Biochemical and Antioxidant effects in crossbred calves fed with *Morinda citrifolia*

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**ABSTRACT**

*Morinda citrifolia* (Noni) is a medicinal plant widely distributed in the tropical regions of India, Indonesia and Malaysia and has a long history of treating a wide variety of diseases such as cancer, atherosclerosis and diabetes. The present investigation was designed to evaluate blood biochemical parameters and antioxidant effects in calves fed with *M. citrifolia*. A total of eight calves were divided into two groups as control (*n* = 4) and treatment (*n* = 4). The calves of treatment group were fed with fresh minced raw fruit (100 g/calf/day) and the calves in control group were fed with placebo. Blood samples were collected at weekly intervals for four weeks for estimation of biochemical parameters and to determine antioxidant activity. The crude extract of noni fruits significantly (*P* < .01) decreased the concentrations of serum total cholesterol, triglycerides, serum glucose and also decreased (*P* < .05) serum creatinine and urea. There was a reduction in lipid peroxidation (LPO) than control; however, superoxide dismutase (SOD) and catalase levels were dramatically increased (*P* < .01) in *morinda*-fed calves. The results of present preliminary study demonstrated hypolipidemic, hypoglycaemic and antioxidant effect of *M. citrifolia* in calves. The findings of this study could be exploited for stress amelioration and management of metabolic diseases in calves and cattle without adverse effects.

**1. Introduction**

*Morinda citrifolia* or noni (family: Rubiaceae) is a medicinal plant called as *Indian mulberry* in India, *nunaakai* in Tamil, *mengkuudu* in Indonesia and Malaysia (Morton 1992). It has been used in folk remedies by Polynesians for over 2000 years, and is reported to have a broad range of therapeutic effects. Generally, the medicinal plants have secondary metabolites which are very effective to cure various diseases (Ghasemzadeh & Jaafar 2011). Almost all the parts of the plant are being used for its medicinal and nutraceutical properties. The noni fruit contains 90% of water and the main components of the dry matter appear to be soluble solids, dietary fibres and proteins (Singh 2012). Biological compounds such as glycosides, polysaccharides, iridoids, alkaloids, lignans, trisaccharide fatty acid esters, anthraquinones, scopoletin, morindin, vitamins and minerals have been isolated from noni fruits, roots and leaves (Yang et al. 2007). These isolated biological compounds exerts various health benefits, including anticancer, antibacterial, antiviral, antifungal, hypotensive, anti-inflammatory, cholesterol regulation, menstrual cycle regulator and immune enhancing effects (Wang et al. 2002; Saminathan et al. 2013). The pharmacologically active compounds derived from noni fruits, leaves and roots are available as readymade capsules, teas and juices, which are most popular (Saminathan et al. 2014). Plants produce various antioxidant compounds to counteract the reactive oxygen species in order to survive. Recently, peroxidation of unsaturated lipids in living organisms is receiving increasing attention in relation to the possible association between lipid oxidation and a wide range of degenerative diseases, including ageing, cancer, diabetes and cardiovascular diseases. Thus, antioxidants are important inhibitors of lipid peroxidation (LPO), not only for food protection but also as a defence mechanism of living cells against oxidative damage (Mohd Zin et al. 2002). Antioxidant plays an important role in cancer treatment by scavenging free radicals produced by cells. Palu et al. (2008) suggested that the fruit extract from *M. citrifolia* modulated the immune system in mice through activation of cannabinoid receptors (CB2), suppression of interleukin-4 production, and by stimulating the production of interferon-γ cytokines. Recently, Brooks et al. (2009) reported that supplementation of juice from *M. citrifolia* enhanced the activation of immune cells in neonatal calves. The immunomodulatory effects of supplementing Morinda-Max® showed an increase in total leucocytes and greater oxidative burst intensity by the primary neutrophil population. This larger pool of leucocytes and greater oxidative burst potential could conceivably improve resistance to disease in a high-risk population of cattle (Ponce et al. 2011). The aim of this preliminary study was to investigate the effect of *M. citrifolia* fruit extract on blood biochemical parameters and antioxidant properties in crossbred calves.
2. Materials and methods

