Author's response to reviews

Title: Aqueous Cinnamon Extract (ACE-c) from the bark of Cinnamomum cassia causes apoptosis in human cervical cancer cell line (SiHa) through loss of Mitochondrial Membrane Potential

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Author's response to reviews: see over
To
The Editor,
BMC Cancer

Dear Sir,

We are thankful to the Editor as well as the Reviewers for their valuable comments towards our manuscript entitled “Aqueous Cinnamon Extract (ACE-c) from the bark of Cinnamomum cassia causes apoptosis in human cervical cancer cell line (SiHa) through loss of Mitochondrial Membrane Potential”. We have tried to satisfy the queries raised by the reviewers as well as incorporate the suggested revisions in the revised manuscript. The queries have been duly answered point-by-point and have been attached with the cover letter. We have made major changes in the style and language of the revised manuscript.

All the changes have been marked in red in the revised manuscript.

We are also providing line by line changes made in the revised manuscript with page and line numbers attached with the cover letter.

Thanking you once again.

Sincerely,

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Answer’s to Reviewer’s Comments

Reviewer 1: Amitava Chatterjee

Query: I have gone through the article very carefully and find the article entitled, ”Aqueous cinnamon extract (ACE-c) from the bark of Cinnamomum cassia causes apoptosis in human cervical cancer cell line (SiHa) through loss of Mitochondrial Membrane Potential” by Soumya J Koppikar et al. I found the article has some new informations and experiments are well done. Though authors may do better and more experiments but in its present form the manuscript may be acceptable after major revisions mainly in its style and language.

Answer: We appreciate the positive remarks of the reviewer towards our work and express our gratitude towards the same.

As suggested by the reviewer, we have revised the style and language of the manuscript.
**Reviewer 2**: Kiran S Panickar

**Major compulsory revisions:**

**Query1.** Mitochondrial calcium level was not measured in this study but the authors suggest that ACE-c increased calcium flux resulting into loss of mitochondrial membrane potential.... (page 13). While it is true that increased calcium is one of the inducers of the mitochondrial permeability transition (mPT), it is not the only one. Several of these polyphenol extracts also have pro-oxidant effects and it possible that the decline in the mitochondrial membrane potential was a result of the pro-oxidant effect of ACE-c. For instance by increasing nitric oxide (which also induces the mPT and thus a decline in mitochondrial membrane potential). The authors themselves have cited Ref #1 which shows a pro-oxidant effect of curcumin. Thus, there is no evidence that increased mitochondrial calcium was responsible for the decline in mitochondrial membrane potential although it is associated with it. This needs to be modified at several places (e.g. Abstract; Intro – page 4; Results page 13; etc). Alternatively, the authors could choose to measure mitochondrial calcium although this is not critical.

**Answer:** We are thankful to the reviewer for his important suggestions.

Since cinnamon increased intracellular calcium levels as well as induced apoptosis in cervical cancer cells, we wanted to know the status of mitochondrial membrane potential in these cells. Moreover, mitochondria are known to accumulate Ca\(^{2+}\) during apoptosis, especially, when the cytosolic calcium level is high [1–3]. Even though we didn’t measure mitochondrial calcium, but there was a decline in the membrane potential observed in ACE-c treated cells. Thus, the increased intracellular calcium induced by ACE-c might be associated with the decline in mitochondrial membrane potential resulting into cellular apoptosis.

As suggested by the reviewer, we have edited the manuscript accordingly.

**References:**

Query 2. Cinnamon polyphenols were reported to attenuate the decline in mitochondrial membrane potential in C6 glioma induced by ischemic injury (Panickar et al., 2009; Experimental Neurology). In general, it seems many polyphenols either depolarize the mitochondrial membrane potential or prevents such depolarization. While not critical, it would be good if a small paragraph was added in the discussion that elucidates these contrasting effects seen with ACE-c or with polyphenols.

Answer: As rightly stated by the reviewer, polyphenols seem to play a dual role in regulating the mitochondrial membrane potential.

Natural products seem to work in a tightly regulated manner wherein they switch their roles either towards protective or therapeutic side depending upon either the amount of the drug being used or upon the cellular phenotype [1]. For example, resveratrol, another polyphenol, has also been shown to play a dual role, either protective [2-4] or therapeutic [5]. Similarly, in our case we find that cinnamon extract, which contains a mixture of polyphenols together with cinnamaldehyde as the major bioactive component, plays a therapeutic role in cervical cancer cells through depolarization of the mitochondrial membrane potential resulting into cellular apoptosis. On the other hand, cinnamon polyphenols have been recently shown by the reviewer to play a protective role by attenuating the decline in mitochondrial membrane potential induced by ischemic injury in C6 glioma cells.

As suggested by the reviewer, we have incorporated a small paragraph in the discussion part elucidating the contrasting effects of plant polyphenols.

References:
Query 3. References 1-5 do not seem very appropriate for what the authors are trying to convey. Please cite appropriate references. As an example, the first sentence of the abstract of Ref #1 (Javvadi et al., 2008) is cited in this text but that is not the correct reference for what the authors what to convey in this manuscript.

Answer: We thank the reviewer for the corrections. We have amended the references accordingly and have incorporated some more references in the revised manuscript.

Minor essential revisions:

Query 4. There are several instances in the manuscript where the authors state “we have reported for the first time”. While these are interesting findings, such statements have no scientific merit and should be used minimally.

Answer: We agree with the reviewer and accordingly have modified our statements.

Query 5. What is the concentration of JC-1 as well as Fluo3 used in this study?

Answer: The concentrations of JC-1 as well as Fluo3 used in this study are 2.5 µg/ml and 5 µM, respectively that have been already mentioned in the manuscript.

Query 6. Page 8 – In the immunoblotting procedure was the protease cocktail tablet used in addition to the aprotinin, leupeptin, pepstatin, etc??

Answer: The protease inhibitor cocktail (Roche) is always used in all our western blotting assays besides addition of other components. This has already been mentioned in the manuscript.

Query 7. In cell culture studies using the SiHa cell line, the authors should mention the passages used for the current studies. Several cell lines are known to behave differently when it exceeds certain passages. To compare results in the future, perhaps the authors can give some more details.

Answer: The passage number for SiHa cell line used in the current study falls between 20-25.
Query 8. Repetition of “#” on page 10 (Statistical analysis).

Answer: The symbol “#” comes on page no. 3 and 8 with Annexin V-FITC apoptosis kit #3, Invitrogen that is labeled on the kit.

Query 9. This is just a suggestion but wouldn’t the flow of this manuscript be better if effects of cinnamon treatment on Her-2 expression in the Results section was moved immediately after the paragraph describing cinnamon’s effects on MMP-2 expression in the Results section?

Answer: We fully agree with the reviewer and thank him for the same. We have accordingly moved the Her-2 result after the MMP-2 result in Materials and Methods, Results and Discussion sections as well as in the Figure part of the manuscript.

