were fixed by immersion in Bouin’s solution for more than 96 hr, and then transferred to 70% alcohol. Heads of selected control (n = 2-3/litter, chosen at random) and all the QS exposed fetuses were detached and cut transversely into 5 blocks using free hand cross section (Fig. 1) guided by external landmarks.<sup>12,13</sup> For the detection of palatal abnormalities, the anterior region of the presumptive hard palate is chosen (Frontal planes 1& 2; Fig 1). They were prepared for light microscopic study by paraffin sections, serially cut at 6 micro meters (µm) in coronal plane. In each block of head every 7<sup>th</sup> section was considered and stained with haematoxylin and eosin and mounted.

## RESULTS

**Palatal abnormalities:** The percentage of palatal defects in the control and different treated groups were mentioned

**Table I: Showing dose of QS administered to different groups at different gestational day.**

Groups	Gestational days (GD) of drug administration	Dose
Control (C)	6-15 day of gestation	Normal saline
Treated (T1)	6-15 day of gestation	50mg/kg bw/day
Treated (T2)	6-15 day of gestation	100mg/kg bw/day
Treated (T3)	6-15 day of gestation	200mg/kg bw/day
Treated (T4)	6-15 day of gestation	400mg/kg bw/day

in the Table II. The palatal abnormalities are produced when elevation and subsequent fusion of the palatal shelves do not take place.

**Control and 50 mg/kg bw (T1) groups:** No abnormalities of the control and T1 treated group heads were noted from the histological examination. Histologically the palatal shelves had fused (and with the nasal septum) and consequently the oral and nasal cavities were separated (Fig. 2A).

**Palatal defects observed in the 100 mg/kg bw (T2), 200 mg/kg bw (T3) and 400 mg/kg bw (T4) treated groups:** The palates exposed to QS were morphologically abnormal, and the fusion patterns were affected in the T2, T3 & T4 groups. The severities of the morphological effects were dependent upon the concentration of QS. Control palatal shelves had elevated to the dorsum of the tongue and fused with the opposite side, separating the nasal cavity from the oral cavity. The palatal defects were more severe in the T4 treated group (37%), followed by T3 and T4 treated groups, which had 10.5% and 5.5% of palatal defects respectively. Palatogenesis is a complex developmental process that requires elevation and fusion

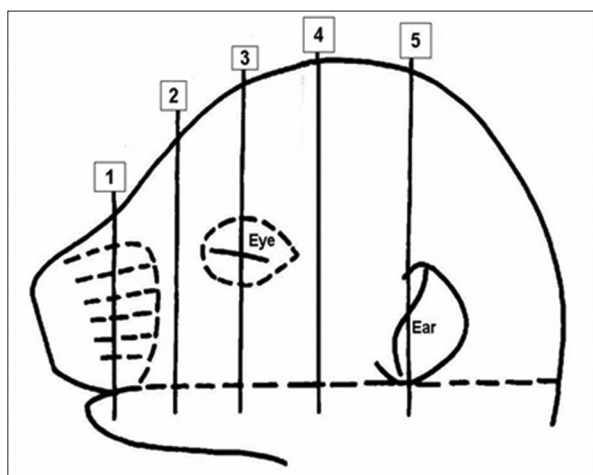
of the palatal shelves. These processes are disrupted in the T2, T3 and T4 treated groups causing varying degrees of clefting of the palate (Figs. 2 B, C, D), from failure of elevation of the palatal shelves (Figs. 3 A, B) to failure of fusion in the midline (Figs. 2 B, C).

## DISCUSSION

Identification of environmental agents that cause damage to unborn children is absolutely necessary. Armed with the knowledge of those drugs, chemicals and foodstuffs that human beings are routinely exposed to that are truly teratogenic, exposures can be avoided, reduced or limited, and the incidence of tragic teratogen-induced birth defects drastically lowered.

Quinine has been used in the treatment of malaria and is still widely used worldwide.<sup>9</sup> It is especially valuable in the treatment of severe or drug resistant Plasmodium falciparum infections.<sup>2</sup> But its effect as a teratological agent is long debated and no conclusive evidence is available. Results of animal experiments so far reported are inconclusive too. To give additional information on the teratological effect of quinine, we performed present experiments using mice and specifically concentrated on its effect on palatal growth.