2.1. Experimental design
A total of eight crossbred (Jersey X Desi) calves (age: 4–6 months; average body weight: 60–70 kg) maintained under standard management condition were selected for the study. The calves were divided into two groups, such as control (n = 4) and treatment (n = 4). M. citrifolia raw fruit was cut into small pieces and minced using an electric mixer. Fresh, minced fruit (100 g/calf/day) was fed orally to the calves of treatment group, and the calves in control group were fed with placebo (distilled water @100 ml/calf/day). Blood samples were collected in tubes with and without anticoagulant ethylene-diaminetetraacetic acid (EDTA) by jugular vein puncture prior to the start of feeding (zero week) and thereafter at weekly intervals for four weeks. Blood serum was separated from coagulated blood for biochemical parameter and antioxidant analysis.

2.2. Estimation of blood biochemical parameters
Total cholesterol (TC), triglycerides (TGL), LDL-Cholesterol, HDL-Cholesterol, serum glucose, total protein, TBARS (thiobarbituric acid reactive substances) assay for LPO, blood urea nitrogen and creatinine were estimated in blood serum spectrophotometrically using commercially available kits (ERBA BIO-SCIENCES). All other chemicals and reagents used were of analytical grade and are commercially available.

2.3. Measurement of antioxidant activity
The superoxide dismutase (SOD) activity was measured by the method of Marklund and Marklund (1974). Briefly, to 0.5 ml of serum, 0.25 ml of ice-cold ethanol and 0.1 ml of ice-cold chloroform (chilled in ice) were added. Then the contents were mixed well and centrifuged at 13,000 rpm for 15 min. Then 0.5 ml of supernatant was taken and 2 ml of tris EDTA buffer was added and the volume was made up to 4.0 ml with distilled water. The reaction was initiated with the addition of 0.5 ml of pyrogallol and the absorbance at 1 min interval for 3 min was measured at 470 nm. One unit of SOD activity was the enzyme concentration required for 50% inhibition of pyrogallol and the absorbance at 1 min interval for 3 min was measured at 470 nm. One unit of SOD activity was the enzyme concentration required for 50% inhibition of pyrogallol auto-oxidation per minute. The SOD activity was expressed as units/mg protein.

Catalase activity was measured using the method of Sinha (1972). Briefly, 3.5 ml of reaction mixture containing 1.0 ml of phosphate buffer solution, 0.4 ml of hydrogen peroxide, 0.1 ml of serum and 2.0 ml of dichromate acetic acid reagent. The activity was measured by keeping the reaction mixture in boiling water bath for 10 min and the absorbance was then read at 570 nm. One unit of Catalase activity was expressed as microgram of hydrogen peroxide consumed per minute per milligram of protein.

2.4. Statistical analysis
Data were analysed using standard statistical methods as per Snedecor and Cochran (1994) and expressed as mean ± SE.

3. Results

3.1. Effect of crude extract of M. citrifolia on Lipid profile
The effects of crude extracts of M. citrifolia fruit (100 g/calf/day) on biochemical parameters are shown in Table 1. There was a continuous decline of the serum TC, TGL, LDL-cholesterol in the treatment group than in the control group and the levels significantly (P < .01) declined gradually till the fourth week of the experiment. However, in case of HDL cholesterol, no significant difference was found throughout the study at specific intervals. The mean concentration of serum TC, TGL, LDL-C and HDL-C in treatment group at 0th week was 129.1 ± 3.50, 123.8 ± 6.87, 68.75 ± 2.87 and 48.93 ± 4.01 and at 4th week was 92.55 ± 1.74, 92.00 ± 6.40, 48.93 ± 4.01 and 58.48 ± 3.39, respectively.

3.2. Effect on glucose and total protein
The serum glucose level was decreased significantly on 4th week of experiment in adult crossbred calves (93.17 ± 7.16) and statistically significant (P < .05) in the treatment group. It did not show any considerable differences till the third week of the experiment; however, the glucose level declined drastically on 4th week. There was a sharp increase in the serum total protein concentration (9.28 ± 0.55) at a specific interval in the treatment group compared to the control.

