

HEPATOPROTECTIVE, ANTIDIABETIC, AND ANTIPYRETIC ACTIVITY STUDIES OF SIDDHA FORMULATION – NILAVEMBU KUDINEERKASIRAJAN N^{1*}, MAHESHWARI B², SUBASHCHANDRAN G², MAANICKHA CHELVI KS³¹Department of Maruthuvam, Tirunelveli, Tamil Nadu, India. ²Department of Maruthuvam, Government Siddha Medical College, Palayamkottai, Tirunelveli, Tamil Nadu, India. ³Department of Clinical Research, Siddha Regional Research Institute, Thiruvananthapuram, Kerala, India. Email: dr.kasibsms@gmail.com

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ABSTRACT

Objective: Traditional medicines like Siddha System of Medicine are one of the most primitive medical system. It plays a major role in treating ailments of humankind. Nowadays, this system flourished throughout India using Nilavembu kudineer (NVK) as a drug to treat in various outbreaks such as dengue, Chikungunya, and other related virus infections. As per literature, NVK indicated many types of fever and various diseases. This study emphasizes on antipyretic, antidiabetic, and hepato protective activities of NVK by In vitro study methods.

Methods: Hepatoprotective effect of NVK on Carbon tetrachloride-induced hepatotoxicity in the rat in five groups, antipyretic activity tested by subcutaneous injection of 10 ml/kg of 15% w/v yeast suspended in 0.5% w/v carboxymethyl cellulose solution for elevation of body temperature of rats in 6 groups. Antidiabetic activity done by administration of streptozotocin dissolved in citrate buffer (pH 4.5) and nicotinamide, intraperitoneal injection of 60 mg/kg streptozotocin, 15 min after the i.p administration of 120 mg/kg of nicotinamide in non-insulin-dependent diabetes, in six groups.

Results: The hepatoprotective effect having the significance of one way ANOVA followed by Dunnett's (n=6); ^{ns}p>0.05, *p<0.05, **p<0.01, and ***p<0.001, calculated by comparing treated groups tumor with the control group. Antidiabetic activity having the significance of Dunnett's ***p<0.001, **p<0.01, and *p<0.05 calculated by comparing the treated group with the control group and antipyretic activity results in Dunnett's *p<0.05 calculated by comparing the treated group with the control group were considered to be significant.

Conclusion: The study shows that NVK having the potency of hepatoprotective and capability of diabetes *in vitro* studies. This study also revealed the potency of antipyretic activity against yeast.

Keywords: Nilavembu kudineer, Hepatoprotective effect, Antidiabetic activity, Antipyretic activity, Statistically significant.

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INTRODUCTION

Medicinal plants continue to be an important therapeutic aid for alleviating ailments of humankind. Search for eternal health and longevity and to seek remedy to relieve pain and discomfort prompted the early man to explore his immediate natural surrounding and tried many plants, animal products, and minerals and developed a variety of therapeutic agents. Over a millennium of years that followed, the traditional therapeutic agents among were selected by the process of trial and error, empirical reasoning, and even by experimental tools. These efforts have gone in history by the name discovery of "medicine." In many eastern cultures such as those of India, China, and the Arab/Persian world, this experience systematically recorded and incorporated into a regular system of medicine that refined, developed, and became a part of the *Materia Medica* of these countries.

Traditional medicines such as Siddha System of Medicine are one of the more primitive medical system. It plays a major role in treating ailments of humankind. The holistic and natural approach toward human illness by Siddha System of Medicine is evident in its use of plants, metals, minerals, and animal products too. This principle plays a major role in diagnosis and treatment and other related areas. Nowadays, this system flourished throughout India using Nilavembu kudineer (NVK) as a drug to treat in various outbreaks such as dengue, Chikungunya, and other related virus infections [1-3] and against other microbes also [4]. The active principle also studied against vector-borne diseases [5]. Hence, the NVK proved for antiviral and antimicrobial activity.

As per Siddha literature, NVK possesses a bitter taste. The bitter taste herbals have more potency to manage diabetes. Most of the antidiabetic

drugs also influence liver function. No relevant studies of NVK published regarding this. NVK's antipyretic activity against yeast to be validated scientifically. Hence, this paper focuses on hepatoprotective and antipyretic hypoglycemic activities.

