EVALUATION OF TOCOLYTIC ACTIVITY OF AQUEOUS SEED EXTRACT OF SYZYGIUM CUMINI ON OXYTOCIN INDUCED PRETERM LABOR

RAMASAMY M, ANU SEBASTIAN*, ASHL Y MERIN GEORGE

Department of Pharmacology, P.S.V College of Pharmaceutical Science and Research, Krishnagiri, Tamil Nadu, India. 2Department of Pharmacology, Nirmala College of Pharmacy, Muvattupuzha, Kerala, India. 3Department of Pharmaceutics, Grace College of Pharmacy, Palakkad, Kerala, India. Email: anusbtn@gmail.com

Received: 11 January 2020, Revised and Accepted: 24 March 2020

ABSTRACT

Introduction: Syzygium cumini is a well-known bioactive plant which has been widely used for the treatment of various diseases in traditional and folk medicine.

Objective: The present investigation was aimed to determine the tocolytic activity of the aqueous seed extract of S. cumini (AESC) in animal models.

Methods: The in vitro antioxidant activity of AESC was evaluated by (1, 1-diphenyl-2- picrylhydrazyl [a,a-diphenyl-β-picrylhydrazyl] and (2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ABTS radical scavenging assay which showed the hydrogen donating and free radical scavenging activity of extract that aids in the prevention of preterm labor. In vivo tocolytic activity of AESC was evaluated. The level of in vivo antioxidant parameters such as catalase, superoxide dismutase (SOD) and glutathione was restored in the treated group compared to the control group. The AESC was subjected to pharmacological testing in vitro on a piece of isolated rat uterus previously pretreated with estradiol valerate, concentrations used were 80 mg/ml and 160 mg/ml.

Results: The study showed the promising radical scavenging activity of the extract due to hydrogen-donating ability of the formulation. In the current study, a significant increase in the rate of preterm delivery (PTD) of the control animals was observed when compared with the normal group. The AESC treated group has shown a significant reduction in the rate of PTD which was comparable with the standard treated group as well as the normal group. The study reveals that the extracts have been able to increase the endogenous antioxidant enzyme activities while reducing the lipid peroxidation. The concentrations (80 mg/ml) and (160 mg/ml) produce 50.9% and 70.9 % inhibition, respectively.

Conclusion: The results indicate the presence of active principles in the AESC which may be responsible for the tocolytic activity.

Keywords: Oxidative stress, Preterm labor, Oxytocin, Syzygium cumini.

INTRODUCTION

Plants have been of great importance to mankind due to their medicinal as well as nutritional properties [1]. Plants have been the source of a wide variety of current drugs which are available in the market today. Natural products, commonly termed as “secondary metabolites,” are an essential, reputable source of successful drug leads which originate from earth’s bio-diverse flora and fauna [2].

The incidence of preterm birth (PTB), or delivery at <37 weeks, increases to continue and accounts for 75% of all neonatal mortality and morbidity. Contractions that do not meet that rate of recurrence or contractions without cervical change several hours after uterine activity starts are called uterine irritability, irregular contractions, Braxton Hicks contractions, or false labor, but have no effect on the cervix are never called preterm labor. To delay or prevent preterm delivery (PTD), true uterine contractions should be detected adequately early (cervical dilatation <4 cm), to allow reasonable success of tocolytic drugs to result in meaningful pregnancy maintenance [3].

Mechanisms of tocolysis

Myometrial contractility is a complex procedure based on myocytes function. It involves the presence of hormonal receptors, ions channels, intercell gap junctions, and regulatory proteins such as oxytocin, endothelin, tachykinin, and angiotensin. The increase of intracellular calcium concentration is necessary for the uterine smooth muscle contraction.
mullerian ducts, commencing with the ostium of the oviduct. In the rat, this ostium forms a complete capsule called the ovarian bursa, which envelop the ovary. The oviducts are small, extremely coiled tubes. The uterus consists of two separated uterine horns, enabling the rat to have multiple offspring. The vagina of the rat opens directly to the exterior.

**Estrous cycle**

The rat estrous cycle is short, lasting 4–5 days. It occurs throughout the year, with no seasonal effect. The first regular estrous cycle occurs about 1 week after the opening of the vaginal orifice, usually 33–42 days after birth. The cycle length increases slightly with age and past about 6 days near the ending of the reproductive life span.

