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Safety evaluation of an antiviral polyherbal drug of *Siddha* Medicine in Wistar rats

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A polyherbal formulation *Nila Vembu Kudineer* (NVK) is traditionally used in the *Siddha* medicine to treat variety of fevers and related illness. This preparation is also claimed to be useful in the treatment of *Dengue* and *Chickungunya*. However, information on its safety and effect on vital organs on prolonged consumption is still lacking. Hence, an attempt had been made to evaluate the safety profile of NVK by investigating its effect on hematology, biochemistry, antioxidant status and histopathology of vital organs of Wistar rats. Three groups of rats (n=4) were considered for the study. Group I rats served as control that received only food and water. Group II and III rats were orally administered with freshly prepared NVK decoction at400 and 800 mg/kg body weight respectively for 28 consecutive days. The body weights were monitored daily and on day 29, overnight fasted animals were sacrificed and the blood was collected to study hematological profile, hepato-renalmarkers and antioxidant parameters. The tissues of liver and kidney were subjected to histopathological examination. Sub-chronic administration of NVK in lower dose to rats did not produce any significant changes in body and organ weights. The hematological parameters as well as hepatic and renaltoxicity markers also showed no significant changes in lower dose. However, with higher dose of NVK, mild sinusoidal congestion and dilatation were noticed in liver tissues while significant elevation of urea in the serum and hydrophic changes in kidney architecture was observed in tissues. The results of the study indicated safety of NVK in the lower dose and possibility of nephrotoxicity with increase in dosage and duration.

Keywords: *Siddha* medicine, *Nila Vembu Kudineer*, Wistar rats, Hematology, Biochemistry, Antioxidant enzymes, Histopathology
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Siddha medicine is one of the oldest traditional medical systems widely practiced in Southern India and parts of Sri Lanka, Malaysia and Singapore. A variety of single and polyherbal formulations are used in the system to treat many diseases. *Nila Vembu Kudineer* (NVK)*,* a polyherbal formulation of *Siddha* medicine is prescribed in the treatment of many types of fevers especially of unknown origin[1]. It is advised in *Siddha* medicine to consume about 25 mL of the decoction (obtained by boiling 3.6 g of the formulation in 25 mL of water), thrice a day for three consecutive days. During the outbreak of *Dengue* and *Chickungunya,* the Government of Tamil Naduhas popularized NVK as a preventive measure in the last few years[2,3]. The decoction of NVK is freely distributed to the public in all hospitals and rural health centers especially to those who reside in epidemic zones to control morbidity and mortality[4,5]. The use of NVK in controlling viral fevers has also been incorporated in the Health Policy Note of the State Government[6].

However, there are apprehensions on NVK's safety due to lack of toxicological profile[7]. Hence, in this study, the effect of the drug on sub-chronic administration in Wistar rats had been carried out by investigating changes in hematology, biochemistry, LPO, antioxidant enzymes and histopathology of liver and kidney tissues.

Materials and methods

Animals

Twelve Wistar rats of either sex weighing about 180±10 g and housed in controlled environmental conditions (temperature: 24±2°C, relative humidity: 50-70% and 12 h light/dark cycle) were used in this

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study. The animals were provided with standard pellet diet and water *ad libitum*. The experiments were performed as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals, after obtaining necessary permission from the Institutional Animal Ethics Committee (Approval No: BRULAC/SDCH/SIMATS/IAEC/02-2018/006)

Drug preparation

NVKwas obtained from Tamil Nadu Medical Plant Farms and Herbal Medicine Corporation Limited (TAMPCOL), Chennai, Tamil Nadu, India. The polyherbal formulation consists of dry powders of the following plants in equal proportions – *Andrographis paniculata* (Burm. f.) Wall. Ex Nees. (Whole plant), *Plectranthus vettiveroides* (Jacob) Singh & Sharma (roots), *Vetiveria zizanioides* L. Nash (roots), *Zingiber officinale* Roscoe.(rhizomes), *Piper nigrum* L. *(*fruits), *Cyperus rotundus* L. (tubers), *Santalum album* L. (heart wood), *Trichosanthes cucumerina* L. (whole plant) and *Hedyotis corymbosa* L. Lam (whole plant)[8]. The decoction for administration to the animals was prepared daily by boiling 3.6 g of NVK with 25 mL of water (The weight of the drug used was calculated on the basis of human dose that is equal to two table spoons of the dry formulation in 200 mL water). The contents were cooled to room temperature, filtered and administered to animals by oral gavages.