METHODS

The following drugs used in this preparation should be procured from authenticated sources and verified,

- Nilavembu (*Andrographis paniculata*)
- Vetiver (*Vetiveria zizanioides*)
- Vilamichai ver (*Plectranthus vettiveroides*)
- Santhana thool (*Santalum album*)
- Peyippudal (*Trichosanthes lobata*)
- Koraikilangu (*Cyperus rotundus*)
- Chukku (*Zingiber officinale*)
- Milagu (*Piper nigrum*)
- Parpadagam (*Hedyotis corymbosa*).

In general, aromatic drugs slightly dried to their aroma and willing properties any adulterants materials such as organic and inorganic material removed from the drugs by strict close inspection. All purified nine ingredients and grind into a coarse powder using machineries, this mixture of powder 100 g mix with 800 ml of water and boiled it. It reduced into 100 ml then filter it.

For all the following pharmacological activities, Wister albino rats weighing 180–200 g collected from Sree Venkateshwara Enterprises, Bengaluru. Animals kept in clean polypropylene cages with 12±1 h light/

dark schedule and fed with normal pellet (Sai Feed P Ltd., Bengaluru., India) rat chow diet and water *ad libitum*. The study protocol approved by the Institutional Animals Ethics Committee.

Hepatoprotective effect of NVK on Carbon tetrachloride (CCl₄)-induced hepatotoxicity in rat

Liver injury due to chemicals (or) infectious agents may lead to progressive liver fibrosis and ultimately cirrhosis and liver failure. CCl₄ is widely used for experimental induction of liver damage. The principal causes of CCl₄ are induced hepatic damage in lipid peroxidation (LPO) and decreased activities of antioxidant enzymes and the generation of free radicals [6-9].

Experimental design

The rats randomly divided into five groups of 6 rats each.

- Group I: Animals served as normal control and received olive oil 1 ml/kg body wt, i.p after every 72 h-3 doses
- Group II: Animals constituted the hepatotoxic group, which received 30% CCl₄ in olive oil (1 ml/kg body wt.i.p) after every 72 h-3 doses
- Group III: Animals received 30% CCl₄ in olive oil (1 ml/kg body wt.i.p) after every 72 h 3 doses + silymarin 100 mg/kg (po) body wt for 10 days to the CCl₄-induced animal
- Group IV: Animals, which received 30% CCl₄ in olive oil (1 ml/kg body wt, i.p) after every 72 h 3 doses + NVK 6 ml/kg (po) body wt for 10 days to the CCl₄-induced animal
- Group V: Animals received 30% CCl₄ in olive oil (1 ml/kg body wt, i.p) after every 72 h 3 doses + NVK 12 ml/kg (po) body wt for 10 days to the CCl₄-induced animal
- Group VI: Animals, which received 30% CCl₄ in olive oil (1 ml/kg body wt, i.p) after every 72 h 3 doses + NVK 18 ml/kg (po) body wt for 10 days to the CCl₄-induced animal.

Antidiabetic activity experimental design

Animals

The animals fed with standard rat pelleted diet and clean drinking water made available *ad libitum*.

Induction of diabetes mellitus

The animal divided into 14 groups of six animals each. The animals maintained overnight fasting to check the initial fasting blood glucose from tip of rat-tail vein. Streptozotocin dissolved in citrate buffer (pH 4.5) and nicotinamide dissolved in normal saline. Non-insulin-dependent diabetes mellitus induced in overnight fasted rats by a single intraperitoneal injection of 60 mg/kg streptozotocin, 15 min after the i.p administration of 120 mg/kg of nicotinamide. Hyperglycemia was confirmed by the elevated levels of blood glucose were determined at 72 h. The animals with blood glucose concentration more than 250 mg/dl selected for the study [9,10].

Study design

Group 1: Control (normal saline)

Group 2: Streptozotocin 60 mg/kg/b.w. (IP) +Nicotinamide 120 mg/kg (po)

Group 3: Streptozotocin (60 mg/kg) +Nicotinamide 120 mg/kg (po) rats treated with Metformin 20 mg/kg (po)

Group 4: Streptozotocin (60 mg/kg) +Nicotinamide 120 mg/kg (po) rats treated with NVK 6 ml/kg (po)

Group 5: Streptozotocin (60 mg/kg) +Nicotinamide 120 mg/kg (po) rats treated with NVK12 ml/kg (po)

Group 6: Streptozotocin (60 mg/kg) +Nicotinamide 120 mg/kg (po) rats treated with NVK 18 ml/kg (po).