The estrous cycle in the rat consists of four stages known as proestrus, estrus, metestrus, and diestrus. Proestrus lasts approximately 12 h; estrus, 9–15 h; metestrus, 21 h; and diestrus (the longest phase), over 57 h Fig. 2.

**Oxidative stress**

Oxidative stress is an imbalance between free radical production and antioxidant defenses and is associated with damage to a wide range of molecular species including lipids, proteins, and nucleic acids. Short-term oxidative stress may occur in tissues injured by trauma, infection, heat injury, hypoxia, toxins, and excessive exercise. These injured tissues produce increased radical generating enzymes (e.g., xanthine oxidase, lipooxygenase, and cyclooxygenase) activation of phagocytes, release of iron, copper ion, or a disruption of the electron transport chains of oxidative phosphorylation producing excess reactive oxygen species (ROS) [7].

### Enzymatic antioxidant defenses

Antioxidant enzymes participate in a complex interaction of reducing and oxidizing molecules that defines the cellular milieu necessary for maintaining cellular, placental, fetal, and postnatal growth. Such enzymes have small activity in preterm infants and cannot balance excessive ROS production. The most significant antioxidant enzymes are copper-zinc superoxide dismutase (SOD), which are found in cytoplasm as well as peroxisomes, and manganese SOD from mitochondria. SOD catalyzes the dismutation of superoxide anion to H$_2$O$_2$. Glutathione peroxidase (GPx) in mitochondria and catalase (CAT) in peroxisomes catalyze the reaction of H$_2$O$_2$ to molecular oxygen and water. These enzymes, together with Vitamin E, play an important role in the peroxidation of polyunsaturated free fatty acids in cell membrane [8].

**Syzygium cumini** L. family Myrtaceae is a well-known common fruit in India. The seed is used in various alternative healing systems such as Ayurveda, Unani, and Chinese medicine for digestive ailments. The literature study done so far revealed that there is a lack of scientific data concerning the tocolytic evaluation of the *S. cumini* seed extract on animal models.

The aim of the current study is to assess the tocolytic effect of the aqueous seed extract of *S. cumini* (AESC) on oxytocin induced preterm labor.

The objectives of the present study include:

- Evaluation of *in vitro* antioxidant activity of the *S. cumini* seed extract
- Tocolytic activity of *S. cumini* seed extract
- Evaluation of *in vivo* antioxidant activity of the *S. cumini* seed extract
- Studies on isolated rat uterus.

The present study examines the efficacy of AESC as a tocolytic agent. The effect of the drug was evaluated on female Wistar rats.

#### METHODS

**Pharmacognostic studies**

*Collection of plant material and authentication*

The plant material was collected and authenticated by Dr. K. Madhava Chetty. Ph. D., Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh. The seeds were processed, powdered coarsely and coarse seeds were used for extraction.

**Extraction of the seeds**

The dried seeds were coarsely powdered. The powdered seeds (200 g) were taken in a round bottom flask and were extracted with water for 48 h at room temperature. After 48 h, the extract was concentrated in a rotary evaporator.

**Preliminary phytochemical analysis of the extract**

The information about the constituents present in the plant clarifies the medicinal uses of the plant. Identification and evaluation of herbal
extracts are a fundamental procedure and parts of quality control protocol. The aqueous extract of *S. cumini* seed was subjected to phytochemical evaluation and identified the various plant constituents present in the test samples by qualitatively and quantitatively. The following studies were carried out in phytochemical analysis.

- Qualitative chemical test
- Estimation of total phenol
- Estimation of total flavonoid.

**Qualitative chemical tests**
The qualitative chemical tests were carried out for the extract and identified the various secondary metabolites present in the aqueous extract of *S. cumini* seed.

**Preparation of test sample**
500 mg of the extract was dissolved in 5 ml of distilled water and then filtered. The filtrate was tested to detect the presence of various phytochemical constituents in the sample.