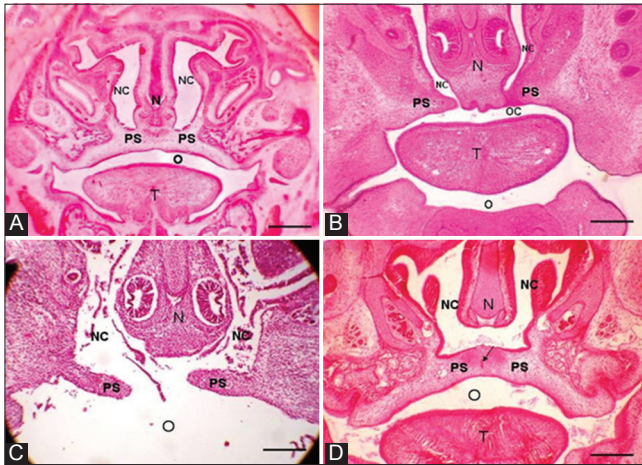
Cleft palate, with or without cleft lip, represents one of the most common birth defects observed in humans, occurring in approximately 1 in 700 live births.<sup>14, 15</sup> The mammalian palate develops from two primordia: the primary palate and the secondary palate. The primary palate represents only a small part of the adult hard palate. The secondary palate is primordium of the hard and soft parts of the palate. Palate develops in a multistep process that involves palatal shelf growth, elevation, midline fusion of palatal shelves, and the disappearance of midline epithelium seam.<sup>16</sup> The palatal structures are composed of the central nervous system (CNS) derived ecto-mesenchyme and pharyngeal ectoderm.<sup>17, 18</sup> The epithelia that cover the palatal shelves are regionally divided into oral, nasal, and medial edge epithelium (MEE).<sup>16</sup> The nasal and oral epithelia differentiate into pseudostratified and squamous epithelia,



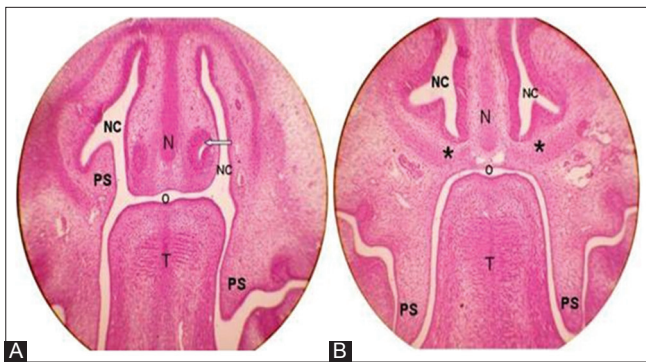
**Figure 1:** Profile of a fetal mouse head demonstrating external landmarks by which free hand transverse cross sections (frontal planes 1-5) were oriented to obtain reproducible sections for histopathological examination. For the detection of palatal abnormalities, the anterior region of the presumptive hard palate is chosen (frontal planes 1 & 2).

Table II: Palatal defects observed in the mice fetuses of the control and quinine sulfate treated groups.					
Groups	Control (%) n=58	T1 (%) n=55	T2 (%) n=54	T3 (%) n=38	T4 (%) n=27
Palatal defects	0	0	3 (5.5)	4 (10.5)	10 (37)
↓					
Unilateral	0	0	1 (1.8)	2 (5.2)	4 (14.8)
Bilateral	0	0	2 (3.7)	2 (5.2)	6 (22.2)

n=number of variable fetuses



**Figure 2:** Palatal shelf growth comparison in the control and treated groups. A: Control; Coronal section of anterior region of the presumptive hard palate. Note the palatal shelves (PS) has been elevated and fused in the midline and with the nasal septum (N), thus separating the nasal cavity (NC) from the oral cavity (O). (T=tongue). B: T3; Coronal section of anterior region of the presumptive hard palate. Note that the left palatal shelf (PS) has fused with the nasal septum (N), but there is no fusion on the right, thus the nasal cavity (NC) and the oral cavity (O) communicating with each other forming unilateral cleft palate. (T=tongue). C: T4; Coronal section of anterior region of the presumptive hard palate. The two palatal shelves (PS) failed to meet and fuse with each other, leaving the gap between the nasal cavity (NC) and oral cavity (O) forming bilateral cleft palate. (T= tongue). D: T4; Coronal section of a more posterior section through the presumptive hard palate. The nasal septum (N) is unattached inferiorly leaving the gap between both the nasal cavities (NC). The palatal shelves are fused. Note the remaining of the midline epithelial seam (arrow). (O=oral cavity; T=tongue). Scale bar 200µm.