Query 10. Photomicrographs need scale bars.

Answer: We are thankful for the suggestion and have put the scale bars in wound healing and confocal photomicrographs.
Reviewer 3: Shozo Nishida

We thank the reviewer for his critical remarks and we have tried to answer the queries to the best of our ability.

Major compulsory Revisions

Query 1. The authors should use individual drugs for each experiment. It is unclear whether purified single drugs in the ACE-c inhibit cell growth and cause apoptosis.

Answer. It is an interesting approach to fractionate the aqueous extract and then carry out experiments with each fraction and find out the best fraction giving the best anticancer activity.

But recently, we have shown that compared to cinnamaldehyde, which is the major bioactive component of cinnamon, the whole aqueous cinnamon extract (containing polyphenols besides cinnamaldehyde.) exhibit more potent cytotoxic activity in various cancer cell lines, including SiHa (Singh et al., 2009). Thus, the main purpose of our study was to test the effect of the whole aqueous extract of cinnamon rather than the individual components on cervical cancer cell line since all the components work synergistically to enhance the antitumor activity, rather than the individual components.

Reference:

Query 2. Did the authors confirm that ACE-c and purified single drugs inhibit tumor growth in vivo? The authors must produce evidence.

Answer: As mentioned already in the manuscript, the anti-tumor activity of cinnamon extract in melanoma has been recently reported in vivo by Kwon HK et al., 2009.

From our recent report (Singh et al., 2009), we had found that the effect of ACE on cervical cancer cells was quite significant and thus we proceeded to elucidate the underlying molecular mechanism in vitro. Moreover, our experiments with the effect of ACE-c in cervical cancer cell line induced tumor growth in mice are underway with interesting results for another manuscript.
Reference:

Query 3. The authors demonstrated that ACE-c inhibits MMP-2 expression. It is unclear that ACE-c inhibit other MMPs expression. The authors should confirm that ACE-c inhibits other MMPs expression, such as MMP-1, MMP-9, and MT1-MMP.

Answer: As rightly suggested by the reviewer, we already have results showing that besides MMP-2, ACE-c inhibits MMP-1 and MMP-3 as well at mRNA level (unpublished data). If required for reference, we can send the data but since it is unpublished we don’t want to put it in public domain as yet.

Query 4. Although the paper describes the inhibition of HER-2 expression by ACE-c, it is important to show that another method of HER-2 expression is capable of suppressing MMP-2 such as by using siRNA to achieve knockdown.

Answer:

There are already reports in the literature showing that Her-2 overexpression results into enhanced signaling pathways that may lead to increased production of gelatinases such as MMP2 and MMP9 (1,2). Thus, at this juncture by using siRNAs to down regulate Her-2 expression and showing downregulation of MMP-2 doesn’t seem to be required.

Our results show that ACE-c could inhibit Her-2 expression that may be responsible for the observed reduction of MMP-2 expression, which goes well with the reported observation showing relationship between Her-2 overexpression and MMP-2 downregulation.

References:
Query 5. The authors demonstrated that ACE-c increased intercellular calcium. However, it is unclear the mechanism of ACE-c-induced up-regulation of intercellular calcium. The authors should confirm the mechanism of ACE-c-induced intercellular calcium.

Answer: Infact, it is very interesting to study how ACE-c increases intracellular calcium but it would form quite an independent study involving ER calcium pumps, ryanodine receptors (RyRs), the G protein-coupled inositol phosphate 3 (IP3) signaling pathway, ionophoric or metabotropic glutamate receptors, and/or voltage-gated calcium channels, etc. Since this doesn't fall under the current purview of the paper, it is a good suggestion raised by the reviewer for future studies.
Changes made in BMC Cancer paper
The page and line numbers are with respect to the revised manuscript that we have uploaded.

Page 1, Line 1:
Title: Aqueous cinnamon extract (ACE-c) from the bark of *Cinnamomum cassia* causes apoptosis in human cervical cancer cell line (SiHa) through loss of Mitochondrial Membrane Potential (*Times New Roman 14*)

**Changed to:**
Aqueous Cinnamon Extract (ACE-c) from the bark of *Cinnamomum cassia* causes apoptosis in human cervical cancer cell line (SiHa) through loss of Mitochondrial Membrane Potential (*Arial 16*)

Page 2:
Abstract: (*Times New Roman 16*) changed to Abstract (*Arial 16*)

**Background:** (*Times New Roman 12*) changed to **Background:** (*Arial 11*)

Line 6: In the present study, we have for the first time analyzed the mechanism of anti-neoplastic activity of cinnamon on cervical cancer cell line SiHa.

**Changed to:**
In the present study, *we have reported* the anti-neoplastic activity of cinnamon *in* cervical cancer cell line, SiHa.

Methods: (*Times New Roman 12*) changed to **Methods:** (*Arial 11*)

Line 10: The aqueous extract of cinnamon (ACE-c) was analyzed for cinnamaldehyde…

**Changed to:**
The *aqueous cinnamon extract* (ACE-c) was analyzed for *its* cinnamaldehyde…

Line 11: HPTLC analysis and the polyphenol content was measured by…

**Changed to:**
HPTLC analysis. *The polyphenol content of ACE-c was measured by*…
**Line 12:** We studied the effect of cinnamon on growth kinetics by performing growth curve, colony formation and soft agar assay. The samples exposed to ACE-c were analyzed. …

**Changed to:**
We studied the effect of cinnamon on growth kinetics by performing growth curve, colony formation and soft agar assays. The cells treated with ACE-c were analyzed. …

**Line 15:** mRNA level by RT-PCR and at protein level by zymography.

**Changed to:**
mRNA and protein level by RT-PCR and zymography, respectively.

**Line 16:**
Her-2 protein expression was also checked in the control and treated samples by western blotting as well as confocal microscopy. Apoptosis studies and calcium signaling assays were analyzed by FACS analysis. Loss of mitochondrial membrane potential ($\Delta\psi_m$) in cinnamon treated cells was studied by JC-1 staining and analyzed by confocal microscopy as well as FACS analysis.

**Changed to:**
Her-2 protein expression was analyzed in the control and ACE-c treated samples by immunoblotting as well as confocal microscopy. Apoptosis studies and calcium signaling assays were analyzed by FACS. Loss of mitochondrial membrane potential ($\Delta\psi_m$) in cinnamon treated cells was studied by JC-1 staining and analyzed by confocal microscopy as well as FACS.