Experimental design

Twelve rats were divided into three groups each containing four animals. Group I rats served as control that received only food and water while Groups II and III rats were administered with 400 and 800 mg/kg body weight of NVK decoction respectively. The doses were selected based on the earlier report[9]. The treatment was continued for 28 consecutive days and on day 29, overnight fasted animals were anaesthetized and sacrificed after cervical dislocation and blood was collected for hematological, biochemical and antioxidant investigations[10-12]. The liver and kidney were quickly removed, washed with normal saline and used for histopathological studies.

Results

Administration of NVK to animals in lower dose (400 mg/kg b. w.) for 28 days did not reveal any perceptible changes in behavior, body and organ weights, hematological parameters and biochemical markers. Aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyltransferase (γ-GT) and bilirubin did not show any significant changes after NVK administration (Fig. 1A, C, D, E). However, with higher dose, significant ($p<0.05$) changes were observed inalanine aminotransferase (ALT) (Fig. 1B) and urea content (Fig. 1F). In the oxidative stress markers, lipid peroxidation (LPO) showed significant ($p<0.001$) reduction with concomitant increase ($p<0.001$) in superoxide dismutase (SOD) activity and catalase showed a significant ($p<0.01$) increase only with lower dose of NVK administration (Fig. 2). In the histopathological investigations, in higher dose group, sinusoidal congestion and dilatation in liver and hydrophic changes in kidney tissues were noticed (Fig. 3).

Discussion

Dengue and *Chickungunya* fevers are caused by re-emergent arbo-viruses that are transmitted by mosquitoes of *Ades* genus. These viruses, over the

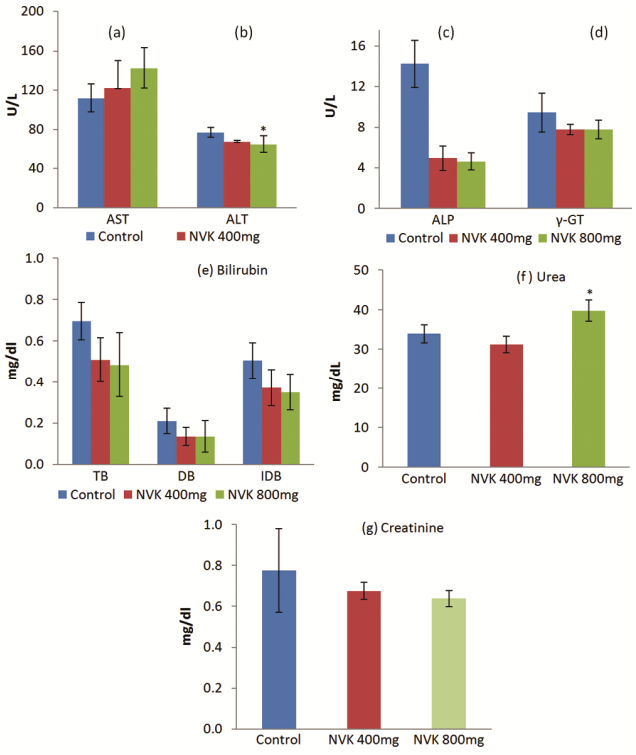

Fig. 1 — NilaVembuKudineer (NVK)-induced changes in hepatotoxic and nephrotoxicitymarkers. Values represent Mean ± S.D of four values; *$p<0.05$ vs control. AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; γ-GT: Gamma glutamyltransferase; TB: Total bilirubin; DB: Direct bilirubin; IDB: Indirect bilirubin.