**Estimation of total phenol content** [10]
The determinations of total phenol content of the extracts were done by Folin–Ciocalteu (F-C) assay with some modifications. The Folin–Ciocalteu reagent produces blue color complex when reacted with polyphenol compounds if present in the sample. The assay relies on the transfer of reducing electrons in the alkaline medium, from phenolic compounds to phosphomolybdic acid complexes, manifested in the formation of blue color that is estimated by ultraviolet-visible spectrophotometer (Thermo Fischer model Evolution 201).

The extract was prepared as 1 mg/ml concentration as stock and prepared volume of 1 ml of each sample was taken in 2 ml centrifuge tube followed by 0.5 ml F-C reagents (1:10 diluted with distilled water) were added and allowed to react for 5 min before adding 0.4 ml 20% Na$_2$CO$_3$. The above solutions were mixed and allowed to stand 15 min at room temperature then measured absorbance of sample at 765 nm. The blank was prepared in similar manner without sample and standard. Calibration curve was plotted using gallic acid as standard (10, 20, 40, 60, 80, and 100 µg/ml). The results were expressed as milligrams of gallic acid equivalents per gram of extract Fig. 3.

**Estimation of total flavonoid content** [11]
Total flavonoid content was estimated for all the extracts by aluminum chloride colorimetric assay with some modifications. 1 ml aliquot of appropriately diluted sample or standard solution of quercetin (10, 20, 40, 60, 80, and 100 µg/ml) was mixed with 50 µl of NaNO$_2$ in 2 ml micro centrifuge tube. After 6 min, 50 µl of a 10% aluminum chloride solution was added and allowed to stand for 6 min, and then 50 µl 1% potassium acetate solution was added to the mixture. The final volume was made up with distilled water to 2 ml, and then the mixture was thoroughly mixed and allowed to stand for another 15 min. The absorbance of the mixture was determined at 510 nm against prepared blank. The blank was prepared in the same above manner omitting sample and standard. All values were expressed as milligrams of quercetin equivalent per 1 g of sample Fig. 4.

**In vitro antioxidant study**
Various methods are used to investigate the antioxidant property of samples. In the present study, the antioxidant properties of the extract were evaluated by *in vitro* methods.

The antioxidant properties could not be concluded based on the single antioxidant test method. It is in practice that generally several *in vitro* test procedures are carried out to conclude the antioxidant properties of the sample. Among various free radical scavenging methods DPPH and ABTS assays were carried out in the present study.

**DPPH radical scavenging activity**
The DPPH assay method is based on the reduction of DPPH, a stable free radical. The free radical DPPH with an odd electron gives a maximum absorption at 517 nm (purple color).

**ABTS radical cation scavenging activity**
ABTS radical scavenging activity of the extract was measured by Rice-Evans method. ABTS was dissolved in water to a 7 mM concentration. ABTS
Fig. 5: Microscopical examination (×100) vaginal smear of mated female rats.

Tocolytic activity

**Experimental design**

Thirty female Wistar albino rats of 150–200 g were used for the study. The rats were divided into five groups of six animals each.

**Procedure**

The rats were divided into five groups of six animals each. The Group I was treated with normal saline, Group II was treated with oxytocin (1 IU Im) only, Group III was treated with atosiban (6 mg/kg), i.p, and Group IV and Group V were treated with 200 and 400 mg/kg of AESC, respectively (Table 1).

**Table 1: Experimental design for oxytocin induced preterm labor**

| Groups  | Treatment          |
|---------|--------------------|
| Group I | Normal saline      |
| Group II| Oxytocin (1 IU), i.m |
| Group III| Atosiban (6 mg/kg), i.p |
| Group IV | AESC (200 mg/kg), p.o |
| Group V | AESC (400 mg/kg), p.o |

**MATING OF RATS**

**Requirements for obtaining vaginal smear:**

Normal saline: The dropper needs only a small volume of normal saline (0.2–0.25 ml) for flushing. Distilled water can also be used without markedly distorting the cells enough to impair identification. Only a drop or two needs to be placed on a slide.

Slides: Microscopic slides can be used.

Microscope: A standard laboratory compound microscope is perfectly sufficient to evaluate a vaginal smear.

Stain: 0.1% methylene blue.

**Procedure**

The thirty (30) female rats were randomly distributed into 10 cages such that each cage contained 3 female rats. Each of the rats was identified with an indelible marker. After the distribution and identification of the female rats, a vaginal smear of each of them was made on a labelled clean glass slide.