**Results:** (Times New Roman 12) changed to **Results:** (Arial 11)

**Line 25:** to down regulation of MMP-2 expression.

**Changed to:**
to downregulation of MMP-2 expression.

**Line 27:** intracellular calcium signaling resulting into loss of mitochondrial membrane potential.

**Changed to:**
intracellular calcium signaling as well as loss of mitochondrial.

**Conclusion:** (Times New Roman 12) changed to **Conclusion:** (Arial 11)

**Line 25:** drug in cervical cancer cells.

**Changed to:**
drug in cervical cancer.

**Page 3:**
**Background:** (Times New Roman 16) changed to **Background** (Arial 16)

**Line 2:** Cervical cancer accounts for the second most….
**Changed to:**
Cervical cancer, which accounts…..

**Line 3:** worldwide and is highly radio resistant,
**Changed to:**
worldwide, is highly radio-resistant, often resulting in local treatment failure [1].

**Line 5:** Complementary and alternative medicine (CAM)…
**Changed to:**
Complementary and Alternative Medicine (CAM)….  

**Line 6:** in cancer therapy [2]. Currently, the plants, vegetables,…..
**Changed to:**
in cancer therapy [2,3]. Currently, plants, vegetables,……

**Line 7:** chemopreventive drugs [3]
**Changed to:**
chemopreventive drugs [4-8].

**Line 9:** drug candidates [5].
**Changed to:**
drug candidates [9-13].

**Line 15:** anti-inflammatory [6], anti-oxidant [7,8], anti-microbial [7,9],…
**Changed to:**
anti-inflammatory [14], anti-oxidant [15,16], anti-microbial [15,17],…..

**Line 16:** anti-diabetic effects [10–12].
**Changed to:**
anti-diabetic effects [18–20].

**Line 17:** shown both in vitro [13-15] and in vivo [5].
**Changed to:**
shown both in vitro [21-23] and in vivo [10].

**Line 19:** and lung tumor cells [16]. Recently, we reported for the first time a comparative analysis….
**Changed to:**
lung tumor cells [24]. Recently, we reported a comparative analysis…

**Line 21:** commercial cinnamaldehyde on a variety of cell lines [15].
**Changed to:**
commercial cinnamaldehyde on a variety of cell lines [23].

**Line 22:** ACE proved to be more cytotoxic compared to the commercial cinnamaldehyde owing to the presence of polyphenolic compounds, besides cinnamaldehyde, that may synergistically act to induce enhanced cytotoxicity.
Compared to the commercial cinnamaldehyde, ACE proved to be more cytotoxic owing to the presence of polyphenolic compounds, besides cinnamaldehyde, that may act synergistically to induce enhanced cytotoxicity.

In the present work, we have reported for the first time the effects as well as putative mechanism of cancer cell growth inhibition by cinnamon aqueous extract, from the bark of *Cinnamomum cassia* (ACE-c) L. family Lauraceae, in a human cervical cancer cell line SiHa.

In the present work, we have reported the putative mechanism of cancer cell growth inhibition by aqueous cinnamon extract (ACE-c), from the bark of *Cinnamomum cassia* L. family Lauraceae, in a human cervical cancer cell line, SiHa.

Cinnamon extract altered the growth kinetics,

…..with (ACE-c) was less compared …..

Cinnamon extract increased the intracellular calcium that might be responsible for the loss of mitochondrial membrane potential ($\Delta\psi_m$), finally leading to cellular apoptosis.

Methods: (Times New Roman 16) changed to Methods (Arial 16)

Tissue culture plasticware was….

Tissue culture plasticware was….

Qualigens fine chemical (Mumbai, India).
**Changed to:**
Qualigens fine chemicals (Mumbai, India).

**Line 21:** …Maharashtra, India. Voucher specimen…..
**Changed to:**
……Maharashtra, India with voucher specimen…..

**Line 22:** Sample was authenticated…
**Changed to:**
The sample was authenticated…

**Line 24:** …. (ratio of cinnamon: water used was 1:16) [17] in a hot water extractor.
**Changed to:**
…. (the ratio of cinnamon: water used was 1:16) in a hot water extractor [25].

**Line 29:** Further, the total polyphenolic content of ACE-c was measured by Folin-Ciocalteau method as described previously [15,18].

**Changed to:**
The total polyphenol content of ACE-c was measured by Folin-Ciocalteau method as described previously [23,26].

**Page 5:**

**Cell culture:**
**Line 2:** The human cervix carcinoma (SiHa) cell line used….

**Changed to:**
The human cervical carcinoma cell line, SiHa, used….

**Line 4:** 2 mM L-glutamine and supplemented with 10% fetal bovine serum…..

**Changed to:**
2 mM L-glutamine supplemented with 10% fetal bovine serum…..

**Line 9:** The antiproliferative activity was determined by MTT dye uptake as described previously [15].

**Changed to:**
The cell viability was determined by MTT dye uptake as described previously [23].

**Line 10:** SiHa cells were seeded at 1 x 10^5/ml density in 96-well plate.

**Changed to:**
SiHa cells were seeded at a density of 1 x 10^5 cells/ml density in 96-well plates.

**Line 12:** ….following concentrations: 10, 20, 40, 80, 160 and 320µg/ml, in each well in triplicates.

**Changed to:**
following concentrations: 10, 20, 40, 80, 160 and 320 µg/ml, in wells in triplicates.

Line 17: optical density (OD) with the ELISA microplate reader.

**Changed to:**
..... density (OD) using the ELISA microplate reader...

**Line 22:** SiHa cells were seeded at a density of $1 \times 10^5$/ml in 24-well plates.

**Changed to:**
SiHa cells were seeded at a density of $1 \times 10^5$ cells/ml in 24-well plates.....

**Line 25:** viability with trypan blue dye exclusion

**Changed to:**
.....viability using trypan blue dye exclusion method.

**Line 29:** various ACE-c concentrations of 0, 10, 20, 40, and 80µg/ml.

**Changed to:**
.....various concentrations of ACE-c: 0, 10, 20, 40, and 80 µg/ml.

**Page 6:**

**Line 1:** with 0.5% crystal violet [19].

**Changed to:**
.....with 0.5% crystal violet [27].

**Line 10:** microscope (Carl Zeiss, Germany) and counted [19].

**Changed to:**
......microscope (Carl Zeiss, Germany) and counted [27].

**Line 13:** Cells were plated at a seeding density of $4 \times 10^5$/ml in 24-well plates..

**Changed to:**
Cells were plated at a seeding density of $4 \times 10^5$ cells/ml in 24-well plates......

**Line 14:** overnight at 37°C in 5% CO₂ incubator.

**Changed to:**
.....overnight at 37°C in 5% CO₂ incubator.

**Line 18:** humidified chamber at 37°C and 5% CO₂ atmosphere.

**Changed to:**
.....humidified chamber at 37°C and 5% CO₂ atmosphere.

**Line 25:** Total cellular RNA from control as well as cells treated with different doses of
ACE-c (10-80µg/ml) was extracted by a 1-step acid guanidine isothiocyanate-phenol
method using TRI reagent (Sigma, St. Louis, MO), precipitated with isopropanol and estimated by spectrophotometry. 10 µg total RNA was used for each RT-PCR reaction. 50 Units of ….

