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Efficacy of hydro-methanolic extract of *Neolamarckia cadamba* bark over hematological & biochemical parameters of Wistar albino rats and against microorganisms

Vishal Khandelwal* & Pradeep Kumar Choudhary

Department of Biotechnology, GLA University, Mathura 281 406, U P, India

E-mail: vishal_k80@rediffmail.com

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Activity of the hydro-methanolic (HM) extract of *Neolamarckia cadamba* bark was studied over hematological and biochemical parameters of Wistar albino rats as well as its antimicrobial potential was tested against certain bacterial (Gram positive: *S. aureus* & *B. subtilis*, Gram negative: *E. coli* & *P. aeruginosa*) and fungal strains (*A. niger* & *C. albicans*). Efficacy of HM extract of *N. cadamba* bark over hematological and biochemical parameters was carried out using four groups containing six Wistar albino rats: Gp-I as control (without fed), Gp-II, Gp-III and Gp-IV were orally fed with HM extract of *N. cadamba* bark with different concentrations of 125 mg/Kg, 250 mg/Kg and 500 mg/Kg bwt, respectively. Study revealed significant increase ($p<0.01$) in level of red blood cells (RBC), haemoglobin (Hb), total leukocyte count (TLC) and packed cell volume (PCV) of Gp-II, III and IV when compared to control (Gp-I). Dose dependent progression in hematological indices values was observed. TLC values of animals of Gp-IV were found to be more increased in comparison with other hematological values of animals of other groups. Dose dependent significant decrease ($p<0.01$) in glucose and total cholesterol value of treated groups was found with respect to control. Concentration of two liver enzymes ALT/SGPT (alanine amino transferase), AST/SGOT (aspartate amino transferase) and amount of albumin, urea, creatinine and bilirubin of treated groups was not significantly different from control. Study suggested significant ($p<0.01$) antimicrobial activity towards *C. albicans*, *A. niger*, *E. coli*, *P. aeruginosa*, *B. subtilis* & *S. aureus*. Study revealed the synergistic efficacy of phytochemicals present in hydro-methanolic extract of *N. cadamba* bark for health benefits.

Keywords: Antibacterial activity, Biochemical parameters, Hematological parameters, *Neolamarckia cadamba*

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Neolamarckia cadamba (Roxb.) Bosser, a member of family Rubiaceae, secured its position in main stream due to its ethnomedicinal uses to treat variety of disorders[1]. *N. cadamba* (commonly known as Kadam) is tropical in distribution and commonly occurr in South Asia, Southeast Asia including Indonesia, Malaysia, Nepal, Thailand and further introduced to Central America and Africa. In India, its distribution ranges from sub-Himalayan tract from Nepal to lower hills of Darjeeling, large streams in Andaman, Karnataka, Kerala, Andhra Pradesh, Orissa, Bihar and low levels in wet places of Western Ghats. *N. cadamba* has been acknowledged with various vernacular names in its respective places; "wild cinchona" in English (commonly called bur-flower), "Kadambah" in Sanskrit, "Kadamb" in Hindi, "Jabon" in Indonesia, "Kalempayan" in Malaysia, "Thkoow" in Cambodia[1]. *Anthocephalus chinesis*, *Anthocephalus macrophygelus*, *Nauclea cadamba*, *Nauclea megaphylla*, *Samma cadamba*, *Sacrocephalus cadamba* and *Anthocephalus cadamba* are some synonymous used by different scientists but *Neolamarckia cadamba* is widely accepted. *N. cadamba* is a large tree with height of 20-45 m with broad umbrella-shaped crown and straight cylindrical bole. Leaves (length: 15-50 cm & width: 8-25 cm) are glossy green, opposite, ovate to elliptical, simple pulvinus base with subsessile to petiolate arrangement and bark is smooth grayish-green in young plants, while rough and grey with longitudinal fissure in old plants[2,3]. Anthraquinone glucosides[4], cadambine[5], chlorogenic acid[6], β-sitosterol[7], neolamarackine A[8], anthocephaline[9], indole alkaloids, triterpenes and saponins[10] are found to be present in different parts of *N. cadamba* and reported for their multifactorial pharmacological activities such as antioxidant, antidiabetic, antimicrobial, anti-inflammatory, analgesic, antipyretic, antiplasmodial and anticancer[11].