The vehicle (saline), standard metformin, and test compounds administered to the respective group animals for 28 days. Throughout the study period, metformin, and test compounds freshly dispersed in normal saline and distilled water before to the administration. The fasting animal body and blood glucose level estimated on 1, 7th, 14th, 21st, and 28th day from tip of rat-tail vein.

Estimation of blood glucose

Blood sample collected from tip of rat-tail vein and glucose levels estimated using glucose oxidase-peroxidase reactive strips and a glucometer (Accu-chek, Roche Diagnostics, USA).

Anti-pyretic activity

Pyrexia or fever caused as a secondary impact of infection, malignancy, or other diseased states. It is the body's natural function to create an environment where infectious agents or damaged tissues cannot survive. Normally, the infected or damaged tissue initiates the enhanced formation of pro-inflammatory mediators (cytokines, such as interleukin 1 β , α , β , and TNF- α), which increase the synthesis of prostaglandin (PGE₂) near hypothalamic area and thereby trigger the hypothalamus to elevate the body temperature.

Procedure

Before yeast injection, the basal rectal temperature of rats recorded, baseline body temperature measured by inserting the digital rectal tele thermometer into the anal cavity of the rat for about 2 min. The steady temperature readings obtained recorded as the pre-temperature. After recording animals given a subcutaneous injection of 10 ml/kg of 15% w/v yeast suspended in 0.5% w/v carboxymethyl cellulose solution for the elevation of body temperature of rats. Rats returned to their home cages. Eighteen hours after yeast injection, rats with elevated body temperature selected for grouping and the NVK and standard drug suspended in CMC and administered by gastric tube.

Animal grouping

Group I- Negative control – injected with 10 ml/kg of 15% w/v yeast given (sc injection)

Group II- Yeast+ Standard paracetamol (150 mg/kg) p.o

Group III- Yeast + NVK 6 ml/kg

Group IV- Yeast + NVK 12 ml/kg

Group V- Yeast + NVK 18 ml/kg.

Rectal temperature was recorded by a digital rectal thermometer at 0, 1, 2, 3, 4, 5, and 6 h after drug administration.

RESULTS

Effect of NVK on body weight and liver weight (physical parameter) (Tables 1 and 2)

The animals in 6 groups treated with CCl₄ and NVK with various doses produce significant changes in body weight and liver weight. Treatment with CCl₄ resulted in rising body weight; NVK administration reduced the weight gain back to normal. Moreover, body weight levels of all groups are near to the control group except CCl₄-treated group. Fatty changes in the liver produced by CCl₄, which resulted in a reduction of liver weight, NVK at 12 ml dose increased the liver weight close to the control group.

Effect of NVK on biochemical parameters (Table 3)

The toxicant CCl₄ administration showed distinct changes in liver enzymes such as serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP), and acid phosphatase (AcP) shown in Table 3, also increased bilirubin level. NVK administration carried out in three dose levels 6 ml, 12 ml, and 18 ml. All three doses range showed a significant reduction in the elevated enzyme levels. At the high dose, 18 ml produced the reduction of enzymes among the three doses.

Effect of NVK on *in vivo* antioxidants (Table 4)

The toxicant CCl₄ injures the hepatic cells and causes significant damage to liver. As a result, there is a significant reduction, 50% lower than the control group shown in Table 4. The effect of NVK on antioxidant status increased the levels superoxide dismutase, glutathione (GSH) peroxidase, GSH, and LPO and restored near to normal levels. NVK significantly restored the depleted GSH and GPX levels, similar to that of the control group at 12 ml and 18 ml doses.

Table 1: Effect of NVK on body weight

| Group | Control (1 ml/kg) | Only CCl ₄ (1 ml/kg) | CCl ₄ + Std (100 mg/kg) | CCl ₄ + NVK low dose (6 ml/kg) | CCl ₄ + NVK middle dose (12 ml/kg) | CCl ₄ + NVK high dose (18 ml/kg) |
|--------------------------|-------------------|---------------------------------|------------------------------------|-------------------------------------------|-----------------------------------------------|---------------------------------------------|
| In body weight (g) | 134±2.129 | 139.7±2.704 | 121.8±0.9098 | 129.3±1.229 | 126.7±0.8028 | 125.3±1.116 |
| 7 th day (g) | 147.8±2.197 | 150±2.933 | 143.2±1.249 | 142.7±1.308 | 141.3±1.202 | 142.5±1.088 |
| 14 th day (g) | 165.7±1.874 | 169.7±2.512 | 152±1.414 | 152.5±1.586 | 151.3±0.7601 | 153±1.528 |