**Changed to:**
The total cellular RNA from control as well as cells treated with different doses of ACE-c (0-80 µg/ml) was extracted by a one-step acid guanidine isothiocyanate-phenol method using TRI reagent (Sigma, St. Louis, MO). RNA was precipitated with isopropanol and the concentration was estimated by spectrophotometer (Biorad, SmartSpec™ 3000). Ten microgram of total RNA was used for each RT-PCR reaction. Fifty units of…..

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**Page 7**

**Line 2:** ….1 h at 42°C followed by incubation at 95°C for 5 min.

**Changed to:**
…1 h at 42°C followed by incubation at 95°C for 5 min.

**Line 16:** The Gelatin zymography was performed to detect the extracellular MMP-2 [20].

**Changed to:**
The Gelatin zymography was performed to detect the presence of extracellular MMP-2 [28].

**Line 18:** The control and treated samples containing an equal amount of total proteins were mixed with sample buffer….

**Changed to:**
Both the control as well as the treated samples containing equal amount of total proteins were mixed with sample buffer….

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**Page 8:**

**Line 2:** Cell extracts were prepared from control and cells dosed with different concentrations of ACE-c.

**Changed to:**
Cell extracts were prepared from control as well as cells treated with different concentrations of ACE-c: (0-80 µg/ml).

**Line 16:** Proteins were visualized with a chemiluminescence kit (Amersham ECL Advance western blotting detection kit, GE Healthcare, UK) and densitometric analysis was performed on Alpha Imager using Alpha Ease FC software, Alpha Innotech.

**Changed to:**
Proteins were visualized using a chemiluminescence kit (Amersham ECL Advance western blotting detection kit, GE Healthcare, UK) and densitometric analysis of X-ray films was performed on Alpha Imager using Alpha Ease FC software, Alpha Innotech.

**Line 21-26:**

*Measurement of Apoptosis*

The cells were plated at a seeding density of 5 x 10^5 per well and treated with different concentrations of ACE-c. After 48 h, the cells were harvested by trypsinization and washed with PBS twice. Cells were stained with Annexin V-FITC (for early apoptosis) and propidium iodide (PI) following the manufacturer’s instructions (Annexin V-FITC apoptosis kit #3, Invitrogen) and analyzed by FACS using CellQuest Software.

**Changed to:**

*Measurement of Apoptosis*

The cells were plated at a seeding density of 5 x 10^5 cells/well and treated with different concentrations of ACE-c: (0-80 µg/ml). After 24 h of treatment, the cells were harvested and washed with PBS twice. Cells were stained with Annexin V-FITC following the manufacturer’s instructions (Annexin V-FITC apoptosis kit #3, Invitrogen) and analyzed for apoptosis by FACS using CellQuest Software.

**Line 28-31:**

*Intracellular calcium measurement by flow cytometry*
Intracellular Ca$^{2+}$ levels were analyzed in control cells as well as cells treated with different doses of ACE-c by flow cytometry [21]. Cells were loaded with 5 μM Fluo-3/AM (Sigma, St. Louis, MO) and 100μg/ml of Pluronic F127(Sigma, St. Louis, MO) in…

**Changed to:**

**Intracellular calcium measurement by flow cytometry**

Intracellular Ca$^{2+}$ levels were analyzed in control cells as well as cells treated with different doses of ACE-c: (0-80 μg/ml) by flow cytometry [29]. Cells were loaded with 5 μM Fluo-3/AM (Sigma, St. Louis, MO) and 100 μg/ml of Pluronic F127 (Sigma, St…..

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**Page 9**

**Line 1-4:** The cells were resuspended approximately every 20 min to ensure even dye loading. The cell pellet were washed twice with 0.9% saline, finally resuspended in 3ml Hank’s Balance salt solution (HBSS) in FACS tubes.

**Changed to**

The cells were resuspended after every 20 min to ensure even dye loading. The cell pellets were washed twice with 0.9% saline and finally resuspended in 3 ml Hank’s Balanced Salt Solution (HBSS) in FACS tubes. Ionomycin (30 μM) was used as a positive control.

**Line 10-26:**

Mitochondrial membrane potential was estimated using the fluorescent dye JC-1 either by confocal microscopy or by flow cytometry. For confocal studies, control as well as cells (1 x 10$^5$) treated with different concentrations of ACE-c (10-80 μg/ml) were seeded in 6-well plates. After 48 h, cells were incubated with culture medium containing JC-1 dye for 30 min at 37°C in the dark. Cells were washed with PBS twice and fixed with 2.5% paraformaldehyde made in 200 mM HEPES buffer for 15 min at room temperature followed by PBS wash. Slides were then mounted in antifade mounting medium
(Ultracruz mounting medium, Santacruz) and analyzed with a Zeiss LSM510 META confocal laser scanning microscope (Zeiss, Thornwood, NY) using LSM Image Examine software. For detection by flow cytometry [22], control as well as cells (5 x 10^5) treated with different concentrations of ACE-c (10-80 µg/ml) were harvested by trypsinization and washed with PBS. The cells were incubated with culture medium containing JC-1 for 30 min at 37°C in the dark. Cells were washed in PBS twice and analyzed for Δψm.

**Changed to**

Mitochondrial membrane potential was estimated using 2.5 µg/ml JC-1 fluorescent dye either by confocal microscopy or by flow cytometry. For confocal studies, cells were seeded at a density of 1 x 10^5 cells/ml on coverslips in 6-well plates. After 24 h, cells were treated with different concentrations of ACE-c: (0-80 µg/ml). Next day, the media was removed and the cells were incubated with fresh culture medium containing JC-1 dye for 30 min at 37°C in the dark. Cells on coverslips were washed with PBS twice and fixed with 2.5% paraformaldehyde made in 200 mM HEPES buffer for 15 min at room temperature followed by PBS wash. The coverslips were mounted in antifade mounting medium containing DAPI (Ultracruz mounting medium, Santacruz) on glass slides. The cells were then analyzed for JC-1 uptake by using Zeiss LSM510 META confocal laser scanning microscope (Zeiss, Thornwood, NY) having LSM Image Examine software. For Δψm detection by flow cytometry [30], 5 x 10^5 cells were plated in 6-well plates. The cells were treated with different concentrations of ACE-c (0-80 µg/ml). Twenty four hours post treatment; the cells were harvested, washed with PBS and incubated with culture medium containing JC-1 for 30 min at 37°C in the dark. Cells were washed in PBS twice and analyzed for Δψm using FACS. FCCP (10 µM) was used as a positive control.

**Line 30-31:**
For immunostaining, 2 x 10^5 cells/ml SiHa cells were plated on coverslips in 6-well plates.

**Changed to**
For immunostaining SiHa cells were plated on coverslips in 6-well plates at a seeding density of 2 x 10^5 cells/ml.