**Figure 3:** Faulty elevation of the palatal shelves: A: T4; Coronal section of the anterior region of the presumptive hard palate. The palatal shelves (PS) are in vertical position lateral to the tongue (T). The nasal septum (N) is unattached inferiorly. The white arrow head indicates Jacobson's organ. (NC=nasal cavity, O=oral cavity). B: T4; Coronal section of the posterior region of the presumptive hard palate. The palatal shelves (PS) are vertically oriented lateral to the tongue (T). The nasal septum (N) has fused laterally with the lateral nasal walls (asterisks), separating the oral (O) and nasal cavities (NC). Magnifications, x4

respectively; the MEE is removed from the fusion line by means of programmed cell death and cell migration.<sup>19, 20</sup>

Palatal defects is one of the most serious congenital anomalies in humans that cause a sucking (breast feeding)

problem in newborn babies and a morphologic deformity that usually leads to death in newborn mouse offspring due to an insufficient ability to suck milk.<sup>21</sup> In humans, the frequency of palatal defects has been reported to increase in pregnant women affected with diabetes mellitus<sup>22</sup> or epilepsy.<sup>23</sup> The incidence of palatal defects are observed when methanol,<sup>24,25</sup> Triamcinolone acetone, a synthetic glucocorticoid,<sup>26</sup> 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), a highly toxic halogenated aromatic hydrocarbon,<sup>27</sup> corticosteroid<sup>28</sup> and retinoic acid,<sup>29, 30</sup> administered in the pregnant rodents.

Anti-malarial drugs and drug combinations like sulfadoxine, pyrimethamine and sulfadoxine-pyrimethamine are known to have caused cleft palate in rodents.<sup>31-33</sup> Mean time there are no such observations made in case of quinine.<sup>2,34</sup> The administration of H-triamcinolone acetone on day 11.5 of the gestation caused a 100% frequency of cleft palate in A/J mice.<sup>35</sup> During morphogenesis of the lip and palate in the mouse fetus, the primary palate (including the upper lip and mucosa) arises from the fusion of the maxillary process, the nasal process, and the hard palate. At the onsets of secondary palate formation, the palatal processes lie vertically beside the tongue. A lowering of the mandible and the tongue allows for a rapid rotation of the palatal processes toward the horizontal position. The horizontally positioned palatal shelves begin to grow toward the midline where they meet and fuse to form a definitive palate.<sup>36</sup> A change in the morphology of the posterior pharyngeal wall also assists in the horizontalization of the palatal shelves. Such a change occurred during palatogenesis, and the oropharyngeal tissues of the tongue and the pharynx might have thus contributed to the completion of palatogenesis.<sup>37</sup>

Palatal defects can be caused at different stages of palatogenesis as a result of incomplete growth, elevation or fusion of the palatal shelves,<sup>30, 38</sup> although the exact mechanism(s) remains unknown. For all the experiments reported in the present study QS treatment was commenced before the formation of palatal shelves, which in the mouse develop between embryonic day (E) 11.5 and 16.5.<sup>16</sup> Treatment with QS was seen not to cause disruptions to the development of the nasal septum, but caused the failure of palatal shelf elevation, shelves to meet following elevation and the medial edge epithelial cell death; although the mechanism(s) by which this occurred are presently unclear, it is possible that the effect of QS treatment on the anterior region of the presumptive hard palate in these specimens might have prevented adequate enlargement of the palatal shelves to allow fusion to occur. The presence of the nasal septum allowed partial separation of the oral and nasal cavities by fusion of the nasal septum and the palatal shelves. More posteriorly, where the nasal septum undercuts and separates from the palatal shelves, the palatal

shelves were close enough to allow fusion. The palatal shelves failed to reorient into a horizontal position only in the specimens treated QS. QS treatment may have caused modification in the extracellular matrix, or disruption of the mesenchymal cells, preventing the organisation of the palatal shelf mesenchyme necessary for elevation,<sup>39</sup> in the present study.

QS induced defects in the developing palate in the present study by disturbing the process of palatogenesis, which is characterized by incomplete growth, elevation or fusion of the palatal shelves.

**Limitation of the study:** Small sample size and lack of inferential statistics.

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**Authors Contribution:**

**SRN-** Concept and design of the study, data collection, manuscript preparation; **LVP-** Concept and design of the study and review of literature; **CVR-** Concept and design of the study and critical revision of the manuscript.

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