3.3. Effect on non-protein nitrogen compounds
Feeding of the calves with M. citrifolia fruit significantly reduced the levels of non-protein nitrogen compounds such as BUN and creatinine. At the 4th week, BUN level was found to decrease (23.66 ± 1.13) markedly and statistically significant (P < .01), whereas the creatinine level was reduced to about 0.82 ± 0.03 and statistically significant (P < .05) at 4th week. At the 4th week, BUN and creatinine levels were reduced significantly in treatment group than in the control.

3.4. Effect on antioxidant system
The mean value of LPO in the serum revealed a little decrease during the crude fruit extract feeding experiment and the decrease was statistically significantly higher (P < .05) in the treatment group. There was a marked increase in enzymatic antioxidants SOD (1.97 ± 0.12) and Catalase (18.58 ± 0.29) in fruit-extract-fed group compared to the control group. The results showed a significant increase (P < .01) in the serum levels of SOD and Catalase.

4. Discussion
The blood is the vital fluid that transports gases and nutrients to the tissues of the body. The biochemistry and functional capacity of the blood is directly linked to the status of the blood components. The M. citrifolia plant and especially its fruit have been used for centuries in folk medicine. Many researchers showed that this fruit contains several nutritional...
Table 1. Effect of *M. citrifolia* on Blood biochemical parameters and antioxidant enzyme activity in Crossbred calves.

| Parameter                          | Zero week | First week | Second week | Third week | Fourth week |
|------------------------------------|-----------|------------|-------------|------------|-------------|
|                                    | Control   | Treatment  | Control     | Treatment  | Control     |
| Triglycerides (mg/dl)              | 124.73 ± 10.43 | 123.8 ± 6.87 | 129.9 ± 9.89 | 114.5 ± 6.32 | 131.7 ± 10.80 |
| LDL-cholesterol (mg/dl)            | 69.68 ± 2.44  | 68.75 ± 2.87 | 68.18 ± 1.91  | 66.25 ± 3.10 | 70.96 ± 2.51  |
| Blood urea nitrogen (mg/dl)        | 27.93 ± 1.44  | 41.57 ± 2.18 | 30.45 ± 0.76  | 38.14 ± 2.49 | 34.61 ± 2.94  |
| Superoxide dismutase (IU/mg protein) | 0.90 ± 0.11 | 0.74 ± 0.12 | 0.90 ± 0.13 | 0.96 ± 0.14 | 0.90 ± 0.11 |
| Catalase (IU/mg protein)           | 13.08 ± 0.73  | 11.41 ± 0.46 | 14.17 ± 1.16 | 13.54 ± 0.49 | 13.92 ± 0.99  |

Notes: Values bearing * and ** differ significantly at P < .05 and P < .01 level, respectively, based on results of ANOVA.

The antioxidant property of natural sources is due to the antioxidants which form free radicals and prevent the tissue from the oxidative damage. The mechanism of hypolipidemic effect of fruit extract is unclear. However, hypolipidemic component present in most of the medicinal plants having a high fibrous content is responsible for reducing the lipid levels due to feeding of *M. citrifolia* fruit in crossbred calves. TC, TGL and LDL-Colesterol was significantly reduced (P < .01) in calves fed with *M. citrifolia* fruit as compared to normal calves. On other hand, HDL-cholesterol was slightly increased but not statistically significant. The findings indicate that the crude extracts of noni fruit are effective in altering blood lipids’ profile. The level of BUN and creatinine may be a result of increased protein catabolism from greater production of proteins (Huber et al. 1976). In the present study, surprisingly BUN and creatinine levels were decreased markedly even with increased level of protein. This could be due to the utilisation of protein for muscle growth. Presence of increased polyphenols in *M. citrifolia* plant extracts could be responsible for anabolic effect on protein metabolism (Yamaguchi et al. 2002).