Page 10

Line 1: After 24 h, the cells were dosed with different concentrations of ACE-c (0-80µg/ml). Cells were washed with PBS after 48 h and fixed with 2.5% paraformaldehyde….

Changed to

Twenty four hours post-treatment; the cells were washed with PBS and fixed in 2.5% paraformaldehyde….

Line 4: ….blocked in 10% FBS in PBS for 1 h.

Changed to

….blocked in 10% FBS (made in PBS) for 1 h.

Line 5: …..cells were incubated with Her-2 antibody….

Changed to

…..the cells were incubated with Her-2 antibody…

Line 9: …..analyzed with a Zeiss LSM510 META confocal laser scanning microscope…

Changed to

….LSM 510 META confocal laser scanning microscope….

Statistical analysis

Line 16: …using 1-way ANOVA.

Changed to:

…..using one-way ANOVA.

Results (Times New Roman 16) changed to Results (Arial 16)

Cinnamon treatment alters growth kinetics of SiHa cells (Times New Roman 12) changed to Arial 11
Aqueous extract of Cinnamon (ACE-c) prepared from *C. cassia*.

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Page 11

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All these data indicate that cinnamon altered the growth kinetics of SiHa cells.

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The latter filled-up the wound gap completely after 15 h whereas in cells treated with ACE-c, particularly at 40 and 80 µg/ml concentrations, the wound gap was not completely filled. Interestingly, at 80 µg/ml, cinnamon treatment significantly decreased
(~1.5-fold; \(p \leq 0.001\)) the migration rate of SiHa cells thereby affecting their migration capability.

Page 12

**Line 1:**
Since MMP-2 is known to play a significant role in the invasive property of tumor cells, we investigated the mechanism behind delay in wound healing by ACE-c by testing the expression of MMP-2 in cells treated with/without cinnamon extract. It was observed that the expression of MMP-2 was significantly down-regulated both at mRNA (Fig. 2C) as well as protein level (Fig. 2D) in a dose-dependent manner compared to the untreated control cells. Interestingly, at 80\(\mu\)g/ml concentration of ACE-c, there was a ~1.6 fold and ~4 fold \((p \leq 0.001)\) down regulation in the expression of MMP-2 in transcript and translation levels, respectively. These data suggest that through down-regulation of MMP-2 expression, ACE-c could induce decrease in the migration of cervical cancer cells.

**Changed to:**
Since MMP-2 is known to play a significant role in the invasive property of tumor cells, we investigated the mechanism behind the delay in wound healing exhibited by ACE-c treated cells. We tested the expression of MMP-2 in cells treated with/without cinnamon extract. It was observed that the expression of MMP-2 was significantly down-regulated both at mRNA (Fig. 2C) as well as protein level (Fig. 2D) in a dose-dependent manner compared to the untreated control cells. Interestingly, at 80 \(\mu\)g/ml concentration of ACE-c, there was a ~1.6-fold \((p \leq 0.001)\) decrease in MMP-2 transcript and ~4-fold \((p \leq 0.001)\) down regulation in the expression of MMP-2 protein. These data suggested that ACE-c induced decrease in the migration of cervical cancer cells through down-regulation of MMP-2 expression.
The Her-2 result that was at the end of Result section has been taken below MMP-2 result and has been

**Changed to:**

*Cinnamon treatment downregulates the expression of Her-2 oncoprotein*  
(Arial 11)

Various studies have shown that a variable proportion of cervical carcinoma tumors overexpress Her-2 oncoprotein [31]. Moreover, there are reports suggesting a correlation between Her-2 overexpression and upregulation of MMP-2 and MMP-9 expression [32,33]. Since we found that cinnamon downregulated the expression of MMP-2, we wanted to examine the status of Her-2 in cinnamon-treated cells and correlate its expression with that of MMP-2. Thus SiHa cells were treated with different concentrations of ACE-c (0-80 µg/ml) followed by immunoblotting of the extracted proteins using Her-2 antibody. Interestingly, we observed that the cinnamon extract down regulated the expression of Her-2 protein in a dose-dependent manner compared to the control cells (Fig. 3A and B), the maximum reduction being at 80 µg/ml (~2.6-fold, p≤0.001). This was further proved by confocal studies wherein at 80 µg/ml of ACE-c treatment, a significant reduction in the expression of Her-2 could be observed (Fig. 3C). These results correlated with decreased MMP-2 expression observed in cinnamon-treated cells, thereby elucidating the potential antineoplastic role of cinnamon in cervical cancer through reduction of Her-2 and MMP-2 expression.

Page 13

**Line 1:** *Cinnamon extract induces apoptosis through increase in intracellular calcium resulting into loss of mitochondrial membrane potential*

**Changed to:**
Cinnamon extract induces apoptosis through increase in intracellular calcium as well as loss of mitochondrial membrane potential (Arial 11)

**Line 6:** .... by flow cytometric analysis (Fig. 3A). It was found that at 80 µg/ml ACE-c concentration, there was ~2.6-fold ($p \leq 0.001$) increase in the population of cells undergoing apoptosis compared to the untreated control cells. Doses below 80 µg/ml concentration could not induce significant apoptosis in cells.

**Changed to:**
.....by flow cytometric analysis (Fig. 4A). It was observed that at concentrations of 40 and 80 µg/ml ACE-c, there was a significant increase in the percentage of cells undergoing apoptosis. Interestingly at 80 µg/ml ACE-c concentration, there was ~2.6-fold ($p \leq 0.001$) increase in the population of cells undergoing apoptosis compared to the untreated control cells.

**Line 12:** Since intracellular Ca$^{2+}$ is a powerful activator of apoptosis, we studied the Ca$^{2+}$ signaling mechanism in cells treated with ACE-c to elucidate the cause of apoptosis [23,24].

**Changed to:**
Since intracellular Ca$^{2+}$ is a powerful activator of apoptosis [34,35], we studied the Ca$^{2+}$ signaling mechanism in cells treated with ACE-c to elucidate the cause of apoptosis.

**Line 14:** ......observed that after treatment of SiHa cells with various concentrations of ACE-c (0-80µg/ml), there was a dose-dependent increase in the intracellular levels of calcium. It was noted that the calcium increase was maximal (~2.64; $p \leq 0.001$) at the concentration of 80µg/ml (Fig. 3B) compared to the control cells. Ionomycin (30µM) was used as a positive control in the experiment.