Values are expressed as mean±SEM, (n=6), comparing treated groups tumor with a control group. Analysis by one way ANOVA followed by Dunnett's test; Significant at p>0.05, *p<0.05, **p<0.01, ***p<0.001. NVK: Nilavembu kudineer. CCl₄: Carbon tetrachloride

Table 2: Effect of NVK on liver weight

| Group | Control (1 ml/kg) | Only CCl ₄ (1 ml/kg) | CCl ₄ + Std (100 mg/kg) | CCl ₄ + NVK low dose (6 ml/kg) | CCl ₄ + NVK middle dose (12 ml/kg) | CCl ₄ + NVK high dose (18 ml/kg) |
|------------------|-------------------|---------------------------------|------------------------------------|-------------------------------------------|-----------------------------------------------|---------------------------------------------|
| Liver weight (g) | 8.09±0.4486 | 4.505±0.2188*** | 4.814±0.3156*** | 4.003±0.6874*** | 5.27±0.4206*** | 4.755±0.2656*** |

Values are expressed as mean±SEM (n=6), comparing treated groups, tumor with the control group. Analysis by one way ANOVA followed by Dunnett's test; significant at p>0.05, *p<0.05, **p<0.01, ***p<0.001. NVK: Nilavembu kudineer. CCl₄: Carbon tetrachloride

Table 3: Effect of NVK on biochemical parameters

| Group | Control (1 ml/kg) | Only CCl ₄ (1ml/kg) | CCl ₄ + Std (100 mg/kg) | CCl ₄ + NVK low dose (6 ml/kg) | CCl ₄ + NVK middle dose (12 ml/kg) | CCl ₄ + NVK high dose (18 ml/kg) |
|------------------------|-------------------|--------------------------------|------------------------------------|-------------------------------------------|-----------------------------------------------|---------------------------------------------|
| Total bilirubin (g/dl) | 0.7733±0.06211 | 1.467±0.1627 | 0.7767±0.0559 | 0.89±0.01897 | 0.8067±0.08628 | 0.8567±0.04104 |
| SGOT (U/L) | 118.3±1.142 | 230.4±4.113*** | 134.7±1.774*** | 165.7±1.79*** | 167.8±1.99*** | 158.4±2.032*** |
| SGPT (U/L) | 46.17±1.515 | 118.4±2.436*** | 61.63±1.312 | 71.53±1.064** | 65.27±1.571* | 64.05±1.957 |
| ALP (U/L) | 63.3±0.9317 | 161.2±1.301 | 72.03±1.468 | 84.3±1.554 | 84.77±1.168 | 79.3±1.43 |
| Acp (U/L) | 5.184±0.283277 | 12.92±0.877724*** | 7.56±0.40694 | 9.1±0.801873* | 9.48±1.347** | 7.9±0.884873 |

Values are expressed as mean±SEM, (n=6), comparing treated groups tumor with control group. Analysis by one way ANOVA followed by Dunnett's test; significant at p>0.05, *p<0.05, **p<0.01, ***p<0.001. NVK: Nilavembu kudineer; SGOT: Serum glutamic-oxaloacetic transaminase, SGPT: Serum glutamic pyruvic transaminase. CCl₄: Carbon tetrachloride

