May 27, 2015

Dear Editor,

We, the authors, express our most sincere appreciation and gratitude to the editorial office and reviewers for their time and invaluable comments.

Please find enclosed the edited manuscript in Word format (file name: 18504-Revised.docx).

Title: Aloesin as a Medical Food Ingredient for Systemic Oxidative Stress of Diabetes

Author: Mesfin Yimam, Lidia Brownell, Qi Jia

Name of Journal: World Journal of Diabetes

ESPS Manuscript NO: 18504

We have revised the full manuscript according to the suggestions and incorporated our response at appropriate sections of the body of the manuscript as follows:

Reviewer #1424366:

This is a comprehensive review of the potential of aloesin as an anti-oxidant in the prevention or better control of type 2 diabetes. It is well written but there are a few places where the generalist reader struggles to follow the narrative.

"1. On page 12 it would be very helpful to start section 3 with an introduction to the plant species from which aloesin is derived, the type of chemical it is and the history of its use in traditional medicine."

Reply: The following has been incorporated into the suggested section:

Aloe vera (*Aloe barbadensis* Miller) is a perennial cactus like succulent plant belonging to the Liliaceal family. It is a biochemically complex plant that includes more than 300 species comprising many biologically active substances with diverse applications[1]. The major components of *Aloe vera* such as anthraquinones, saccharides, vitamins, enzymes, and Low molecular weight substances, collectively, have been reported to possess immunomodulatory, anti-inflammatory, ultraviolet radiation protective, antiprotozoal, and wound/ burn-healing promoting properties[2]. While polysaccharides, in specific, have been described to show anti-inflammation, anticancer, and immunomodulation activities, biological activities such as cell growth stimulation, melanin synthesis inhibitions and antioxidant functions were documented for aloesin[3]. Structurally, the aloe whole leaf encompasses three main distinctive sections each with specific function. These parts are categorized as the green rind or cuticle, the outer leaf pulp and the gel fillet. Polysaccharides are mainly located within the mucilaginous gel from the parenchymatous tissue whereas aloesin is housed inside the exudate of the leaf pulp.

" 2. As an anti-oxidant it is likely that aloesin has been used to treat other inflammation-based diseases. Is there such a literature and can it be used in multiple chronic diseases, which would be a major advantage?"

Reply: While it is speculated that the wound healing effect of aloe is as a result of aloesin and other active components of aloe, preclinical activities of Aloesin as anti-inflammatory[4, 5] and anti-tumor [6] has been described in the literature.

"3. How does aloesin compare with other nutraceutical approaches in the control of type 2 diabetes such as the hypoglycemic actions of curcumin or mistletoe extracts?"

Reply: Efficacy and/or application comparisons were not conducted in this review as it was beyond the scope of the subject.

"4. Some Tables or Figures would help the reader to grasp the main messages of the review, such as a comparison of aloesin with other nutraceuticals used in diabetes, a list of major clinical studies with a brief summary of the findings, or a diagram of how ROS are generated and can interfere in insulin signaling, and where aloesin is most likely to interfere."

Reply: The following has been included at the end of the body of the manuscript:

