Trop J Nat Prod Res, February 2019; 3(2):54-57 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Involvement of Anti-Inflammatory Mechanism in the Antidepressant Activity of Methanol Stem Bark Extract of *Adansonia digitata* L. (Malvaceae)

Aishatu Shehu*, Sagir Mustapha, Isah A.Mansir, Mohammed G. Magaji

Department of Pharmacology and Therapeutics, Ahmadu Bello University, Nigeria

ARTICLE INFO

Article history:
Received 12 February 2019
Revised 22 February 2019
Accepted 25 February 2019
Published online 01 March 2019

ABSTRACT

The plant *Adansonia digitata* has been reported to possess antidepressant activity mediated via monoaminergic, neuroendocrine and neurotrophic pathways. This study investigated the involvement of anti-inflammatory mechanism in the antidepressant activity of methanol stem bark extract of *A. digitata*. Acute oral median lethal dose (LD_{50}) was estimated using Organization for Economic Co-operation and Development (OECD 425) method. Depression was induced using Bacillus Calmette-Guerin (BCG, 0.2 mg/kg, *i.p.*) in mice and readings taken at 0, 4, 24 and 48 hours post BCG administration. Subsequently the antidepressant activity of the extract (250-1000 mg/kg) was assessed using tail suspension test (TST) and open field test (OFT). The extract significantly ($p < 0.05$) decreased the rectal temperature of mice following BCG-induced sickness 4 and 48 hours post induction. The extract also significantly ($p < 0.05$) decreased the duration of immobility in the TST at 4 and 48 hours post BCG administration. The extract significantly ($p < 0.05$) increased the total number of line crossedby mice at 4, 24 and 48 hours post BCG administration. The ability of the extract to ameliorate BCG-induced depression suggests the possible involvement of anti-inflammatory mechanismin its antidepressant activity.

Keywords: Depression, *Adansonia digitata*, Bacillus Calmette-Guerin, Inflammatory mechanism

Introduction

Depressive disorders are very common in patients afflicted with conditions such as cardiovascular diseases, type 2 diabetes and rheumatoid arthritis that are associated with chronic inflammation.[1,2] In some other conditions such as cancer and hepatitis C, immunotherapy has been confirmed to be responsible for depression in the patients.[3-5] Neurovegetative and somatic symptoms of depression such as flu-like symptoms, fatigue, anorexia, pain and sleep disorders are observed in these patients.[6] Later on, psychological symptoms like mild cognitive alterations, depressed mood, anxiety and irritability follows.[7] Treatment with existing antidepressant were reported to have little or no effect on the symptomatology of depression in these patients.[8,9] In addition, treatment resistant depression (TRD) is associated with pro-inflammatory pathways and it poses significant challenge in depression therapy.[10,11] Treatment failures occur in TRDs because most existing antidepressant drugs do not stimulate this pathway, thus, symptoms consequential to the activation of inflammatory pathways are not ameliorated.[12] All these necessitated the screening of alternative therapies that act beyond monoaminergic, neuroendocrine and neurotrophic pathways. Medicinal plants are used traditionally in the management of depression. One of such medicinal plants which enjoys wide patronage among traditional practitioners is *Andasonia digitata* (AD).[13] Its antidepressant potential has been validated[14] and reported to be mediated via monoaminergic pathways.[15] It has also been reported to possess anti-inflammatory activity in animal model of hyperalgesia.[16]In furtherance of our attempt to establish the mechanism of action of the plant, the antidepressant effect was investigated on BCG models of depression.

*Corresponding author. E mail: pharmaishatu@gmail.com
Tel: +234-803-6028298

Citation: Shehu A, Mustapha S, Mansir IA, Magaji MG. Involvement of Anti-Inflammatory Mechanism in the Antidepressant Activity of Methanol Stem Bark Extract of *Adansonia digitata* L. (Malvaceae). Trop J Nat Prod Res. 2019; 3(2):54-57. doi.org/10.26538/tjnpr/v3i2.6

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Materials and Methods

Plant Collection and Extraction

The stem bark, leaves and fruits of AD were collected in a bushy area of Zaria Local Government Area of Kaduna State in June, 2018. The plant was identified by Mr Namadi Sanusi of the Department of Botany, Ahmadu Bello University, Zaria where it was authenticated by comparing with existing voucher specimen (2512).