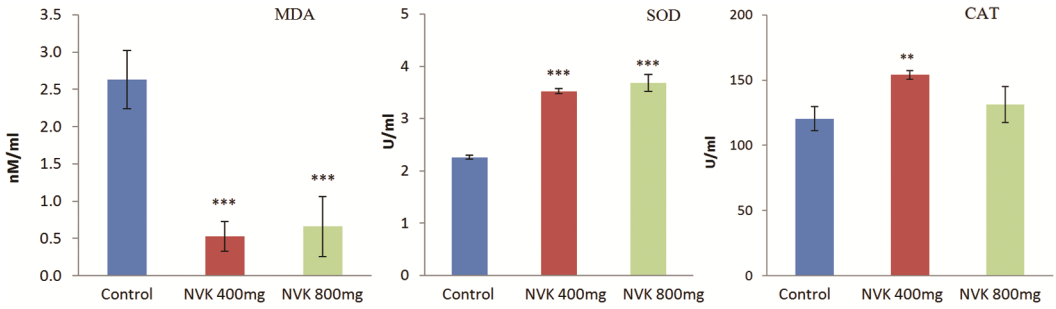

Fig. 2 — NilaVembuKudineer (NVK)-induced changes in oxidative stress markers. Values represent Mean ± S.D of four values; $^{**}p<0.01$ and $^{***}p<0.001$ vs control. MDA: Malondialdehyde; SOD: Superoxide dismutase; CAT: Catalase