The smear was collected by carefully with droppers, although moistened cotton swabs have also been used. Dropper tips should be smooth and tapered. If a single dropper is used for more than one animal, it should be thoroughly rinsed between lavages to remove any residual cells from the dropper wall. When inserting the tip of the dropper into the vaginal orifice, it is important that the penetration be relatively shallow, approximately 1 cm.

Immediately after withdrawal from the vaginal cavity, the content was smeared onto a labelled clean glass slide. The smear was observed grossly to check for the presence of protein coagulates (remnants of the copulatory plug).

After the initial smears were collected from the female rats, the male rats were introduced into 10 cages, such that the male: female ratio in these 10 cages was 1:3. After the introduction of males into the 10 mating cages, vaginal smears were made as described above for each of the females in all the 10 cages daily in the morning, and the smeared slides were observed grossly for protein coagulates. The observation of grossly visible protein coagulates on the vaginal smear of each female was recorded as evidence of mating. The mating was also confirmed by the presence of sperms in the vaginal smear. The sperm was observed by staining the smear with 0.1% methylene blue shown in Fig 5.

The presence of sperm in the vaginal smear or observation of a vaginal plug indicates the occurrence of mating. The day that sperm is detected in the vaginal smear is designated as day 1 of gestation. After 10 days of gestation, the fetuses can be palpated, but palpation is more accurate after day 12. By day 13 of gestation, the abdominal enlargement is visible, and mammary development and nipple enlargement can be observed on day 14 of gestation. Once the protein coagulates and sperms were observed on the vaginal smear of each rat, the rat was thereafter weighed at four-day intervals to check the progress of the pregnancy.

**In vivo antioxidant activity**

**Blood collection**

After the end of the treatment period, the animals were anaesthetized with diethyl ether (inhalation) and its blood was collected by retro-orbital puncture without adding ethylenediaminetetra acetic acid (EDTA).
Procedure
0.1 ml sample was made up to 1 ml with distilled water. 5 ml of alkaline solution was added, mixed well and allowed to stand for 10 min. Then, 0.5 ml Folin’s reagent was added, mixed well and incubated at room temperature for another 10 min. The blue color developed was measured at 640 nm against blank. Bovine serum albumin (1 mg/ml) served as the standard and from the standard graph the amount of protein in the sample was calculated.

Enzymatic antioxidant activity

Estimation of SOD
To 0.5 ml of the sample, 1.5 ml of carbonate buffer and 0.5 ml of 0.1 mM EDTA were added and mixed. To this, 0.4 ml of adrenaline was added at the time of measurement of the optical density at 480 nm. The antioxidant activity of SOD enzyme was expressed as units/min/mg protein.

Estimation of CAT

To 1 ml of sample, 4 ml of hydrogen peroxide, and 5 ml of phosphate buffer were added and mixed. From this, 1 ml of solution was taken and mixed with dichromate acetic acid reagent and allowed to incubate for 30 min at room temperature. The absorbance was measured at 570 nm. The activity of CAT was expressed as umole of H₂O₂ consumed/min/mg protein.

Non enzymatic antioxidant activity

Estimation of reduced GSH activity
To 1 ml of the sample, 1 ml of trichloroacetic acid (TCA) solution was added and centrifuged. The supernatant was collected and the precipitate formed was removed. To 0.5 ml of supernatant, 2 ml DTNB was added, the volume was made up to 3 ml with phosphate buffer. Then, absorbance was read at 412 nm. The amount of GSH was expressed as µg/mg protein.

Determination of LPO
To 0.1 ml of sample, 2 ml of TBA-TCA-HCL reagent (ratio of 1:1:1) was added, mixed, and kept in a water bath for 15 min. Afterward, the solution was cooled and supernatant was removed and absorbance was measured at 535 nm against reference blank. The level of lipid peroxides was given as moles of MDA formed/mg protein.