The stem bark was air-dried and reduced to fine powder using mortar and pestle. The powdered AD was subsequently extracted with 2.5 L of methanol via soxhlet extraction over 48 hours' period. The filtrate obtained was concentrated on a water bath at 45ºC, where it afforded a brownish sticky mass subsequently referred to as methanol stem bark extract of AD and stored in desiccators until needed. An aqueous solution was freshly prepared for each study using distilled water.

Animals

Swiss Albino mice of both sexes (18-22 g) were obtained from the animal house facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. They were housed in improvised propylene cages and kept under natural day and light cycle. The mice were fed on standard laboratory animal diet and water freely available. Experiments were carried out in accordance with the Ahmadu Bello University Animal Ethics Committee (ABUCAUC/2017/022).

Acute Toxicity Study

Median lethal dose (LD_{50}) was determined using Organization for Economic Co-operation and Development (OECD 425) guidelines in mice. Briefly, the limit test (at a dose of 5,000 mg/kg) was employed. Mice were fasted 2 hours before and one hour after extract administration. A dose of 5,000 mg/kg was administered to one

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

mouse and observed for 48 h. On survival, two additional mice were also given same dose of the extract. Each mouse was observed within 30 minutes of treatment and then variably over 24 hours for 14 days. Animals were monitored for behavioural changes and death.[17]

Groupings and Treatments
Forty mice were divided into 5 groups of eight mice each and administered with 0.2 mg/kg Bacillus Calmette Guerin vaccine (BCG) through intraperitoneal route.

Effect of AD on Body Temperature following BCG-induced Depression
Three hours post BCG administration, mice in group 1 were administered distilled water (10 mL/kg, p.o.), group 2 mice were treated with fluoxetine (20mg/kg, p.o.), groups 3, 4 and 5 mice were treated with 250,500 and 1,000 mg/kg methanol stem bark extract of AD, respectively. A time interval of 15 minutes was allowed between treatment groups. Rectal temperatures of mice in all the groups were taken at time 0, 4, 24 and 48 hours post BCG injection.

Behavioural Studies
Tail suspension test
Mice in all the groups were subjected to tail suspension test one-hour post treatment as earlier described.[18]An adhesive tape 16 cm in length was placed 1 cm from tip of each mouse tail, each mouse was hung for a period of 6minutes on a shelf. The duration of immobility was then recorded for each mouse. TST was done at 4, 24 and 48 hours post BCG induction.

Open field test
Locomotor activity was assessed by placing each mouse individually in a plexiglass apparatus that has16squares with one centre square (15×15 cm). Locomotor activity was measured by counting the number of line crosses over a six minutes' period with a counter and result recorded.[19] The apparatus was cleaned with ethanol between each testto remove olfactory cues. OFT was done at 4, 24 and 48 hours post BCG administration.

Statistical Analysis
All values were expressed as mean ± SEM. The mean rectal temperature was analysed using repeated measures ANOVA while mean of immobility from TST and mean number of line crossed from OFT were analysed by one-way ANOVA followed by Bonferroni post-hoc test using SPSS version 20.0, a value of $p<0.05$ was considered significant.