**Changed to:**
.....was observed that after treatment of SiHa cells with various concentrations of ACE-c (0-80 µg/ml), there was a dose-dependent increase in the intracellular levels of calcium. It was noted that the calcium increase was maximal (~2.64-fold; $p \leq 0.001$) at the concentration of 80 µg/ml (Fig. 4B) compared to the control cells.
**Line 19-31:** It is well known that increase in mitochondrial levels of calcium Ca$^{2+}$ induces apoptosis through the loss of Δψ$_m$ [25]. To analyse whether the increased intracellular Ca$^{2+}$ induced by ACE-c treatment resulted into mitochondrial dysfunction, we stained the cells with Δψ$_m$ indicator, JC-1. By both confocal as well as flow cytometry assays, we observed that the cells exposed to ACE-c exhibited a dose-dependent decrease in JC-1 staining (Fig. 3C and 3D, respectively). This indicates loss of mitochondrial membrane potential after treatment with cinnamon, which approaches the loss of potential observed after treatment with the positive control agent, FCCP (10µM) (Fig. 3D). As clearly observed from the figure, cinnamon induces significant depolarization at 80µg/ml ACE-c concentration wherein there was ~5-fold reduction in the ratio of red-green fluorescence intensity (p≤0.001). Taken together, all these results suggest that cinnamon extract exhibited……

**Changed to:**

It is known that increase in intracellular calcium might be one of the factors responsible for disrupting the mitochondrial membrane potential resulting finally into cell apoptosis [36-39]. To measure the collapse of electrochemical gradient across the mitochondrial membrane, we stained the cells with JC-1 dye that aggregates into healthy mitochondria and fluoresces red. By confocal as well as flow cytometry assays, we observed that the cells exposed to ACE-c exhibited a dose-dependent decrease in JC-1 staining (Fig. 4C and 4D, respectively) compared to the untreated control cells. This indicated that there was a loss of mitochondrial membrane potential in cinnamon-treated cells, which approached the loss of potential observed after treating the cells with the positive control agent, FCCP (Fig. 4D). As clearly observed from the figure, cinnamon induced significant depolarization at 80 µg/ml ACE-c concentration wherein there was ~5-fold reduction in the ratio of red-green fluorescence intensity (p≤0.001). Taken together, all these results suggested that cinnamon extract exhibited……
Line 1: …cervical cancer cells through increase in calcium flux resulting into loss of mitochondrial membrane potential,

Changed to:
…cervical cancer cells through increase in intracellular calcium flux as well as through loss of mitochondrial membrane potential,

Line 5: Discussion (Times New Roman 16) changed to Discussion (Arial 16)

Line 6: Dietary constituents may display promising chemopreventive and chemotherapeutic anti-cancer potential and thus ameliorate…

Changed to:
Dietary constituents may display promising chemopreventive and chemotherapeutic potential and thus ameliorate ….

Line 9: …..treatment of cancer patients [27].

Changed to:
…..treatment of cancer patients [2,3,9-13,40].

Line 12-21:
In the present study, we have reported the anti-cancer potential of cinnamon extract in vitro in human cervical cancer cell line and have elucidated the possible underlying mechanism. The anti-tumor activity of Cinnamon has been reported in vitro [13-15] as well as in vivo [5]; however, its role in cervical cancer remained to be elucidated. We found that the aqueous cinnamon extract affected the growth rate of SiHa cells in a dose-dependent manner with a significant reduction in growth kinetics. This data was further supported by results from colony formation and soft agar assays, which demonstrated statistically significant reduction in the number of colonies in ACE-c treated cells compared to the untreated control cells. Thus Cinnamon could be proposed as a suitable candidate that could be used for restricting the growth of cervical cancer cells.

Changed to:
In the present study, we have reported the anti-cancer potential of cinnamon extract in vitro in a human cervical cancer cell line and have elucidated the possible underlying mechanism. The anti-tumor activity of cinnamon has been reported in vitro [21-23] as well as in vivo [10]; however, its role in cervical cancer remained to be elucidated. We found that the aqueous cinnamon extract significantly affected the growth rate of SiHa cells in a dose-dependent manner. This data was further supported by results from colony formation and soft agar assays, which demonstrated statistically significant reduction in the number of colonies in ACE-c treated cells compared to the untreated control cells. Thus, cinnamon could be proposed to be a promising candidate for restricting the growth of cervical cancer cells.

Line 23-28:
It is well known that metastasis, being one of the major causes of mortality in cancer, involves various steps such as cancer cell adhesion, invasion, and migration [28]. Thus to know the effect of Cinnamon extract on migration of SiHa cells, wound healing assays were performed on untreated control and ACE-c treated cells. Interestingly, Cinnamon inhibited the migration of cancer cells in a highly significant manner (~1.5 fold), further strengthening its potential use as an anti-cancer drug in cervical cancer.

Changed to:
It is well known that metastasis, being one of the major causes of mortality in cancer, involves various steps such as cancer cell adhesion, invasion, and migration [41]. Thus, to examine the effect of cinnamon extract on migration of SiHa cells, wound healing assays were performed on untreated control and ACE-c treated cells. Interestingly, cinnamon reduced the migration of cancer cells in a significant manner, further strengthening its potential use as an anti-cancer drug in cervical cancer.

Page 15:
Line 1-9:
One of the key steps in the invasive progress of cancer cells is the degradation of extracellular matrix (ECM) proteins by a family of zinc-binding enzymes called as matrix metalloproteinases [29]. To elucidate the reason behind the poor migration of ACE-c treated cells, we tested the expression of MMP-2 (gelatinase) in control as well as cinnamon extract treated cells. A significant decrease in the expression of MMP-2 (gelatinase) was observed at both mRNA as well as protein levels in ACE-c treated cells that resulted into their reduced migration compared to the control cells. Thus, inhibition of MMP-2 expression by Cinnamon could be regarded as a rational approach towards metastatic disease therapy in cervical cancer.

**Changed to:**

One of the key steps in the invasive progress of cancer cells is the degradation of extracellular matrix (ECM) proteins by a family of zinc-binding enzymes called as matrix metalloproteinases [42]. To elucidate the reason behind the poor migration of ACE-c treated cells, we tested the expression of MMP-2 (gelatinase) in control as well as cinnamon-treated cells. A significant decrease in the expression of MMP-2 was observed both at mRNA as well as protein levels in ACE-c treated cells that could be the reason for their reduced migration capability compared to the control cells. Thus, downregulation of MMP-2 expression by cinnamon could be regarded as a rational approach towards metastatic disease therapy in cervical cancer.

**Line 11-20:** It is well-known that Her-2/Erb2, a transmembrane receptor protein with tyrosine kinase activity from EGF3-receptor family, is a critical marker of cervical and breast cancer. Moreover, Her-2 is known to be overexpressed in a number of tumors [26]. Interestingly, we found for the first time that cinnamon could effectively and significantly down-regulate the expression of Her-2 in SiHa cells. It has been shown that HER2 overexpression is related with the invasion capacity of the tumor cells that is related partly with the up-regulation of MMP-2 and MMP-9 expression as well as proteolytic activity [36]. Thus, downregulation of MMP2 expression by cinnamon could be linked with the reduction in the expression of Her-2 oncoprotein. These leads could be explored
in detail to further establish the antineoplastic activity of cinnamon in cervical cancer that would in turn emphasize the chemopreventive potential of natural products.