LPO has been defined as the biological damage caused by free radicals that are formed under oxidative stress (Zin et al. 2006). The antioxidants’ levels increase to compensate this oxidation reaction and prevent the tissue from the oxidative damage. The antioxidant property of natural sources is due to
the presence of antioxidant compounds in the plants. Many natural antioxidants are found in fruit, root and leaves of various plants. Most of these compounds are normally phenolic or polyphenolic compounds in nature. These compounds as an antioxidant may act by scavenging the radicals directly or sustaining the activity of antioxidant enzymes or inhibiting the activity of oxidising enzymes. In present study, there is an increased level of enzymatic antioxidants such as SOD and catalase, which could be due to alkaloids, flavanoids and some glycosides such as citrifolin. Serafini et al. (2011) investigated the antioxidant activity of aqueous extract from *M. citrifolia* leaves against LPO, hydroxyl and nitric-oxide-induced radicals. Dussossoy et al. (2011) showed that Noni's antioxidant activities are possibly due to phenolic compounds, iridoids and ascorbic acid. The seed extract exhibited significant antioxidant potential against various types of free-radical-induced damage (West et al. 2011). *M. citrifolia* is well documented that noni fruit showed effective antioxidant activity (Mohd Zin et al. 2002). Besides, LPO activity, was measured by the amount of malondialdehyde (MDA) found in serum which is formed from decomposition of peroxides by oxidative damage. The levels of LPO gradually decreased at specific intervals, which could be due to the elevated levels of enzymatic serum antioxidants in calves fed with *M. citrifolia* fruit.

5. Conclusion

Feeding of *M. citrifolia* fruit extract to crossbred calves showed altered biochemical constituents (viz. hypolipidemic, hypoglycaemic) and enhanced antioxidant activity in calves. The findings of this study could be exploited for the stress amelioration and management of metabolic diseases in calves without adverse effects.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

Brooks VJ, Schafer M, Sharp P, Xu J, Cai J, Keuler NS, Godbee RG, Peek SF, Schultz RD, Suresh M, Darien BJ. 2009. Effects of *Morinda citrifolia* (Noni) on CD4 + and CD8 + T-cell activation in neonatal calves. Prof Anim Sci. 25:262–265.

Chan-Blanco Y, Vaillant F, Perez AM, Reyes M, Brillouet JM, Brat P. 2006. A review of agricultural research, nutritional and therapeutic properties. J Food Comp Anal. 19: 645–654.

Dussossoy E, Brat P, Bony E, Bouard F, Pouceret P. 2011. Characterization, anti-oxidative and anti-inflammatory effects of Costa Rican noni juice (*Morinda citrifolia* L.). J Ethnopharmacol. 133:108–115.

Ghasemzadeh A, Jaafar HZE. 2011. Anticancer and antioxidant activities of Malaysian young ginger (*Zingiber officinale* R) varieties grown under different CO2 concentration. J Med Plant Res. 5:3247–3255.

Goh SH, Chuah CH, Mok JSL, Soepadmo E. 1995. Malaysian medicinal plants for the treatment of cardiovascular diseases. Kuala Lumpur, Malaysia: Peladuk Publications. ISBN 967–978–515–7. 162 pg.

Hadjiah H, Ayub MY, Zaidah H, Normah A. 2008. Hypolipidemic activity of an aqueous extract of *Morinda Citrifolia* fruit in normal and streptozotocin-induced diabetic rats. J Trop Agric and Fd Sc. 36(1):77–85.

Huber JT, Boman RL, Henderson HE. 1976. Fermented ammoniated condensed whey as a nitrogen supplement for lactating cows. J Dairy Sci. 59: 1936–1940.

Luo Q, Cai Y, Yan J, Sun M, Corke H. 2004. Hypoglycemic and hypolipidemic effects and antioxidant activity of fruit extracts from Lycium barbarum. Life Sci. 76(2):137–149.

Mandukhail SUR, Aziz N, Gilani AH. 2010. Studies on anti-diabetic effects of *Morinda citrifolia* (Noni) fruit, leaves and root extracts. Lipids Health Dis. 9:88–93.

Marklund S, Marklund G. 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 47(3):469–474.