*Corresponding author

Ethnomedical announcement revealed that bark of *N. cadamba* has been used in treating sour throat, gum related troubles, eye infection, skin diseases, inflammation and fever[11]. There is a need for scientific validation of ethnomedicinal implications of bark of *N. cadamba*, therefore above work has been evolved to explore the effect of hydro-methanolic (HM) extract of *N. cadamba* bark over hematological & biochemical indices of Wistar albino rats and its antimicrobial efficacy.

Materials & Methods

Sample collection and extract preparation

Bark of *N. cadamba* was collected from Mathura (U.P.) and Vrindavan (27°33'39" N- 77°41'14'' E), at the month of November 2019. Identification and authentication of plant material was done by Dr. A. K. Agrawal, Dept. of Microbiology, BSA College, Mathura (U.P). Bark was comprehensively collected and followed by washing and grinding to obtain coarse powder.HM extract (80:20, v/v) of *N. cadamba* was prepared at 65°C for 4-5 h using soxhlet apparatus.

***In vivo* experimentation**

Wistar albino rats of both sexes having weight ranging from 60-100 g were procured from animal house of GLA University, Mathura with the IAEC (Institutional Animal Ethics Committee) approval vide GLAIPR/CPCSEA/IAEC/2014/Biotech/02. Study was designed into four groups: Group I, II, III & IV containing six animals in each. Animals of Gp-I (control) were orally fed with rat pellets and water *ad libitum*. Gp-II, III & IV were respectively fed with 125/250/500 mg/Kg body weight (bwt) of HM extract of *N. cadamba* bark for 21 days along with water and libitum.

Microorganisms

Staphylococcus aureus (MTCC-9160), *Bacillus subtilis* (MTCC-121), *Escherichia coli* (MTCC-1563), *Pseudomonas aeruginosa* (MTCC-0872), *Aspergillus niger* (MTCC-872) and *Candida albicans* (MTCC-227) were used for antimicrobial assays. Strains were identified and procured from Institute of Microbial Technology (IMTECH), Chandigarh, India.

Effect of HM extract over hematological and biochemical indices of Wistar albino rats

Manual methods[12,13] include the use of improved Neubauer haemocytometer for measuring RBC as well as TLC values and Sahli's haemoglobinometer for determination of haemoglobin. Blood from retro orbital plexus of animals of Gp-I, II, III & IV was collected after 21 days, in ethylenediaminetetraacetic acid (EDTA) coated tubes for quantitation of hematological parameters. Hb, RBC, PCV & TLC of animals of different groups were measured by using three parts automated haematology analyzer, Nihon-Kohden (Celltac α, Model No: MEK-6420P), USA.

Traditional method[14] was routinely used for estimation of ALT and AST activities. Serum from test groups (Gp-II, III & IV) and control group (Gp-I) rats were taken in collection tube for determination of biochemical parameters. Glucose, urea, creatinine, cholesterol, albumin, bilirubin, alanine amino transferase (ALT or SGPT) and aspartate amino transferase (AST or SGOT) were estimated by UV-visible double beamspectrophotometer using span diagnostic kit.

Antibacterial activity

Disc diffusion method[15] was used for antibacterial assay. One-two loopful growth from different bacterial cultures were inoculated into 5.0 mL of nutrient broth and were subjected to incubation at 37°C for 6 h, followed by centrifugation at 30000 rpm for 10 min. Bacterial pellets were washed with normal saline, followed by suspension of it in 5 mL of normal saline. Bacterial concentration was adjusted to 5×10^6 CFU/mL (Standardized 0.5 McFarland Nephelometer). Discs seeded with different concentrations (1.25, 2.5, 5.0 and 10.0 mg) of HM extract of *N. cadamba* were placed at even distance over nutrient agar medium containing test microorganisms. Ofloxacin (10 µg/disc) were taken as positive control. Nutrient agar plate containing different concentrations (1.25/2.5/5.0/10.0 mg/disc) of extract were kept in incubator at 37°C for 24 h. Determination of antibacterial activity was carried out by measuring the zone of inhibition. Each experiment was performed in triplets.