In vitro study of isolated rat uterus
Female non-pregnant Wistar rats were pre-treated subcutaneously with estradiol valerate 0.1 mg/kg of 24 h before the actual experiment for uterine sensitization. The rats were killed by cervical dislocation and exsanguinations. The abdomen was opened and the two horns of the uterus carefully isolated, freed of mesenteric fat, and a strip of the horn about 1–2 cm was cut out. A thread was then attached to one end of the isolated strip of uterus and was tied to the aerator tube in the organ bath containing 25 ml De Jalon’s physiological salt solution having the following chemical composition: NaCl – 9 g/L, NaHCO₃ – 0.5 g/L, D-glucose – 0.5 g/L, KCl-0.402 g/L, and CaCl₂ × 2 H₂O-0.08 g/L. Another thread was attached to the other end of the isolated uterus and fixed to a lever system. The tissue was aerated and temperature was maintained at 30–32°C, with a pH of 7.4.

The tissue was allowed to equilibrate for 30 min before the start of the experiment and placed under tension of 0.500 g. A lever system was used to record the uterine contractions and relaxations on a smoked glossy paper in kymograph drum, moved at 2.5 mm/s speed which was varnished after and calculated the data. The response of uterine tissue to oxytocin (0.01 IU/ml) before and after incubation with AESC (25 and 50 mg/ml) for 1 min was recorded along with standard drug atosiban (0.5 IU).

Statistical analysis
The data of all the parameters were analyzed using the GraphPad 5.0 software. Analysis of variance (ANOVA); one-way ANOVA followed by Tukey comparison test was performed. The values were expressed as Mean±SEM.

RESULTS

Extractive yield
The percentage yield of AESC: Coarsely powdered seeds of S. cumini were extracted with water and the percentage yield was found to be 14.5% w/w.

Preliminary phytochemical analysis
Qualitative chemical tests were conducted and found with positive results.

Quantification of total phenol and flavonoids
The total phenol content present in AESC was found to be 46.04 mg/g equivalent to gallic acid and the total flavonoid content in AESC was found to be 47.93 mg/g of extract calculated as quercetin equivalent.

In vitro antioxidant study

DPPH radical scavenging activity
The percentage inhibition of DPPH radical by quercetin and AESC was found to be 6.24 µg/ml and 33.15 µg/ml, respectively. Figs. 6 and 7.

ABTS radical scavenging activity
The percentage inhibition of ABTS radical by quercetin and AESC was found to be 0.1895 µg/ml and 4.149 µg/ml. Table 2.

Table 2: Percentage inhibition of ABTS radical by quercetin and AESC

| Sample     | Concentration (µg/mL) | % Inhibition | IC₅₀ (µg/mL) |
|------------|-----------------------|--------------|--------------|
| Standard   | 0.25                  | 63.4         | 0.1895       |
| (quercetin)| 0.5                   | 67.38        |              |
|            | 0.75                  | 79.79        |              |
|            | 1                     | 87.95        |              |
|            | 1.25                  | 99.41        |              |
|            | 1.5                   | 99.5         |              |
| AESC       | 5                     | 59.79        | 4.149        |
|            | 10                    | 69.39        |              |
|            | 15                    | 77.43        |              |
|            | 20                    | 86.53        |              |
|            | 25                    | 98.63        |              |
|            | 30                    | 98.94        |              |

Fig. 6: (1, 1-diphenyl-2-picrylhydrazyl (α,α-diphenyl-β-picrylhydrazyl) radical scavenging activity of quercetin
Tocolytic activity
A significant increase in the rate of PTD of the control animals was observed when compared with the normal group. The AESC treated group has showed a significant reduction in the rate of PTD which was comparable with the standard treated group as well as the normal group Table 3 and Fig. 8.

In vivo antioxidant study
The study reveals that the extracts had shown significant in vivo antioxidant activity and the results are expressed in Table 4 and Figs. 9-13.

In vitro study of isolated rat uterus
The results of in vitro study showed that the aqueous extract of S. cumini at 25 mg/ml and 50 mg/ml produced significant inhibition of oxytocin, induced contractions of the uterine smooth muscle in non-pregnant rats Fig. 14.