Table 4: Effect of NVK on *in vivo* antioxidants

| Group | Control (1 ml/kg) | Only CCl ₄ (1 ml/kg) | CCl ₄ + Std (100 mg/kg) | CCl ₄ + NVK low dose (6 ml/kg) | CCl ₄ + NVK middle dose (12 ml/kg) | CCl ₄ + NVK high dose (18 ml/kg) |
|-------------------------|-------------------|---------------------------------|------------------------------------|-------------------------------------------|-----------------------------------------------|---------------------------------------------|
| SOD (unit/min/mg) | 5.8±0.2352 | 6.167±0.08433*** | 3.117±0.1851*** | 4.467±0.2565*** | 4.767±0.09888 | 4.8±0.2309 |
| Catalysis (μmol/min/mg) | 89.33±1.687 | 90.33±1.542*** | 53.33±0.9545 | 74.33±2.642*** | 78±0.9661*** | 78.17±1.493 |
| GPX (μmol/min/mg) | 44.5±0.922 | 49.83±0.7923*** | 22.83±0.7032 | 34.5±1.057*** | 40.83±0.7923 | 41.33±1.909 |
| GSH (μg/mg) | 32±0.5164 | 15.83±0.7032 | 38.83±0.6009 | 21.83±0.4773 | 32.33±0.6667*** | 33.5±0.9574*** |
| LPO (μg/mg) | 9.833±0.3073 | 40.17±0.4773 | 15.33±0.4216 | 26.5±0.5627 | 23.33±0.4944 | 22.5±0.7638 |

Values are expressed as mean±SEM, (n=6); comparing treated groups tumor with control group. Analysis by one way ANOVA followed by Dunnett's test; significant at p>0.05, *p<0.05, **p<0.01, ***p<0.001. NVK: Nilavembu kudineer; SOD: Superoxide dismutase, GPX: Glutathione peroxidase, LPO: Lipid peroxidation. CCl₄: Carbon tetrachloride

Effect of NVK on body weight in normal and diabetic rats (Table 5)

Streptozotocin- and nicotinamide-induced diabetic rat's body weight results showed in Table 5. The body weight of streptozotocin and metformin group increased in the first 2 weeks and gradually decreased after that. The NVK administration at various doses resulted with the same weight after 2 weeks as a control group throughout the study period and statistically significant.

Effect of NVK on blood glucose level in normal and diabetic rats (Table 6)

Streptozotocin- and nicotinamide-induced diabetes result in a significant rise of blood glucose levels. NVK treatment produces a significant effect in the reduction of blood sugar level when compared to standard medicine; this effect is highly appreciated at 12 ml and 18 ml doses (p<0.01). The later days of treatment effectively controlled by NVK than initial days.

Effect of NVK on Brewer's yeast-induced pyrexia in rats (Table 7)

The treatment with yeast produced the desired effect of fever, which is controlled by the NVK administration and produced a significant reduction in body temperature within 30 min (p<0.05). The lower dose 6 ml of NVK showed a marked reduction in temperature when compared to the paracetamol treated group. The trial drug NVK proved good antipyretic activity at low dose.

DISCUSSION

Either human beings exposed to various chemicals and chemical products directly or indirectly, lifestyle habits such as alcohol, which causes liver damage. The clinical symptoms and significant changes in the liver functions will be noticed in advance liver damage of hepatocytes. Liver damage may lead to hepatitis, non-alcoholic liver disease, portal hypertension, cirrhosis, Wilson's disease, and hepatocellular carcinoma [11-15]. CCl₄ is one of the most commonly used hepatotoxins in the experimental study of liver diseases for its active metabolite and trichloromethyl radical [16,17]. CCl₄ administered rats show increased levels of bilirubin, increased levels of SGOT, SGPT, and ALP were a strong indication of the cellular leakage and loss of cell membrane integrity. When treated with NVK, inhibited the LPO, reduced the cellular damage, and protected and stabilize the functions of cell, maintain the enzymes, and bilirubin level normal limits. Since the polyphenols are known to inhibit the activity of GST enzymes, there may be a competition between the two for the active site of the enzyme [18]. This pathway also confirmed by the NVK effect in antioxidant levels results and formulation had polyphenols. NVK with a medium dose level gave the desired effect compared to other doses. Likewise, CCl₄ treatment reduced body weight due to hepatotoxicity fatty changes. NVK prevents hepatocellular damage and improves the function of liver leads to increase liver weight and metabolism also enhanced.

Table 5: Effect of NVK on body weight in normal and diabetic rats

| Groups | Initial body weight (g) | Body weight 1 st wk (g) | Body weight 2 nd wk (g) | Body weight 3 rd wk (g) | Body weight 4 th wk (g) |
|--------------------------------|-------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|
| Control | 114.83±1.424 | 122.5±1.310 | 130.33±1.666 | 141±1.527 | 148±1.06458 |
| Only STZ (60 mg/kg) | 133±0.856 | 142.17±1.275 | 132±0.894 | 123.5±0.957 | 120.333±1.3081 |
| STZ + Glibenclamide (20 mg/kg) | 123.33±1.229 | 133.16±1.514 | 122.33±0.954 | 131±1.527 | 138±2.129 |
| STZ + NVK.L.D (6 ml/kg) | 127±1.632 | 133.33±1.837 | 139.16±1.815 | 143.5±1.648 | 148.67±1.145 |
| STZ + NVK.M.D (12 ml/kg) | 122±1.549 | 130±1.632 | 136.16±1.275 | 142.67±1.115 | 150.83±1.046 |
| STZ + NVK.H.D (18 ml/kg) | 117.5±0.5 | 123.66±1.201 | 134.17±1.275 | 143±1.154 | 148.67±1.358 |