Results and Discussion

Bacillus Calmette Guerin administration was able to induce change in core body temperature of mice measured at 4, 24 and 48 hours post administration. There was significant ($p<0.001$) decrease in rectal temperature and a dose-dependent decrease in the duration of immobility of mice following treatment with methanol stem bark extract of AD at all tested doses (250, 500 and 1,000 mg/kg) 4 hours post BCG injection when compared with the rectal temperature and duration of immobility of the distilled water administered group. Similarly, there was a significant ($p<0.05$) decrease in rectal temperature and duration of immobility at dose of 1,000 mg/kg AD 24 hours post BCG injection. So also, there was a decrease in rectal temperature at all tested doses of AD and fluoxetine (20 mg/kg) 48 hours post BCG injection when compared to the distilled water administered group.However, there was only significant ($p<0.05$) decrease in the duration of immobility of AD at dose of 500 and 1,000 mg/kg and fluoxetine (20 mg/kg) 48 hours post BCG injection when compared to the distilled water group (Figures 1 and 2). Increase in rectal temperature, fever and weight loss has been reported to be associated with BCG-administration.[17] These occur due to the release of pro-inflammatory markers such as tumour necrosis factor (TNF), interleukin (IL)-1β, IL-6, C-reactive protein (CRP) activated by BCG through the inflammatory pathway.[20,21] The release of inflammatory markers causes the reduction of monoamine availability by increasing the expression and function of the presynaptic reuptake pumps to dopamine, serotonin and norepinephrine.[22] Inflammation also decrease relevant monoamine precursors by activating enzyme indoleamine deoxygenase which breaks down tryptophan into kynurenine and then to quinolinic acid that lead to an excess excitatory amino acid glutamate.[23] Excessive glutamate in circulation leads to decreased brain derived neurotrophic factor (BDNF) and excitotoxicity.[24] All these cascades of events initiated by BCG is responsible for the increase in rectal temperature, sickness behaviour and depressive episodes observed in mice. The ability of extract AD to decrease the rectal temperature as well as decrease the duration of immobility of mice is an indication of its antidepressant activity. The extract might have acted to neutralize the effect or prevent the release of the inflammatory markers caused by BCG. Previously, the plant *Adansonia digitata* was reported to possess marked anti-inflammation in formalin-induced pedal swelling in rodents further ascertaining the involvement of inflammatory pathways as mechanism of action of the plant.[25]The beneficial effects of AD on BCG-induced depression may also be due to inhibition of nuclear factor *kappa* B (NF-κB) activation, which suppresses expression of the pro-inflammatory inducible nitric oxide synthase (iNOS) gene, resulting in decreased nitric oxide (NO) production as reported to be the mechanism of anti-inflammation in RAW264.7 cells.[16] Thus, this further ascertained the involvement of inflammatory mechanism in the antidepressant activity of AD.
BCG administration caused a decrease in locomotor activity of mice in this study. Treatment with methanol stem bark extract of AD (250, 500 and 1,000 mg/kg) significantly ($p< 0.001$) increased the number of line crossed in mice at 4 and 24 hours post BCG injection. However, only the highest dose of the extract (1,000mg/kg) and fluoxetine (20 mg/kg) significantly ($p < 0.05$) increased the number of line crossing 48 hours post BCG administration (Figure 3).In addition to the fever, weight loss and sickness behaviour caused by BCG induction, the locomotor activity of mice was reported to be impaired.[26,27] The extract AD was able to ameliorate the impaired locomotion in mice at all the tested doses. The activity of extract AD was more prominent at the early hours of BCG administration than lately. This is similar to other findings which showed that locomotion and exploration behaviour following BCG administration varies with time.[28]

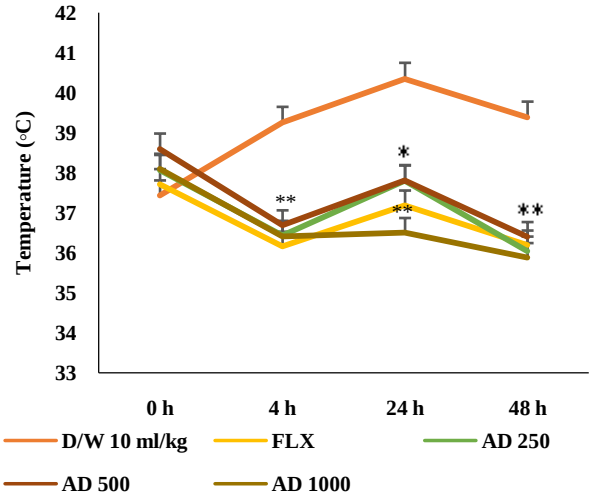

Figure 1: Effect of Methanol Stem Bark Extract of Adansonia digitata on Mice Rectal Temperature following BCG Induction.
Data represents the mean ± S.E.M. of 8 mice. Data was analysed using repeated measures ANOVA followed by Bonferroni post-hoc test, $**p\leq 0.001$ and $*p\leq 0.05$, significantly different from distilled water group at various time. AD=*Adansonia digitata*, DW= Distilled water, FLX= Fluoxetine, h= hours.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