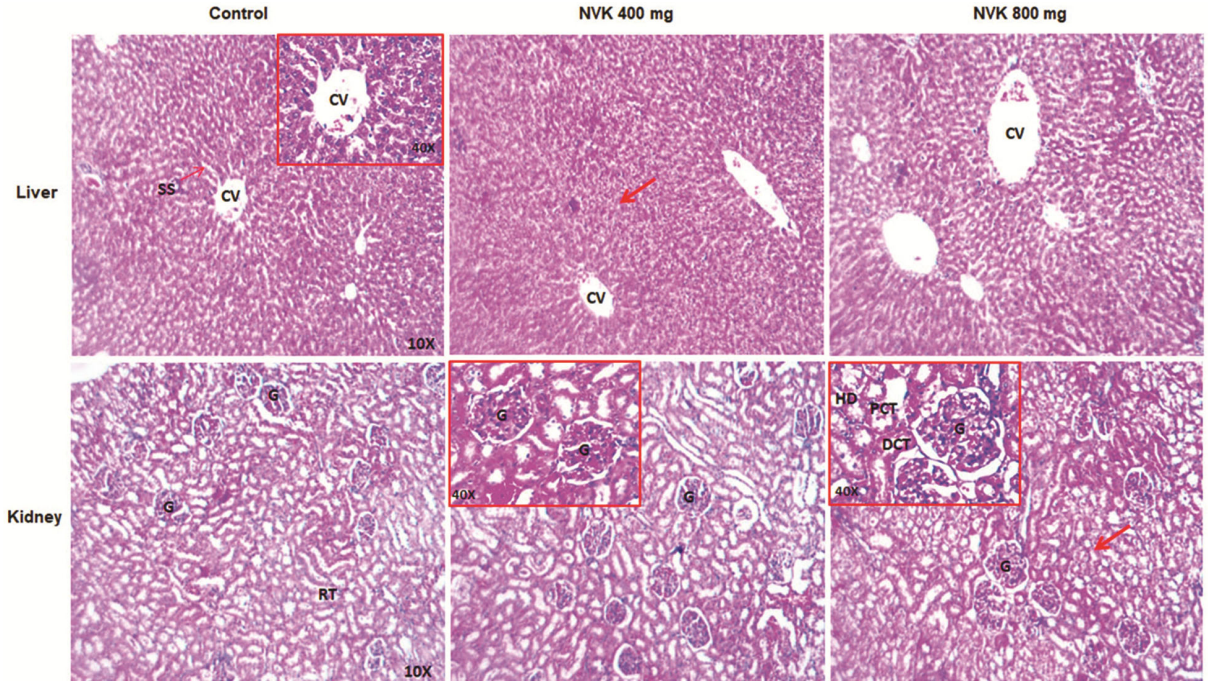

Fig. 3 — Histopathology of Liver and Kidney tissues; (a): Control; (b) NVK: 400 mg (c) NVK: 400 mgCV: Central vein; SS: Sinusoidal space; G: Glomerulus; RT: Renal tubules; HD: Hydropic degeneration; PCT and DCT: Proximal and Distal Convoluted Tubule

years, were responsible for thousands of deaths every year worldwide. The incidence of *Dengue* infection has increased nearly thirty folds in the past fifty years. Approximately 50-100 million new infections are reported to occur annually in more than hundred endemic countries and also further spread to areas that were earlier unaffected resulting in about 20,000 deaths[13]. *Chickungunya* fever was first reported in 1952 along the borders of Tanzania and Mozambique[14,15]. In 2006, the virus was responsible for an epidemic for unprecedented magnitude in Indian Ocean countries[16]. In this scenario, new therapeutic approaches are needed to check these incidents with cost-effective and readily available natural materials. The traditional medical systems that are practiced in many countries are explored of late to overcome such maladies. India is blessed with age old medical practices such as Ayurveda, *Siddha* and *Unani* besides Homoeopathy and folklore medicines.

In *Siddha* system of medicine, many formulations of single and polyherbal combinations are extensively employed to treat diseases. NVK is one such polyherbal formulation that is prescribed in the treatment of a variety of fevers. In the recent years, it has been tried as a prophylactic measure during the outbreak of *Dengue* and *Chikungunya* in Tamil Nadu State[2,3]. As there is no scientific validation available on date on its toxicological profile, the present study has been undertaken in rat model. Sub-chronic

administration of NVK to the animals did not change their body and organ weights as well as hematology, hepatic and renal parameters in lower dose. However, with higher dose, mild sinusoidal congestion and dilatation were noticed in liver tissues. It also caused significant elevation of urea in the serum with hydropic changes in kidney architecture.

Liver and kidney are detoxifying organs and the enzymatic changes occur in them are revealed in their parameters (AST, ALT, ALP, bilirubin, γ-GT, urea and creatinine). The toxic substances that induce lipid peroxidation through oxidative stress are supposed to be responsible for such changes. The enzyme γ-GT is found in many organs throughout the body, with highest concentrations in liver[17]. Its biological role is to reconstitute glutathione (GSH), which is the main intracellular antioxidant. When the concentration of γ-GT goes above normal level, it catabolizes the non-enzymatic GSH leading to its depletion[18]. The increased SOD and catalase activities observed in the study suggested that these enzymes might have a major role in the inhibition of LPO. Hence the level of MDA was reduced probably due to increase in scavenging rate of free radicals and thus enhancing non-enzymatic defences[19]. With respect to renal markers, creatinine did not show any significant alteration in both doses of NVK. However, a significant increase in urea level has been observed in high dose of NVK treatment suggesting a possible adverse effect on long term administration of this formulation.

Histopathological changes in liver showed mild sinusoidal congestion and dilatation with higher dose group of animals while the kidney revealed hydrophic degeneration-like changes and loss of tubular architecture. Andrographolide, a constituent of *Andrographis paniculata*, has been reported to cause acute kidney injury[20]. The latter is one of the ingredients of NVK formulation. The increase in urea level at higher dose of NVK lends support to this observation. However, further studies are warranted to substantiate this.

Conclusion

On the basis of observed results, it is concluded that NVK had no adverse effect on biochemical parameters and tissues of liver and kidney at lower dose suggesting that the drug might be consumed following dose and duration restrictions as mentioned in the classical *Siddha* literature.

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Conflict of interest

No conflict of interests for all the authors.

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