Values expressed as the mean±S.D; statistical significance (p) calculated by one-way ANOVA followed by Dunnett's ***p<0.001, **p<0.01, *p<0.05 calculated by comparing treated group with control group. NVK: Nilavembu kudineer

Table 6: Effect of NVK on blood glucose level in normal and diabetic rats

| Groups | Initial blood sugar | Blood sugar fasting 72 h | Blood sugar fasting 10 th day | Blood sugar fasting 15 th day | Blood sugar fasting 28 th day |
|---------------------|---------------------|--------------------------|------------------------------------------|------------------------------------------|------------------------------------------|
| Control | 76.3333±2.66667 | 71.333±5.407 | 72.33±5.070 | 72.33±5.07061 | 70.6667±2.36173 |
| Only STZ | 80±4.131 | 463.33±47.164*** | 368.33±25.353*** | 326.67±23.8979*** | 306.66±24.44*** |
| STZ + Glibenclamide | 85.33±5.919 | 426.66±52.578*** | 331.67±36.001** | 248.33±17.966*** | 128.333±11.3774* |
| STZ + NVK.L.D 6 ml | 77.5±4.232 | 376.67±52.451** | 288.33±40.85* | 226.67±24.720*** | 148.33±17.207** |
| STZ + NVK.M.D 12 ml | 77.5±4.256 | 430±57.677*** | 303.333±37.475** | 201.67±15.5813** | 126.67±12.56* |
| STZ + NVK.H.D 18 ml | 79.33±5.607 | 413.33±56.312*** | 335±50.711*** | 198.33±9.45751* | 141.67±11.377** |

Values expressed as mean±SD, comparing treated group with control group. Analysis by one way ANOVA followed by Dunnett's test; significant at *p<0.05. NVK: Nilavembu kudineer

Table 7: Effect of NVK on Brewer's yeast-induced pyrexia in rats

| Group | Rectal temperature | Rectal temperature (8 h) after induction with yeast | | | |
|-----------------------------------|--------------------|-----------------------------------------------------|-----------------------|-----------------------------------------------------|----------------------|
| | | Body temperature after 8 h induction with yeast (F) | | Body temperature after 8 h induction with yeast (F) | |
| | | Initial (A) | 8 th h (B) | 30 th min | 60 th min |
| Only yeast (10 ml/kg of 15 % w/v) | 32.5±1.708 | 39.5±1.5 | 36.25±0.8539 | 32.75±0.4787 | 32±0.8165 |
| Yeast + Std (150 mg/kg) | 33±1.291 | 33±1.291 | 32.5±1.258 | 32.75±0.8539 | 32±0.4082 |
| Yeast + L.D (6 ml/kg) | 32.25±1.652 | 34.5±1.708 | 31±1.291 | 31.75±1.652 | 31.5±1.555 |
| Yeast + M.D (12 ml/kg) | 34±0.8165 | 33.5±1.5 | 34.5±1.708 | 33.75±1.493 | 34±0.9129 |
| Yeast + H.D (18 ml/kg) | 33±1.291 | 34.25±2.323 | 37±0.4082 | 36.25±0.8539 | 33.75±0.4787 |

Values expressed as mean±SD, comparing treated group with control group. Analysis by one way ANOVA followed by Dunnett's test; significant at *p<0.05. NVK: Nilavembu kudineer

Metabolic activation of CCl₄ by cytochrome P450-dependent mixed oxidase in the endoplasmic reticulum to form a trichloromethyl free radical (CCl₃) leads to severe damage to liver cells. It also combines with cellular lipids and proteins in the presence of oxygen to induce LPO [18,19]. In states of oxidative stress, GSH converted to GSH disulfide and depleted, leading to LPO. Therefore, the role of GSH as a reasonable marker for the evaluation of oxidative stress is important [18-20]. Since the polyphenols are known to inhibit the activity of GST enzymes, there may be a competition between the two for the active site of the enzyme NVK inhibited LPO and elevated depleted serum GSH levels to normal limits. NVK formulation had constituent of andrographolide derived from *A. paniculata* possess liver protection under various experimental conditions of treatment with galactosamine [21]. Among the remaining eight herbals, ginger, pepper, and nutgrass may possess add on effect for this antioxidant property.