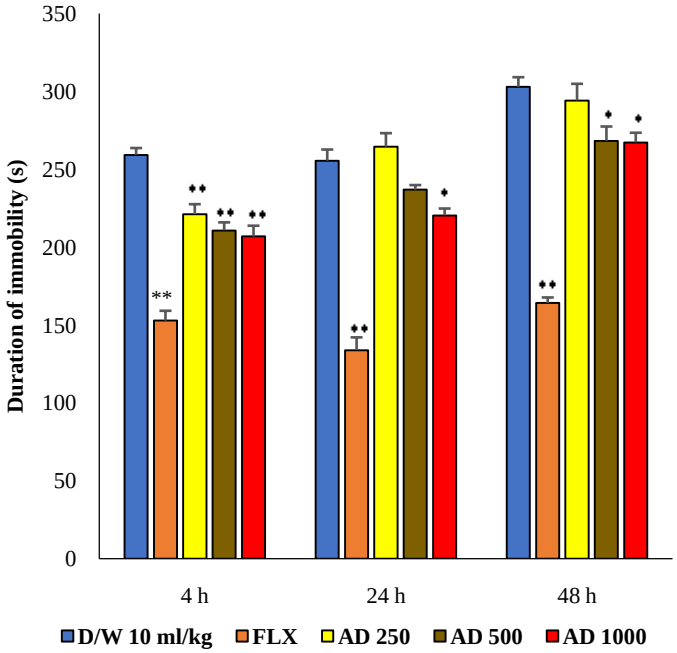

Figure 2: Effect of Methanol Stem Bark Extract of *Adansonia digitata* on Duration of Immobility Following BCG-induced Depression in Mice.
Values are mean ± SEM (n = 8). Data were analysed using one-way ANOVA followed by Bonferoni post-hoc test, *p< 0.05 and **p<0.001 significant difference as compared to distilled water group. DW = distilled water (10 mL/kg, *p.o.*), AD= *Adansonia digitata* (250, 500 and 1, 000 mg/kg, *p.o.*), FLX= Fluoxetine (20 mg/kg, *p.o.*).

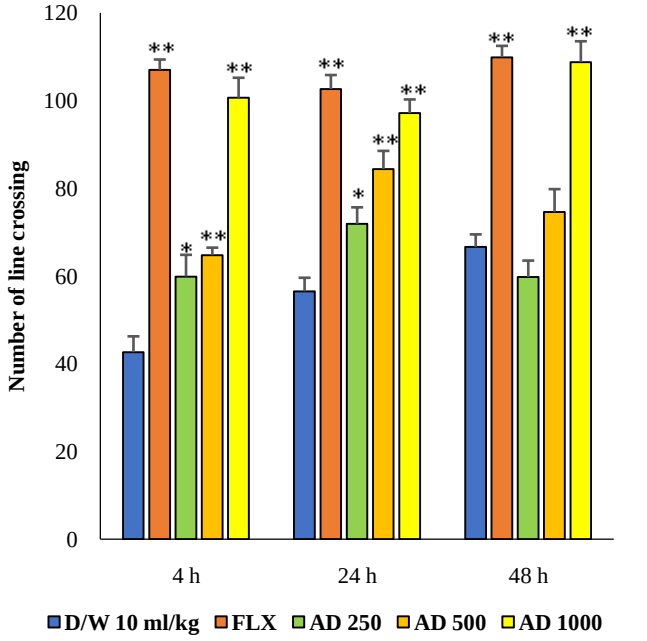

Figure 3: Effect of Methanol Stem Bark Extract of *Adansonia digitata* on Line Crossing Behaviour of Mice following BCG-induced Depression.
Data were analysed using one-way ANOVA followed by Bonferoni post-hoc test, expressed as mean ± SEM (n = 8). ∗p< 0.05 and ∗∗p<0.001 significant differences as compared with distilled water group. DW = distilled water (10 mL/kg, *p.o.),* AD= *Adansonia digitata*(250, 500 and 1,000 mg/kg, *p.o.*),FLX= Fluoxetine (20 mg/kg, *p.o.*) h= hours.