**Changed to:**

It is well-known that Her-2/Erb2, a transmembrane receptor protein with tyrosine kinase activity from EGF3-receptor family, is a critical marker of cervical and breast cancer. Moreover, it has been shown that Her-2 overexpression is related with the invasion capacity of the tumor cells that is related partly with the up-regulation of MMP-2 and MMP-9 expression as well as proteolytic activity [32,33]. Interestingly, we found that cinnamon could effectively and significantly down-regulate the expression of Her-2 in SiHa cells. Thus, downregulation of Her-2 oncoprotein expression by cinnamon could be correlated with the reduction in the expression of MMP-2 protein. These leads could be explored in detail to further establish the antineoplastic activity of cinnamon in cervical cancer that would in turn emphasize the chemopreventive potential of natural products.

**Line 22-29:** Apoptosis plays a key role in the regulation of normal tissue homeostasis and participates in the elimination of abnormal cells. Most of the antitumor drugs kill the cancer cells by stimulating the apoptotic pathway [30]. It is known that mitochondria play an important role in the regulation of apoptosis. To test whether Cinnamon could induce apoptosis in cervical cancer cell line SiHa, we carried out apoptosis studies in control as well as cells exposed to ACE-c. At an effective concentration of 80µg/ml, a significantly higher population of cells was observed to undergo apoptosis compared to the control cells. To further elucidate the mechanism of apoptosis, we tested whether there is any disruption of calcium signaling mechanism as it is known to be one of the main causes of apoptosis.

**Changed to:**

Apoptosis plays a key role in the regulation of normal tissue homeostasis and participates in the elimination of abnormal cells. Most of the antitumor drugs kill the cancer cells by stimulating the apoptotic pathway [43]. To test whether cinnamon could induce apoptosis in cervical cancer cell line SiHa, we carried out apoptosis studies. At an effective ACE-c
concentration of 80 µg/ml, a significant proportion of cells were observed to undergo apoptosis compared to the control cells. To further elucidate the mechanism of apoptosis, we tested whether cinnamon could modulate calcium flux as the latter is known to be one of the major mediators of apoptosis.

Page 16

**Line 2:** Cytosolic calcium, (Ca$^{2+}$)$_c$, is usually maintained at lower level (f100 nmol/L) compared to the extracellular concentration (f1 mmol/L).

*Changed to:*  
Cytosolic calcium, (Ca$^{2+}$)$_c$, is usually maintained at lower level (~100 nmol/L) compared to the extracellular concentration (~1 mmol/L).

**Line 5-6:** …the mitochondria [31].

*Changed to:*  
…..the mitochondria [42].

**Line 8:** ……(Ca$^{2+}$)$_c$ level is high [24].

*Changed to:*  
………(Ca$^{2+}$)$_c$ level is high [35, 37-39].

**Line 10-11:** …the outer mitochondrial membrane [24]. Interestingly, we found that at 80µg/ml concentration of cinnamon extract….

*Changed to:*  
…..of the outer mitochondrial membrane [36]. Interestingly, at 80 µg/ml concentration of cinnamon extract,….
Since cinnamon extract led to increase in calcium flux in cells, it was obvious that it would result into mitochondrial dysfunction. Thus, we tested the $\Delta \psi_m$ in cinnamon treated cells by using the fluorescent dye, JC-1 that aggregates into healthy mitochondria and fluoresces red. When the mitochondria collapses in apoptotic cells, the JC-1 no longer accumulates and instead, it is distributed throughout the cell resulting into decrease in red fluorescence. In accordance with this, we found that ACE-c indeed disrupted the mitochondrial membrane potential as observed by decrease in the red fluorescence. FCCP, a drug known to disrupt the transmembrane potential of mitochondria [33,34], was used as a positive control. Conclusively, all these data strongly implicate cinnamon as a potent antineoplastic agent in cervical cancer cells wherein it could induce apoptosis in cells through increase in calcium flux leading to loss of $\Delta \psi_m$ [35].

**Changed to:**

Since cinnamon increased intracellular calcium levels as well as induced apoptosis in cervical cancer cells, we wanted to know the status of mitochondrial membrane potential in these cells. Moreover, mitochondria are known to accumulate Ca$^{2+}$ during apoptosis especially when the cytosolic calcium level is high [37–39]. Thus, we tested the $\Delta \psi_m$ in cinnamon treated cells by using the fluorescent dye, JC-1 that aggregates into healthy mitochondria and fluoresces red. Upon mitochondrial collapse in apoptotic cells, JC-1 dye no longer accumulates and instead is distributed throughout the cell resulting into decrease in red fluorescence. In accordance with this, we found that ACE-c indeed disrupted the mitochondrial membrane potential as observed by decrease in the intensity of red fluorescence. FCCP, a drug known to disrupt the transmembrane potential of mitochondria [45], was used as a positive control. Thus, the increased intracellular calcium induced by ACE-c might be associated with the observed decline in mitochondrial membrane potential.
Dietary polyphenols have recently invited a great deal of attention owing to their chemopreventive properties [46-48]. They can modulate the process of carcinogenesis through several mechanisms. Interestingly, polyphenols seem to play dual roles (either protective or therapeutic) under different situations. For example, cinnamon polyphenols…….

Page 17

Line 1-14: ...have been recently shown to play a protective role by attenuating the decline in mitochondrial membrane potential induced by ischemic injury in C6 glioma cells [49]. On the other hand, in our case, we observe that cinnamon extract, which contains a mixture of polyphenols together with cinnamaldehyde as the major bioactive component, plays a therapeutic role in cervical cancer cells through depolarization of the mitochondrial membrane potential resulting into cellular apoptosis. These natural products seem to work in a tightly regulated manner wherein they switch their roles either towards protective or therapeutic side depending upon either the amount of the drug being used or upon the cellular phenotype [50]. For example, resveratrol, another polyphenol, has also been shown to play a dual role, either protective [51-53] or therapeutic [54]. Based on all these observations, our data strongly implicates that cinnamon could be proposed to be a potent antineoplastic agent in cervical cancer wherein it could induce apoptosis through increase in calcium flux as well as through loss of mitochondrial membrane potential.

Line 16: Conclusion (Times New Roman 16) changed to Conclusion (Arial 16)
Line 21: ......natural sources such as plants, marine organisms and microorganisms [37,38].

Changed to
...... natural sources such as plants, marine organisms and microorganisms [55,56].

Line 23-26: Several studies have been conducted on herbs that possess anticancer properties and have been used as potent anticancer drugs [39]. The antineoplastic potential of the spice cinnamon in cervical cancer has been addressed to for the first time in the present work.

Changed to
Several studies have been conducted on herbs that possess anticancer properties and have been used as potent anticancer drugs [57]. The present work has addressed the antineoplastic potential of the spice cinnamon in cervical cancer.

Line 29-30: Collectively, these data suggest that cinnamon extract could be proposed to be a potent anticancer drug candidate in cervical cancer cells.