The antidiabetic activity of NVK well established from above streptozotocin-induced diabetes. The NVK treated diabetic rats study showed that NVK results in a marked decrease in blood glucose in diabetic rats after 28 days of treatment. This may be due to an increase in the release of insulin from β-cells of pancreas. Loss of body weight in a diabetic is an indication of damage of tissue proteins in abnormal levels. Decreased body weight observed in the streptozotocin-treated group. Treatment with NVK improved body weight, indicating that control over tissue protein damage and muscle wasting resulted from glycemic control. The herbals of this formulation such as green chiretta, ginger, and nutgrass have active metabolites that possess antidiabetic activity [22-24]. This suggests the hypoglycemic effect of NVK in diabetic rats.

The NVK effect against viral fevers like those that dengue and Chikungunya proved scientifically by various *in vitro* and *in vivo* studies [25,26]; however, no data available regarding the antipyretic effect against yeast-induced fever. Hence, this study showed that NVK has a significant effect of reducing fever. This may be due to COX₂ inhibition and binding with PGE₂ [26,27]. It may be confirmed by clinical trials in the future.

CONCLUSION

In the above studies, hepatoprotective activity carried out using CCl₄-induced hepatotoxicity in rat. The trial drug NVK having significant hepatoprotective activity also reveals the effectiveness of NVK in treating liver diseases. Hypoglycemic activity of the trial drug NVK on streptozotocin and nicotinamide induced type-II diabetic rats. This study reveals NVK decoction possesses good control of blood sugar levels and significant antidiabetic activity. Antipyretic activity of the trial drug NVK carried out using the yeast-induced method. The drug NVK showed potent antipyretic activity. Overall study results torching light in the treatment of diabetes, hepatoprotective effect, and reducing the fever.

CONFLICTS OF INTEREST

The author declares no conflicts of interest.

AUTHORS' CONTRIBUTIONS

Kasirajan N – Conception of the work and data collection. Maheshwari B – Conception of the work and data collection. Subashchandran

G – Drafting the article. Maanickha Chelvi K S – Data analysis and interpretation.