Mohd Zin Z, Abdul-Hamid A, Osman A. 2002. Antioxidative activity of extracts from Mengkudu (*Morinda citrifolia* L) root, fruit and leaf. Food Chemistry. 78:227–231.

Morton JF. 1992. The ocean-going Noni, or Indian mulberry (*Morinda citrifolia*, Rubiaceae) and some of its ‘colourful’ relatives. Economic Botany. 46:241–256.

Palu AK, Kim AH, West BJ, Deng S, Jensen J, White L. 2008. The effects of *Morinda citrifolia* L. (Noni) on the immune system: Its molecular mechanism of action. J Ethnopharmacol. 115:502–506.

Ponce CH, Ballou MA, Godbee RG, Hulbert LE, DiLorenzo N, Quinn MJ, Smith DR, Galyean ML. 2011. Case Study: Effects of *Morinda citrifolia* extract on performance and morbidity of newly received beef heifers. Prof Anim Sci. 27:269–275.

Saminathan M, Rai RB, Dharma K, Jangir BL, Suresh S, Ranganath GJ, Sophia I, Karuppanasamy K, Barathiraja S, Gopalakrishnan A. 2014. Effect of *Morinda citrifolia* (Noni) Fruit Juice on Antioxidant, Hematological and Biochemical Parameters in N-Methyl-N-Nitrosourea (NMU) Induced Mammary Carcinogenesis in Sprague-Dawley Rats. Int J Pharmacol. 10:109–119.

Saminathan M, Rai RB, Dharma K, Tiwari R, Chakraborty S. 2013. Systematic review on anticancer potential and other health beneficial pharmacological activities of novel medicinal plant *Morinda citrifolia* (Noni). Int J Pharmacol. 9:462–492.

Serafini MR, Santos RC, Guimaraes AG, Dos Santos JP, Santos DC. 2011. Morinda citrifolia Linn leaf extract possesses antioxidant activities and reduces nociceptive behavior and leukocyte migration. J Med Food. 14:1159–1166.

Singh AS, Pal DT, Mandal BC, Singh P, Pathak NN. 2002. Studies on changes in some of blood constituents of adult cross-bred cattle fed different levels of extracted rice bran. Pakistan Journal of Nutrition. 1(2):95–98. doi:10.3923/pjn.2002.95.98

Singh DR. 2012. *Morinda citrifolia* L. (Noni): a review of the scientific validation for its nutritional and therapeutic properties. J Diabetes Endocrinol. 3:77–91.

Sinha KA. 1972. Colorimetric assay of catalase. Ann Biochem. 47:389–394. doi:10.1002/0003-2697(72)90132-7

Snedecor GW, Cochran WG. 1994. Statistical methods. 6th Ed. Ames, Iowa: Iowa State University Press.

Wang MY, West BJ, Jensen CJ, Nowicki D, Chen S, Palu A. 2002. *Morinda citrifolia* (Noni): A literature review and recent advances in noni research. Acta Pharmacologica Sinica. 23:1127–1141.

West BJ, Jensen CJ, Palu AK, Deng S. 2011. Toxicity and antioxidant tests of *Morinda citrifolia* (Noni) seed extract. Adv J Food Sci Technol. 3:303–307.

Yamaguchi S, Ohnishi J, Sagawa M, Maru I, Ohata Y, Tsukada Y. 2002. Inhibition of angiotsensin I converting enzyme by noni (*Morinda citrifolia*) juice. J Ap Soc Food Sci Technol. 49:624–627. doi:10.3136/nssk.49.624

Yang J, Paulino R, Janke-Stedronsky S, Abawi F. 2007. Free-radical-scavenging activity and total phenols of noni (*Morinda citrifolia*) fruit, leaves and root extracts. Food Chemistry. 102:302–308. doi:10.1016/j.foodchem.2006.05.020

Zin ZM, Hamid AA, Osman A, Saari N. 2006. Antioxidative activities of chromatographic fractions obtained from root, fruit and leaf of Mengkudu (*Morinda citrifolia* L.). Food Chemistry. 94:169–178. doi:10.1016/j.foodchem.2004.08.048