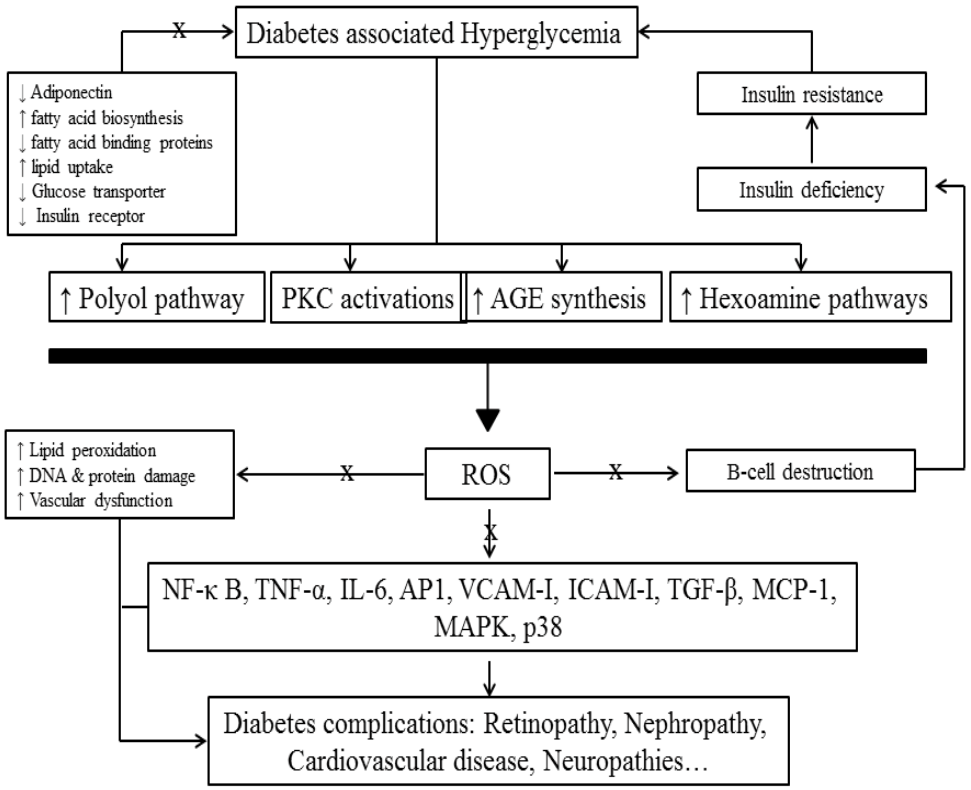

Figure 1: Oxidative stress and its possible pathways leading to diabetes complications. "X" potential sites where aloesin may likely interfere. PKC: Protein kinase C; AGE: Advanced glycation

end-products; ROS: Reactive oxygen species; NF-κB: nuclear factor-kappaB ; TNF-α: tumor necrosis factor alpha; IL-6: Interleukin 6; AP1: activating protein-1; VCAM-1: vascular cell adhesion molecule-1; ICAM-1: Intercellular Adhesion Molecule 1; TGF-β: Transforming growth factor beta; MCP-1: monocyte chemotactic protein; MAPK, p38: Mitogen-activated protein kinases, p38.

Reviewer #506304

General comments Yimam et al. have reviewed the potential application of aloesin and formulated aloe-derived medicinal products for diabetic patients. In general, this is a well-written comprehensive review article. Some minor specific comments are as follows. Specific comments
"1. On pages 4–9, the pathophysiology of DM should also be presented in a diagram, which includes how aloe products/other antioxidant supplements antagonize each specific mechanism."

Reply: We have summarized the possible mechanisms in diagram as shown above.

"2. On page 10–11, each category of antioxidants should be described under subheading (e.g., Vitamins, Polyphenols, Flavonoids, etc.). "

Reply: Suggestions adapted.

"3. A figure that shows cross-sectional structure of aloe leaf may be included in the paper. This will help readers who are not familiar with aloe plant to understand where the inner leaf gel comes from."

Reply: The following has been included to give more clarity:

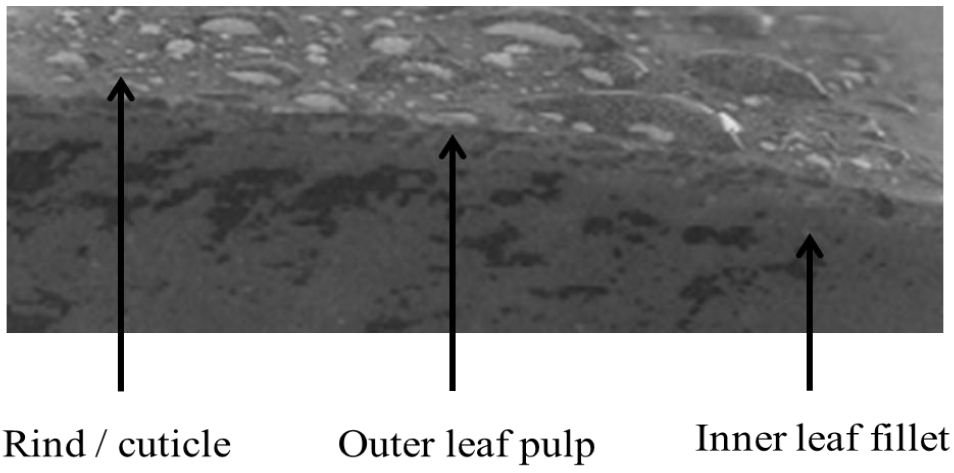

Figure 2: Cross-section of Aloe

"4. The authors should minimize using the trade names in the article. For example, Loesyn in the conclusions section should be changed to "formulated aloe products"."

Reply: Maximum efforts were made to minimize the use of trade name.

"5. Please correct typographical errors (e.g., page 16: "adipokin marker" should be "adipokine

marker").”

Reply: Corrected.