![Fig. 7: (1, 1-diphenyl-2-picrylhydrazyl (α,α-diphenyl-β-picrylhydrazyl) radical scavenging activity of AESC](image)

![Fig. 8: Effect of AESC on preterm labor](image)

Table 3: Effects of AESC and atosiban on oxytocin induced preterm labor on rat model

| Drug                     | Total number of rats | Numbers of rat delivered | Rates of term delivery | Delivery day (Mean±SEM) |
|--------------------------|----------------------|--------------------------|------------------------|-------------------------|
|                          |                      | Preterm | Term |                      |                         |
|                          |                      | Day 17 | Day 18 | Day 20 | Day 21 | Day 22 | Day 23 |  |
| Normal                   | 6                    | 0       | 0       | 0       | 0       | 6       | 100    | 23±0   |
| Control (oxytocin 1 IU i.m.) | 6              | 4       | 2       | 0       | 0       | 0       | 0       | 17.3±0.210*** |
| Control+Atosiban (6 mg/kg, i.p) | 6              | 0       | 0       | 0       | 3       | 3       | 100    | 22.5±0.341*** |
| Control+AESC (200 mg/kg, p.o) | 6              | 0       | 0       | 0       | 2       | 2       | 2       | 22±0.365*** |
| Control+AESC (400 mg/kg, p.o) | 6              | 0       | 0       | 0       | 1       | 4       | 83     | 22.5±0.223*** |

***p<0.001. Data are expressed as Mean±SEM (n=6 animals in each group)

Table 4: Effect of AESC on enzymatic and non‑enzymatic antioxidant levels

| Group                     | Total protein (mg/dl) | SOD (Unit/mg protein) | CAT (μmol of H₂O₂ consumed/mg protein) | GSH (μg/mg) | LPO (nmol of MDA/mg protein) |
|---------------------------|-----------------------|-----------------------|----------------------------------------|-------------|-----------------------------|
| Normal                    | 3.9±0.089             | 7.4±0.24              | 53.3±0.37                              | 12.8±0.09   | 2.8±0.09                    |
| Control oxytocin (1 IU)   | 9.8±0.447***           | 2.4±0.19***           | 28.8±0.12***                           | 6.7±0.19*** | 8.8±0.11***                 |
| Control+Atosiban (6 mg/kg) | 2.6±0.547***           | 6.9±0.077***          | 51.4±0.43***                           | 11.9±0.10***| 3.7±0.16**                  |
| Control+AESC (200 mg/kg)  | 4.8±0.216**            | 4.8±0.122**           | 44.4±0.4**                             | 10.8±0.09*  | 6.8±0.09***                 |
| Control+AESC (400 mg/kg)  | 3.9±0.044***           | 5.8±0.13***           | 48.8±0.09***                           | 11.8±0.122**| 5.9±0.02**                  |

***p<0.001, **p<0.01, *p<0.05; Data are expressed as Mean±SEM; (n=6 animals in each group). SOD: Superoxide dismutase, GSH: Glutathione

DISCUSSION
PTB is the most common cause of neonatal morbidity and mortality worldwide. Almost 75% of perinatal deaths occur in infants born before 37 weeks gestation.
The rationale for treating preterm labor is to reduce perinatal morbidity and mortality by increasing the gestational age at delivery. Assuming tocolysis is beneficial, the choice of drug remains controversial. Magnesium sulfate, calcium channel blockers, prostaglandin synthase inhibitors, NO donors, β-sympathomimetics, and oxytocin antagonists have all been suggested to be effective. Given the problems of efficacy and adverse effects with each tocolytic, particularly in view of the potentially devastating consequences of PTD, it is not surprising that new drugs have been developed and tested in clinical trials.

### Table 5: Effect of AESC on oxytocin induced contraction in rat uterus

| Group | Drug                  | Concentration | Height of contractions (cm) | % Inhibition |
|-------|-----------------------|---------------|----------------------------|--------------|
| I     | Oxytocin              | 0.01 IU/ml    | 5.5                        | 0%           |
| II    | AESC extract+oxytocin | 25 mg/ml+0.01 IU/ml | 2.5                      | 50.9         |
| III   | AESC extract+oxytocin | 50 mg/ml+0.01 IU/ml | 1.5                      | 72.7         |
| IV    | Atosiban+oxytocin     | 0.5 IU+0.01 IU/ml | 0.57                     | 89.6         |
Phenolics and flavonoids normally scavenge the free radicals and play an essential role in preventing oxidative stress related diseases [14]. In the present study, it was observed that there was a crucial depletion in GSH levels in control animals when compared with the normal group. The AESC treated group has showed a significant reduction in the rate of PTD of the same. It seems promising that these data obtained from the AESC showed significant tocolytic activity which was evident with reduction in the incidence of PTD. Hence, from the present study, it can be concluded that the AESC has the potential to prevent PTD as well as it can reduce the complications of the same. It seems promising that these data obtained from the study can be further validated in the future studies which eventually can be developed as a formulation that offers a high degree of protection from PTD.