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REFERENCES

- Jain J, Kumar A, Narayanan V, Ramaswamy RS, Sathiyarajeswaran P, Devi MS, et al. Antiviral activity of ethanolic extract of nilavembu kudineer against dengue and chikungunya virus through *in vitro* evaluation. *J Ayurveda Integr Med* 2018;1:76-80.
- Sathya B, Velpandian V, Ramani M, Kumar A. Primitive approach on review of siddha herbs, herbo-mineral formulation exhibiting antiviral activity. *Int J Pharma Bio Sci* 2014;5:138-47.
- Christian GJ, Subramanian M, Periyasami D, Manickavasakam K, Gunasekaran P, Sivasubramanian S, et al. Protective effect of polyherbal siddha formulation-nilavembu kudineer against common viral fevers including dengue-a case control approach. *IJPSR* 2015;6:1656-60.
- Uma KS, Jacob M, Arumugam G, Kalpana S, Natarajan M. *In vitro* Antimicrobial activity of the siddha drugs seenthil Sarkarai and nilavembu kudineer against *Leptospira*. *Int J Pharm Pharm Sci* 2012;4:75-8.
- Sahabudeen S, Nag H, Madhavan S, Chatterjee A, Dan P, Bhattacharya M, et al. Fecundity studies of *Andrographis paniculata* on *Drosophila melanogaster*. *Int J Pharm Pharm Sci* 2019;11:23-7.
- Maiti K, Mukherjee K, Murugan V, Saha BP, Mukherjee PK. Enhancing bioavailability and hepato protective activity of andrographolide from *Andrographis paniculata*, a well-known medicinal food, through its herbo some. *J Sci Food Agric* 2010;90:43-51.
- Castro JA, De Ferreyra EC, De Castro CR, De Fenos OM, Sasame H, Gillette JR. Prevention of carbon tetrachloride-induced necrosis by inhibitors of drug metabolism-further studies on their mechanism of action. *Biochem Pharmacol* 1974;23:295-2.
- Parola M, Leonarduzzi G, Biasi F, Albono M, Biocca G, Dianzani MU. Vitamin E dietary supplementation. Protects against CCl₄ induced chronic liver damage and cirrhosis. *Hepatology* 1992;16:1014-21.
- Poli G. Liver damage due to free radicals. *Br Med Bull* 1993;49:604-20.
- Marudamuthu AS, Leelavinothan P. Effect of pterostilbene on lipids and lipid profiles for diabetes, in streptozotocin-nicotinamide induced Type 2 diabetes mellitus. *J Appl Biomed* 2008;6:31-7.
- Singh A, Bhat TK, Sharma OP. Clinical biochemistry of hepatotoxicity. *J Clin Toxicol* 2011;4:210-7.
- Pandit A, Sachdeva T, Bafna P. Drug-induced hepatotoxicity: A review. *J Appl Pharm Sci* 2012;2:233-43.
- Kumar A. A review on hepatoprotective herbal drugs. *IJRPC* 2012;2:92-2.
- Seeff LB, Lindsay KL, Bacon BR, Kresina TF, Hoofnagle JH. Complementary and alternative medicine in chronic liver disease. *Hepatology* 2001;34:595-3.
- Dhingra M, Nain P, Nain J, Malik M. Hepatotoxicity v/s hepato protective agents-a pharmacological review. *IRJP* 2011;2:31-7.
- Johnston DE, Kroening C. Mechanism of early carbon tetrachloride toxicity in cultured rat hepatocytes. *Pharmacol Toxicol* 1998;83:231-9.
- Srivastava SP, Chen NO, Holtzman JL. The *in vitro* NADPH dependent inhibition by CCl₄ of the ATP-dependent calcium uptake of hepatic microsomes from male rats. Studies on the mechanism of inactivation of the hepatic microsomal calcium pump by the CCl₃ radical. *J Biol Chem* 1990;265:8392-9.
- Recknagel RO, Glende EA, Hruszkewycz AM. Free Radicals in Biology. Vol. 3. New York: Academic Press; 1977. p. 97-32.
- De Groot H, Noll T. The crucial role of low steady state oxygen partial pressures in haloalkane free-radical mediated lipid peroxidation. *Biochem Pharmacol* 1986;35:15.
- Al-Harbi MS. Hepatoprotective effect and antioxidant capacity of naringenin on arsenic-induced liver injury in rats. *Int J Pharm Pharm Sci* 2016;8:103-8.
- Saxena S, Jain DC, Gupta MM, Bhakuni RS, Mishra HO, Sharma RP. High-performance thin-layer chromatographic analysis of hepatoprotective diterpenoids from *Andrographis paniculata*. *Phytochem Anal* 2000;11:34-6.
- Putta W, Chedalawada V. Dose dependent effect on hypoglycemic and antihyperglycemic activities of chloroform extract of physalis minima in streptozotocin induced diabetic rats. *Int J Curr Pharm Res* 2014;6:61-5.
- Raut NA, Gaikwad NJ. Antidiabetic activity of hydro-ethanolic extract of *Cyperus rotundus* in alloxan induced diabetes in rats. *Fitoterapia* 2006;77:585-8.
- Al-Amin ZM, Thomson M. Anti-diabetic and hypo lipidaemic properties of ginger (*Zingiber officinale*) in streptozotocin-induced diabetic rats. *Br J Nutr* 2006;96:660-6.
- Calabrese C, Berman SH, Babish JG, Ma X, Shinto L, Dorr M, et al. A Phase I trial of andrographolide in HIV positive patients and normal volunteers. *Phytother Res* 2000;14:333-8.
- Suebsasana S, Pongnaratorn P, Sattayasai J, Arkaravichien T, Tiamkao S, Aromdee C. Analgesic, antipyretic, anti-inflammatory and toxic effects of andrographolide derivatives in experimental animals. *Arch Pharm Res* 2009;32:1191-200.
- Vedavathy S, Rao KN. Antipyretic activity of six indigenous medicinal plants of Tirumala Hills, Andhra Pradesh, India. *J Ethnopharmacol* 1991;33:193-6.