Antifungal activity

Small amount of *A. niger* and *C. albicans* was taken from stock culture. Each were inoculated in to 5 mL Potato dextrose broth and incubated at 37°C for 6 h. The broth culture was then centrifuged at 3000 rpm for 10 min. Pellet was washed twice with normal saline. Finally, the pellet was suspended in 5 mL of normal saline and store at 4°C for further use. The final concentration of culture used in the study was 5×10^6 CFU/mL. Each reference fungal culture (0.5 mL) with approximately 5×10^6 CFU/mL was

swabbed on the top of the potato dextrose agar (PDA) medium. Discs seeded with different concentrations of HM extract were placed at even distance over PDA medium with test microorganisms placed on culture plates. Standard disc of Amphotericin-B (10 µg/discs) against fungi was taken as positive control. The culture plates with discs were placed in incubator at 37°C for 24 h. The antifungal activity of HA extract at different concentrations was determined by measuring the diameter of zone of inhibition.

Statistical analysis

Analysis of statistical data was done by using one way analysis of variance (ANOVA) using SPSS version 20.0 software and DMRT at $p<0.01$ in order to determine any significant differences among treated means. Value are expressed as mean±SEM

Result

Effect of HM extract of *N. cadamba* bark on hematological and biochemical parameters of Wistar albino rats

Hematological and biochemical parameters of Gp-I, Gp-II, Gp-III &Gp IV of Wistar albino rats has been represented in Table 1 and Table 2. HME fed rats exhibited increase in the values of Hb, PCV, RBC and TLC as compared to control group (Table 1). Among the various hematological parameters studied, maximum increase was found in TLC value of Group IV orally fed with 500 mg/Kg bwt of HM extract of *N. cadamba* bark.

Mean glucose concentration in animals of Gp-II/Gp-III/Gp/IV was $97.67^{c}\pm 1.05$, $94.50^{b}\pm 0.67$ and $88.33^{a}\pm 0.84$, respectively as compared to Gp-I ($103.33^{d}\pm 0.76$) as shown in Table 2. Mean value of total cholesterol concentrations Gp-II/Gp-III/Gp/IV was $106.33^{b}\pm 1.76$, $101.83^{a}\pm 1.01$ and $98.67^{a}\pm 1.60$, respectively as compared to Gp-I ($112.33^{c}\pm 0.98$) (Table 2). HM extract treated rats did not exhibit any significant differences in the values of urea, creatinine, albumin, bilirubin, AST and ALT with respect to control (Gp-I). More significant decrease ($p<0.01$) was found in glucose concentrations of animals treated with different doses of HM extract of *N. cadamba* bark as shown in Table 2.

Antimicrobial activity

Different doses of HM extract i.e., 1.25, 2.5, 5.0 and 10.0 mg/disc possess significant ($p<0.01$) antibacterial activity against different bacterial strains (Table 3). Among the different bacterial strains used, maximum zone of inhibition (mm) was found against *S. aureus* ($17.73^{c}\pm 0.48$) at 10.0 mg/disc HM extract (Table 3). Significant ($p<0.01$) antifungal activity of disc seeded with different concentrations of HM extract was found against *A. niger* and *C. albicans* as given in Table 4.

Discussion

Percentage yield through soxhlet extraction was found to be 14-15% with dark brown crystals. The

Table 1 — Effect of various concentrations of HM extract of *N. cadamba* bark over hematological indices of Wistar albino rats