The LD_{50} of AD was found to be 5,000 mg/kg suggesting its relative safety as described by Lorke.[29]The safety and non-toxic effect could be due to its use as food with high nutritional value reported.[30] Theplant AD with relative safety will be highly beneficial in developing lead compounds for the management ofdepression.

Conclusion

The methanol stem bark extract of *Adansonia digitata* ameliorates BCG-induced depression due to involvement of anti-inflammatory mechanisms.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

The technical help of Mr. Bashir Shehu and Mr. Abubakar Balarabe of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria is acknowledged.

References

1. Steptoe A. Depression and Physical Illness. Cambridge University Press 2007; ISBN 13978-0-521-60360-7 Pp 421.
2. Saleh LA, Hamza M, El Gayar NH, Abd El-Samad AA, Nasr EA, Masoud SI. Ibuprofen suppresses depressive like behaviuor induced by BCG inoculation in mice: role of nitric oxide and prostaglandin. Pharmacol Biochem Behav. 2014; 125:29-39.
3. Capuron L, Ravaud A,Dantzer R. Early depressive symptoms in cancer patients receiving interleukin 2 and/or interferon alfa-2b therapy. J Clin Oncol. 2000; 18:2143–2151.
4. Capuron L, Gumnick JF, Musselman DL, Lawson DH, Reemsnyder A, Nemeroff CB, Miller AH. Neurobehavioral effects of interferon-alpha in cancer patients: phenomenology and paroxetine responsiveness of symptom dimensions. Neuropsychopharmacol. 2002; 26:643–652.
5. Constant A, Castera L, Dantzer R, Couzigou P, de Ledinghen V, Demotes-Mainard J, Henry C. Mood alterations during interferon-alfa therapy in patients with chronic hepatitis C: evidence for an overlap between manic/hypomanic and depressive symptoms. J Clin Psych. 2005; 66:1050–1057.
6. Li M, Chen Z, Liu Z, Zhang N, Liu J, Wang H, Wang W, Liang Y, Chen J, Liu Z, Li Y, Zhai S. Twelve Chinese herbal preparations for the treatment of depression or depressive symptoms in cancer patients: a systematic review and meta-analysis of randomized controlled trials. BMC Complement Altern Med. 2019; 19:28.

7. Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosci. 2008; 9(1):46–56.
8. Saeedi S, Arefidoust A, Yaftian R, Dehpour AR. 17*α*-ethinylestradiol attenuates depressive-like behavior through GABAA receptor activation/nitrergic pathway blockade in ovariectomized mice. Psychopharmacol (Berl). 2016; 233(8):1467-1485.
9. Rana P, Sharma AK, Jain S, Deshmukh P, Bhattacharya SK, Banerjee BD, Mediratta PK. Comparison of fluoxetine and 1-methyl -L-tryptophan in treatment of depression-like illness in Bacillus Calmette-Guerin-induced inflammatory model of depression in mice. J Basic Clin Physiol Pharmacol. 2016; 27(6):569-576.
10. Fried EI. Problematic assumptions have slowed down depression research: why symptoms, not syndromes are the way forward. Front Psychol. 2015; 6:1–11.
11. Fried, EI. Moving forward: how depression heterogeneity hinders progress in treatment and research. Expert Rev Neurother.2017; 17(5):423-425.
12. Vijaya KK, Rudra A, Sreedhara MV, Siva ST, Prasad DS, Das ML, Murugesan S, Yadav R, Trivedi RK, Louis JV. Bacillus Calmette-Guérin vaccine induces a selective serotonin reuptake inhibitor (SSRI)-resistant depression like phenotype in mice. Brain, Behav Immun. 2014; 42:204-211.
13. Shehu A, Magaji MG, Yau J, Abubakar A. Ethno-botanical survey of medicinal plants used for the management of depression by Hausa tribes of Kaduna State, Nigeria. J Med Plants Res. 2017; 11(36):562-567.
14. Shehu A, Magaji MG, Yau J, Mahmud B, Abubakar A. Antidepressant effect of methanol stem bark extract of *Adansonia digitata* L. (Malvaceae) in mice. Trop J Nat Prod Res. 2018; 2(2):87-91.
15. Shehu A, Magaji MG, Yau J, Mahmud B,Ahmed A. Antidepressant effect of methanol stem bark extract of *Adansoniadigitata* L.: involvement of dopaminergic, adrenergic, nitric oxide and serotonergic pathways presented at 15TH Annual Conference and General Meeting, Neuroscience Society of Nigeria (NSN); BAZE University Abuja, 2017. 58 p.
16. Ayele Y, Kim J, Park E, Kim Y, Retta N, Dessie G, Rhee S, Koh K, Nam K, Kim HS. A Methanol Extract of *Adansoniadigitata* L. Leaves Inhibits Pro-Inflammatory iNOS Possibly via the Inhibition of NF-κB Activation. Biomol Ther (Seoul). 2013; 21(2):146–152.
17. Organization for Economic Cooperation and Development-OECD Guideline for Testing of Chemicals, Acute Oral Toxicity- Fixed Dose Procedure 425, Adopted 17th December, 2008.
18. Steru L, Chermat R, Thierry B, Simon P. Tail Suspension test: a new method for screening antidepressants in mice. Psychopharmacol. 1985; 85:367-370.
19. Rex A, Voigt JP, Voits M, Fink H. Pharmacological evaluation of a modified open field test sensitive to anxiolytic drugs. Pharmacol Biochem Behav. 1998; 59:677-683.
20. Painsipp E, Köfer MJ, Aitak FA, Dischinger US, Sinner F, Herzog H, Peter HP. (2013). Neuropeptide Y and peptide YY protect from weight loss caused by Bacille Calmette–Guérin in mice. Br J Pharmacol. 2013; 170:1014–1026.
21. Sanders B, Skansén-Saphir U, Damm O, Håkansson L, Andersson J, Andersson U. Sequential production of Th1 and Th2 cytokines in response to live bacillus Calmette–Guérin. Immunol. 1995; 86:512–518.
22. Moreau M, Andre C, O'Connor JC, Dumich SA, Woods JA, Kelley KW, Dantzer R, Lestage J, Castanon N. Inoculation of Bacillus Calmette-Guerin to mice induces an acute episode of sickness behaviour followed by chronic depressive-like behavior. Brain Behav Immun. 2008; 22:1087-1095.
23. Haroon E, Raison CL, Miller AH. Psychoneuroimmunology meets neuropsychopharmacology: translational implications of the impact of inflammation on behaviour. Neuropsychopharmacol. 2012; 37:137–162.
24. O'Connor JC, Lawson MA, André C, Briley EM, Szegedi SS, Lestage J. Induction of IDO by bacilleCalmette–Guérin is responsible for development of murine depressive-like behaviour. J Immunol. 2009; 182:3202–3212.
25. Ramadan A, Harraz FM, El-Mougy SA. Anti-inflammatory, analgesic and antipyretic effects of the fruit pulp extract of *Adansonia digitata*. Fitoterapia 1994; LXV(5):418-422.
26. Painsipp E, Herzog H, Holzer P. Implication of neuropeptide-Y Y2 receptors in the effects of immune stress on emotional, locomotor and social behaviour of mice. Neuropharmacol. 2008; 55:117-126.
27. Painsipp E, Herzog H, Holzer P. Evidence from knockout mice that neuropeptide-Y Y2 and Y4 receptor signalling prevents long-term depression-like behaviour caused by immune challenge. J Psychopharmacol. 2010; 24:1551–1560.
28. Painsipp E, Herzog H, SperkG,Holzer P. Sex-dependent control of murine emotional–affective behaviour in health andcolitis by peptide YY and neuropeptide Y. Br J Pharmacol. 2011; 163:1302–1314.
29. Lorke DA. A new Approach to Practical acute toxicity testing. Arch Toxicol. 1983; 45:275-87.
30. Chadare FJ, Linnemann AR, Hounhouigan JD, Nout MJ, Van Boekel MA. Baobab food products: a review on theircomposition and nutritional value. Crit Rev Food Sci Nutr. 2009; 49:254-274.