Reviewer #2446589

In this review the authors discuss potential use of Aloesin or other products of aloe vera as medical food to manage systemic oxidative stress and/or high blood glucose of diabetes. This possibility is important and cost effective strategy due to progressive increase in diabetic patients all over the world. However there are some drawbacks about this issue, -As they explained extensively, oxidative stress has been considered as a unique mechanism which connects almost all of the complicated destructive biochemical pathways induced by hyperglycemia in diabetic patients. However this may not be real since most of the antioxidant therapies or reducing agents experienced through many years have not provided significant improvement in diabetes and related diseases. Can oxidative stress be a result of disease which develops secondary to impaired insulin regulation instead of cause? Accordingly they noticed that new therapeutic approaches by administration of anti-oxidants or modulation of the oxidative-inflammatory cascade have been proposed for many years; however there are limited clinical evidences that suggest dramatically improved systemic oxidative stress that can be achieved from oral administration of common anti-oxidants. Moreover in most cases the complication persists even in the case of reduced oxidative stress, in contrast to animal studies. How do authors explain that? -In further is it possible to think whether the suggested beneficial effects of aloe vera may arise due to insulin-like or insulin mimetic effect? Lastly, I think the beneficial effects of foods are complicated story, because they include many chemical substances that may modulate different cellular pathways. Consistently the mentioned beneficial effect of aloe vera in diabetic patients may be through other mechanisms or they may be achieved via currently undefined chemical components of plant.

Reply: We totally agree with the reviewer's insight. Despite all the research on diabetes and recent advances in diabetes treatments, the inconvenient fact is that there is no true cure for diabetes and its devastating complications. Here we are trying to establish an association between one of the most complicated disease with the most complicated plant ever know to human kind. While we still believe that oxidative stress plays a central role in diabetes and its complications, obviously, that is not the whole story. There is no single way to cure a multifactorial disease like diabetes which may, at least in part, explain why mechanism or target specific interventions have yielded insufficient benefit. This has been reflected especially as a result of sub-optimal disease management where far too many patients with diabetes experiencing an increased risk for disease-associated complications such as nephropathy, neuropathy, retinopathy, cardiovascular disease and sometimes hypoglycemia. We believe that aloesin in combination with aloe polysaccharides could potentially be considered to mitigate some of the complications associated with diabetes. However, that doesn't exclude the need for proper disease management as diabetes is a progressive disease caused by a combination of genetic and environmental risk factors which could relapse anytime when there is a fail in the whole management system. At the same time, taking the intricate nature of the disease into considerations, we cannot rule out the possibility of oxidative stress as being an outcome of the disease and vice versa. Plus, intervention with natural products that contains ranges of bio-actives such as aloe may lead to interference at multiple pathways to impose the desired outcome.

Reviewer #503221

"Should be clear in the discussion that the treatment of diabetic nephropathy. The new pathways involved authophagy impairment and new treatments such as rapammun must be mentioned Must be aware with the conclusions regarding this treatment."

Reply: While refraining from discussing the use of the mentioned specific drug, the following paragraph has been inserted for diabetic nephropathy:

Currently, diabetic nephropathy is largely considered as the leading cause of end-stage renal disease in the western world. Hyperglycemia-mediated alterations of intracellular metabolism, including oxidative stress are major contributing factors to the pathogenesis of diabetic nephropathy. Despite the fact that interventions such as intensive lifestyle modification coupled with aggressive therapeutic management of glycemic control, blood pressure control, and inhibition of the renin-angiotensin-aldosterone system have shown promise to slow down progression of the disease, the number of patients with diabetes that ultimately develop end-stage renal disease have become significantly high. These highly predictive consequences suggest that there still is an urgent need to further understand the pathogenesis of the disease in order to establish new therapeutic strategies and promote enhanced clinical management for a better prognosis. In this respect, in the past few years, significant evidences from pre-clinical and clinical studies have been documented to link impaired autophagic activity in the pathogenesis of diabetic renal disease[7]. Autophagy is a fundamental homeostatic cellular process that plays a critical role in maintaining functional integrity during normal or diseased state[8]. It is believed that increase in ROS can induce autophagy, presumably as an adaptive response to cellular stress, and in turn autophagy could lead to reduction of ROS to protect the kidney under diabetic conditions. In fact a recent study has shown this association in a way that exposure of podocytes to a high glucose challenge resulted in an increase in ROS generation and hence autophagy inductions within 24 hours. Interestingly, treatment with antioxidant acetylcysteine inhibited the high glucose-induced autophagy[9].

References:

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7. Ding Y, Choi ME. Autophagy in diabetic nephropathy. J Endocrinol. 2015 Jan;224(1):R15-30. doi: 10.1530/JOE-14-0437. [PMID: 25349246]
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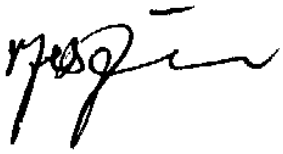
Thank you for considering our manuscript for the World Journal of Diabetes.

Regards,



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