Hematological parameters	Gp-I (Control)	Gp-II (125 mg/Kg)	Gp-III (250 mg/Kg)	Gp-IV (500 mg/Kg)
Hb (g/dL)**	$12.20^{a}\pm0.12$	$12.45^{ab}\pm0.10$	$12.82^{b}\pm0.07$	$13.37^{bc}\pm0.18$
PCV (%)**	$32.84^{a}\pm0.41$	$35.10^{b}\pm0.50$	$35.37^{bc}\pm0.20$	$36.35^{c}\pm0.18$
RBC ($x10^{6}/mm^{3}$)**	$6.5^{a}\pm 0.09$	$6.92^{ab}\pm0.07$	$7.31^{b}\pm0.22$	$7.88^{c}\pm0.17$
TLC ($x10^{3}/mm^{3}$)**	$6.42^{a}\pm0.28$	$8.05^{b}\pm0.32$	$8.57^{b}\pm0.27$	$9.60^{c}\pm0.38$

Data refers the mean ± SEM of six Wistar albino rats with significant result at **$p<0.01$. ^{a,b,c} shows significant difference between groups.

Table 2 — Effect of various doses of HM extract of *N. cadamba* bark over biochemical indices of Wistar albino rats

Biochemical parameters	Gp-I (Control)	Gp-II (125 mg/Kg)	Gp-III (250 mg/Kg)	Gp-IV (500 mg/Kg)
Glucose (mg/dL)**	$103.33^{d}\pm0.76$	$97.67^{c}\pm1.05$	$94.50^{b}\pm0.67$	$88.33^{a}\pm0.84$
Urea (mg/dL)^{NS}	36.53±1.58	38.83±3.44	35.53±2.40	31.90±3.53
Creatinine(mg/dL) ^{NS}	0.85±0.01	0.83±0.00	0.84± 0.01	0.84±0.00
Cholesterol (mg/dL)**	$112.33^{c}\pm0.98$	$106.33^{b}\pm1.76$	$101.83^{a}\pm1.01$	$98.67^{a}\pm1.60$
Albumin(mg/dL) ^{NS}	3.23±0.15	3.16±0.16	2.99±0.10	2.89±0.11
Bilirubin (mg/dL) ^{NS}	0.77±0.02	0.82±0.03	0.81±0.01	0.79±0.02
AST (IU/L) ^{NS}	66.61±1.24	66.51±3.71	67.71±2.27	68.45±1.60
ALT (IU/L) ^{NS}	27.22±0.37	27.63±0.27	28.41±0.42	27.30±0.49

Data refers the mean ± SEM of six Wistar albino rats with significant result at **$p<0.01$, NS = non significant. ^{a,b,c} shows significant difference between groups.

Table 3 — Effect of various concentrations of HM extract of *N. cadamba* bark against different bacterial strains

Organism	Zone of inhibition (mm)				
	1.25 mg HM/disc	2.50 mg HM/disc	5.0 mg HM/disc	10.0 mg HM/disc	Ofloxacin 10 µg/disc
*S. aureus***	13.17^{a}±0.60	14.5^{ab}±0.60	15.80^{bc}±0.52	17.73^{c}±0.48	31.5^{d}±1.04
*B. subtilis***	9.30^{a}±0.32	11.87^{b}±0.50	13.20^{bc}±0.34	14.27^{c}±0.29	30.33^{d}±0.66
*E. coli***	8.10^{a}±0.36	10.60^{b}±0.41	11.73^{bc}±0.43	12.87^{c}±0.08	31.06^{d}±0.68
*P. aureginosa***	7.0^{a}±0.45	9.00^{b}±0.26	10.53^{c}±0.37	11.93^{c}±0.24	33.37^{d}±0.73

Data refers the mean ± SEM of six Wistar albino rats with significant result at **p<0.01.^{a,b,c} shows significant difference between groups.

Table 4 — Effect of various concentrations of HM extract of *N. cadamba* bark against different fungal strains

Organism	Zone of inhibition (mm)				
	1.25 mg HM/disc	2.50 mg HM/disc	5.0 mg HM/disc	10.0 mg HM/disc	Amphotericin-B 10 µg/disc
*A. niger***	7.0^{a}±0.45	8.5^{b}±0.36	9.03^{b}±0.24	10.20^{c}±0.41	11.13^{c}±0.24
*C. albicans***	4.2^{a}±0.23	5.23^{b}±0.20	6.20^{c}±0.17	7.3^{d}±0.25	11.01^{e}±0.40