Changed to
Collectively, these data suggest that cinnamon extract could be proposed to be a potent anticancer drug candidate in cervical cancer.

Page 18

List of abbreviations used (Times New Roman 16) Changed to
List of abbreviations used (Arial 16)

Line 2-4: ACE-c: Aqueous cinnamon extract from C. cassia; CAM: Complementary and Alternative medicine; Δψ\textsubscript{m}: Mitochondrial membrane potential; MMP-2: Matrix metalloproteinase-2; MTT:4,5-dimethylthiazol-2-yl-2,5-diphenylthiazolium bromide....

changed to
ACE-c: Aqueous Cinnamon Extract from C. cassia; CAM: Complementary and Alternative Medicine; \( \Delta \psi_m \): Mitochondrial membrane potential; MMP-2: Matrix Metalloproteinase-2; MTT: 4,5-dimethylthiazol-2-yl-2,5-diphenylthiazolium bromide; 

**Acknowledgements** (Times New Roman 16) changed to

**Acknowledgements** (Arial 16)

**Line 24:** In this regard, we would like to thank Ms. Hemangini Shikhara,…..

**Changed to**
In this regard, we would like to thank Dr. Limaye, Ms. Hemangini Shikhara,…..

**Page 19**

**References** (Times New Roman 16) changed to

**References** (Arial 16)

**Following References:**


Cinnamomum zeylanicum bark with commercial cinnamaldehyde on various cell lines. *Phar Bio* 2009, Epub ahead of print.


**Have been changed to:**  
(with new references marked as red)


28. Rangaswami H, Bulbule A, Kundu GC: Nuclear factor-inducing kinase plays a crucial role in osteopontin-induced MAPK/IκBα kinase-dependent nuclear


Page 24:

**Line 9: Figure Legends (Times New Roman 16) Changed to**

**Figures (Arial 16)**

Figure 1 Cinnamon alters growth kinetics of cervical cancer cells. (Times New Roman 12)

Changed to

Figure 1 Cinnamon alters growth kinetics of cervical cancer cells. (Arial 11)

**Line 11: ….concentrations (0-80µg/mL) of ACE-c…. Changed to**

…..concentrations (0-80 µg/ml) of ACE-c…. 
**Line 14:** .....represent means ±SD of five....

**Changed to**

..... represent mean ±SD of five....

**Line 15:** .....concentrations (0-80µg/mL) of ACE-c....

**Changed to**

....concentrations (0-80 µg/ml) of ACE-c.....

**Line 18:** .....concentrations (0-80µg/mL) of ACE-c....

**Changed to**

....concentrations (0-80 µg/ml) of ACE-c.....

**Line 20:** .....data represent means ±SD of five....

**Changed to**

......data represent mean ±SD of five....

**Line 22:** Figure 2 Cinnamon inhibits migration potential. (Times New Roman 12)

**Changed to**

Figure 2 Cinnamon reduces migration potential. (Arial 11)

**Line 22:** (A) Time-Lapse image at the end of 15 h in a wound-healing assay in cells treated with different concentrations (0-80µg/mL) of ACE-c treatment.

**Changed to**

(A) Photomicrographs of time-lapse image at 0 and 15 h in a wound-healing assay in cells treated with different concentrations (0-80 µg/ml) of ACE-c.

**Page 25:**

**Line 1:** .....the distance travelled by the cells....

**Changed to**

.....the distance traveled by the cells.....
**Line 8:** (D) Gelatin zymography showing down regulation of MMP-2 expression in SiHa cells at 80µg/ml ACE-c treatment compared to the untreated control cells.

**Changed to**

(D) Gelatin zymography showing **downregulation** of MMP-2 expression in SiHa cells at 80 µg/ml ACE-c treatment compared to the untreated control cells.

**Line 13-22:** Figure 3 Cinnamon induces apoptosis in SiHa cells through dysregulation of mitochondrial membrane potential.

**Replaced by**

**Figure 3 Cinnamon decreased the expression of Her-2 oncoprotein** (Arial 11)

(A) Western blot analysis showing decrease in Her-2 expression in SiHa cells treated with different concentrations of ACE-c (0-80 µg/ml). Equal amounts of protein were loaded on 7.5% SDS-gel and immunoblotted with anti-Her-2 antibody. Tubulin was used as a loading control. Densitometric analysis of Her-2 expression was performed using phosphorimager. The data represents mean ±SD of five independent experiments. (B) Confocal images of the cells treated with indicated concentrations of ACE-c showing decrease in Her-2 expression. The cells were stained indirectly for Her-2 using Cy3 antibody (Panel II) and counterstained with DAPI (Panel I). Panel III represents the merge images.

**Page 26:**

**Line 1-20:** Figure 4 Cinnamon decreased the expression of Her-2 oncoprotein

**Replaced by**
Figure 4 Cinnamon induces apoptosis in SiHa cells through dysregulation of mitochondrial membrane potential. (Arial 11)

(A) SiHa cells were treated with different concentrations of ACE-c (0-80 µg/ml) followed by Annexin V-FITC and PI staining to analyze the effect of cinnamon in apoptosis. This was determined by FACS analysis showing the percentage of early (lower right quadrant) and late (upper right quadrant) apoptotic cells. (B) Flow cytometric analysis of the rapid calcium release in SiHa cells after treatment with cinnamon. Cells (5 x 10^3 cells) were treated with different doses (0-80 µg/ml) of ACE-c for 24 h. This was followed by loading the cells with Fluo-3/AM for 1 h before analyzing in calcium-free HBSS. Ionomycin was used as a positive control. Fluorescence intensities were measured with FACS Calibur flowcytometer. The data represents mean ±SD of five independent experiments. (C) Confocal images showing mitochondrial membrane depolarization induced by cinnamon. Control and cinnamon-treated SiHa cells were stained with JC-1 and the staining pattern was monitored by confocal laser scanning microscopy. For detection of J-aggregate form (red) (Panel II) and J-monomer alone (green) (Panel I), Argon-Krypton laser line was excited at 590 nm and 527 nm, respectively. Panel III represents the merge images. (D) Flow cytometric analysis with JC-1 dye showing decrease in red to green fluorescence ratio. Control (5 x 10^5) and cells treated with various concentrations (0-80 µg/ml) of ACE-c were stained with JC-1 dye for 30 min. Fluorescence intensities were measured with FACS Calibur flowcytometer. The data represents mean ±SD of five independent experiments.
Page 27

**Line 1:** Additional Files (Times New Roman 16)

*Changed to*

**Additional Files (Arial 16)**

**Line 4:** Title: Biochemical Analysis and cytotoxic activity of Aqueous Cinnamon Extract (ACE-c)

*Changed to*

Title: Biochemical Analysis and *Cytotoxic Activity* of Aqueous Cinnamon Extract (ACE-c)