AUTHOR CONTRIBUTIONS

M. Ramasamy: He came with the idea of this research work and contributed his idea throughout the work.

Anu Sebastian: Reviewed many articles, started the work with full confidence and maintained the animals throughout the work.
CONFLICTS OF INTEREST

No conflicts of interest from authors.

REFERENCES

1. Adhikari BS, Babu MM, Saklani PL, Rawat GS. Medicinal plants diversity and their conservation status in wildlife institute of India (WII) campus, Dehradun. Ethnobotanical Leaf 2010;14:46-83.
2. Sharma S, Mehta BK. Comparative study of alcoholic and aqueous extracts of *Syzygium cumini* on carbon tetrachloride-induced hepatotoxicity in Wistar rats. Asian J Pharm Clin Res 2016;9 Suppl 3:1-4.
3. Jaybhaye DL, Varma S, Chaudhary P, Bonde V, Gite A. Tocolytic plant *Tectona grandis* Linn. Extended study on other systemic effect. J Mahatma Gandhi Inst Med Sci 2016;21:122-9.
4. Kumar SP, Soni K, Saraf MN. The In Vitro tocolytic activity of *Sarcostemma* brevistigma Wight. Indian J Pharm Sci 2006;68 Suppl 2:190-4.
5. Menon R. Oxidative stress damage as a detrimental factor in preterm birth pathology. Front Immunol 2014;5 Suppl 557:1-14.
6. Priya SH, Prakasan N, Parusothaman J. Antioxidant activity, phenolic-flavonoid content and high-performance liquid chromatography profiling of three different variants of *Syzygium cumini* seeds: A comparative study. J Int Ethnopharmacol 2017;6 Suppl 1:112-4.
7. Singh K, Kaur R, Kaur AP. Studies on antioxidant and antimicrobial potential of *Syzygium cumini* leaves. Res J Pharm Biol Chem Sci 2016;7 Suppl 2:677.
8. Pradhan M. Phytochemistry, pharmacology and novel delivery-applications of *Syzygium cumini* (L.). Int J Pharm Pharm Res 2016;7 Suppl 1:659-75.
9. Ratajczak CK, Fay JC, Muglia LJ. Preventing preterm birth: The past limitations and new potential of animal models. Dis Models Mech 2010;3 Suppl 1:407-14.
10. Lakshmanashetty RH, Nagaraj VB, Hiremuth MG, Kumar V. *In vitro* antioxidant activity of *Vitex negundo* L. Leaf extract. Chiang Mai J Sci 2010;37 Suppl 3:489-97.
11. Kokate CK. Practical Pharmacognosy. 4th ed. Pune: Pragati Books Pvt. Ltd; 1994. p. 108-9.
12. Yesufu HB, Bassi PU, Khaz IZ, Abdulrahaman FI, Mohammed GT. Phytochemical screening and hepatoprotective properties of aqueous root bark extract of *Sarcocephalus latifolius* (smith) Bruce (African peach). Arch Clin Microbiol 2010;1 Suppl 2:1-5.
13. Lowry OH, Rosebrough NJ, Farr LA, Randall AJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951;193:265-75.
14. Sun Y, Oberly LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clin Chem 1988;34 Suppl 3:497-500.
15. Sharma S, Mehta BK, Mehta D, Nagar H, Mishra A. A review of pharmacological activity of *Syzygium cumini* extracts using different solvent and their effective doses. Int Res J Pharm 2012;3 Suppl 12:54-8.
16. David B, Wolfender JL, Dias DA. The pharmaceutical industry and natural products: Historical status and new trends. Phytochem Rev 2015;14 Suppl 2:299-315.