Data refers the mean ± SEM of six Wistar albino rats with significant result at **p<0.01.

results of effect of HM extract of *N. cadamba* over hematological parameters are given in Table 1. Present study suggested significant increase (p<0.01) in Hb, PCV, RBC and TLC values at 250 and 500 mg/Kg bwt of HM extract of *N. cadamba* bark, which are in agreement with previous findings[16]. Dose dependent increase in Hb, PCV, RBC and TLC values was found with increase in concentration of HM extract of bark of *N. cadamba*. Maximum increase (p<0.01) in hematological parameters was found at dose of 500 mg/Kg bwt. Enhanced hematological parameters of Wistar albino rats might possibly be due to the presence of tannins, phenols, alkaloids, saponins, flavonoids and glycosides in the bark of *N. cadamba*[17]. Result supports haematopoietic potential of HM extract of bark of *N. cadamba*, since presence of ingredients in plants enhanced rapid synthesis of blood cells[18]. Enhanced value of TLC at 125/250/ 500 mg/Kg bwt of HM extract of *N. cadamba* in comparison with control, suggested immuno-stimulatory potential which support previous findings[19,20]. There is a need to characterize biological ingredients responsible for enhanced Hb, PCV, RBC and TLC indices.

The effect of HM extract of *N. cadamba* bark over biochemical parameters of Wistar albino rats is given in Table 2. Result depicts significant decrease (p<0.01) in glucose and total cholesterol values at different doses of HM extract of *N. cadamba* bark with respect to control. Glucose and total cholesterol concentrations were found to decrease in dose dependent manner. Phytochemical analysis of *N. cadamba* revealed the presence of cadambine and chlorogenic acid[11], which cause decrease in glucose concentration[9,21]. Results of present study signify hypoglycemic potential of HM extract of bark of *N. cadamba*, in concurrence with earlier studies[22]. The exact mechanism of glucose lowering potential of *N. cadamba* is yet to be explored but it seems that antidiabetic efficacy of extract might be by modulating pancreatic secretions of insulin or by enhancing glucose uptake[23,24]. More significant decrease (p<0.01) in total cholesterol value was noticed in animals of group IV, orally fed with 500 mg/Kg bwt (Table 2). Results suggested lipid lowering efficacy of HM extract of *N. cadamba* bark, probably due to presence of phenolic compound and chlorogenic acid in *N. cadamba* extract[25-27].

Results suggested probable non toxic effect of HM extract of bark of *N. cadamba* over liver & kidney of experimental animals, since no significant differences in urea, creatinine, albumin, bilirubin, AST and ALT values of orally fed groups with respect to control were observed.

Significant (p<0.01) antibacterial and antifungal activity was exhibited by HM extract against *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa*, *A. niger* and *C. albicans* as shown in Table 3 & Table 4. Among the test microorganisms, *S. aureus* was found to be more sensitive. Similar findings were previously reported[28,29]. Presence of secondary metabolites viz., alkalkoids, saponins, terpenes & flavonoids in *N. cadamba* extract[30] might have inhibitory effect on microbial growth[31,32]. Ethnomedicinally, bark of *N. cadamba* has been used by many communities against skin disease, cholera, eye and throat infection suggesting its significant antimicrobial activity[11].

Conclusion

Hydromethanolic extract of *N. cadamba* bark brings about increase in Hb, RBC, PCV and TLC values, suggesting its probable role in stimulation of haematopoietic system which supports the traditional use of *N. cadamba* against anemia and blood disorders. HM extract of *N. cadamba* extract lowers glucose and total cholesterol content suggesting its probable hypoglycemic and hypolipidomic efficacy. Significant antibacterial activity of this plant does offer many ethnomedicinal uses.

There is a need to isolate and characterize bioactive compounds from *N. cadamba* at molecular level, exhibiting health benefits.

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Conflict of Interest

The authors have no conflict of interest to declare.

Authors' Contributions

VK and PKC contributed towards planning and execution of present research study. VK performed lab work with experimental animals and prepare final manuscript. PKC carried out antimicrobial work, result analysis and calculations.

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