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RESEARCH ARTICLE

ANALGESIC AND ANTI-DIABETIC POTENTIAL OF *ROYSTONEA REGIA*

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ABSTRACT

Background: Plants are the rich source of nutrients and contain biological and pharmacological potential. *Roystonea regia*, a plant of Aceraceae family has been traditionally used in different countries especially in Asia. **Objectives:** The aim of the study was to evaluate its analgesic and anti-diabetic potential by hot plate method and Alloxan induced method in Swiss albino female rats weighing 150 to 200 gm respectively. **Methodology:** Shoots of *Roystonea regia* plant was extracted using n-hexane, chloroform and ethanol in Soxhlet apparatus. Oral acute toxicity test showed the different mortality rates of all extracts. **Results:** The crude *n*-hexane and ethanol extract showed increase in the latency time till 120 min and were found to have significant (p<0.001) analgesic potential at 100 and 200 mg/kg dose. However, different anti-diabetic potential was shown by all three extracts. Chloroform extract showed maximum reduction in blood glucose level (BGL) and found significant (p<0.001) as compared to negative control till 21st day at the dose of 250 and 500 mg/kg of body weight. **Conclusion:** Further study is required to understand the complete mechanism involved in both activities. In future, purified fractions and column chromatography can be conducted for their complete characterization and their toxic effects to establish a proper therapeutic dose for further trials.

Keywords: *Roystonea regia*, Alloxan induced rats, Mortality rate, Diabetes

INTRODUCTION

Analgesics are the medications that are used to relieve pain without sleep and loss of consciousness. Internationally there is no agreed classification of analgesics. The basic division is the opioid (narcotic) and non-opioid (non-narcotic) analgesics. According to the world health organization (WHO), world is looking forward for plant based drugs due to the severe adverse effects of opiates and 80% of world population rely on natural products (1).

Stage at which blood glucose level of a person increase either in the state of fasting or postprandial condition is called the diabetes (2). The exact cause of this diabetes is still unknown. Lot of factors affect this condition such as obesity, infections, autoimmune diseases and environmental changes (3). Many medicinal plants play an important role in controlling the diabetes such as *Acacia arabica*, which shows the hypoglycemic effects by releasing the insulin from pancreatic cells. *Allium cepa*, which not only shows the anti-hyperglycemic effects but also used as antioxidant and hypolipidemic agent (4). Basically, it is of three types diabetes mellitus 1 (occurs due to damage of pancreatic cells and there is no production of insulin), diabetes mellitus 2 (due to the inability of pancreatic cells to produce sufficient insulin) and gestational diabetes (diabetes at the stage of pregnancy) (5).

Roystonea regia belonging to the family of Aceraceae and class of Magnoliopsida is also known as Cuban royal palm (6). *Roystonea elata* is no longer the scientific name for this species, which is now known as *Roystonea regia*. As much as 20–30 meters tall, the plant has a stem diameter of approximately 47 cm. Trees may grow to a height of four meters and have approximately 15 leaves. Flower, anthers are bright red, with a white flower center. Spherical or elliptical in form, the fruits measure 8.9 to 15 millimeters in length and breadth, respectively (7). Fruit pulp of *Roystonea regia* has lot of therapeutic uses as it contains alkaloids and is traditionally used as analgesic medicine (8). *Roystonea regia* contain the polysaccharides and is used as immunoprotected agent due to its ability to reduce the growth of pathogens (9). The antimicrobial property of lichen material of *Roystonea regia* is reported by Vinitha M. Thadhani *et al*; (10). This plant is traditionally used in the treatment of diabetes in Asian countries such as China, Bangladesh, India etc. (11). The lipid extract which is derived from fruit of *Roystonea regia* is much effective in prostate hyperplasia (12). A study reported in 2013 revealed that lipid extract is much effective as an anti-inflammatory agent in rats containing prostate cancer (13). The most active constituents found in lipid extract D-004 are oleic, lauric and palmitic acid (14).

D-004 shows antioxidant efficacy in active liver microsomes. It was discovered that when D-004 was given at doses ranging from 250 to 1000 mg/mL (15). Para hydroxy benzoic acid is one of the constituents derived from this plant contains the antitussive, anti-mutagenic as well as the anti-inflammatory properties (16). The traditional uses of plant such as an analgesic, antipyretic, anti-diabetic agent is still not proven scientifically and studies are in progress to explore it as a crude drug in market.

MATERIAL AND METHODS

Chemicals

Ethanol, n-hexane, and Chloroform (Sigma Aldrich), Paracetamol (GSK), Diclofenac sodium (Sigma England), tween-80, Alloxan (sigma England), normal saline (Freshly prepared). All the chemicals used were of analytical grades.

Plant material

Roystonea regia plant was collected from local flora and identified by Chairman Botany department, Government College University, Lahore, Pakistan. A specimen of *Roystonea regia* was submitted in GCU Dr. Ahmed Sultan herbarium and its voucher number (GC-herb-BOT-3779) was issued by authority. Plant was dried under shade and ground to powder to preserve it in tight container.

Extract preparation

Almost 500 gm of plant powder was taken and was packed in a tight thimble of filter paper. Extraction process was done sequentially by Soxhlet apparatus (Quickfit, England) with three different solvents i.e., n-hexane, chloroform, ethanol (according to the polarity) respectively. Temperature of Soxhlet apparatus (Quickfit, England) was set according the boiling point of each solvent. The process continued until the solvent become colorless. Rotary evaporator (SLN 53 STD, Poland) was used to concentrate the extract by evaporating the extra solvent. At the end a thick, gummy and viscous material obtained which was further dried in an oven at 40^0C and stored in glass vials for further use (17).

Oral acute toxicity test

Following the organization for economic cooperation and development (OECD) 423 guidelines, the acute oral acute toxicity test was performed. Rats were divided into three different groups and each group contained three rats. Animals were given access water to drink. Extract dose of 100 mg/kg was administered and animals were kept under observation for any unusual response.

If two to three animals were found dead then this dose was considered toxic but if no animal died this dose was repeated for confirmation. In case of no mortality, extract dose was given in high amount such as 400 mg/kg, 700 mg/kg, 1000 mg/kg etc. and observed in the similar manner as described above (18).

Experimental animals for the study of analgesic, and anti-diabetic activity

Swiss albino female rats of weight ranging from 150 mg - 200 mg were used for these activities. Approval was taken from animal research ethical committee of The University of Lahore (UOL) vide project file no IREC-2021-25. Animals were divided into various groups and acclimatized according to the conditions of the animal's house before the analgesic and anti-diabetic activity to minimize any physiological, psychological, central nervous system as well as cardiovascular changes for 7 days (19). Cages of standard size along with suitable length and of proper space were used. Temperature of the animal's house was set at 25^0C. The fine wood cage bed and water bottles were changed after every 24 hours. Before and during the anti-diabetic activity the rats were given free access to standard food that was taken from the animal's food store. Dose of extract was chosen according to the oral acute toxicity test of extract (20).

Analgesic activity

Rats were divided into four groups such as negative control (1% of 10 ml/kg of tween-80 solution in water), positive control (Diclofenac sodium 10 mg/kg), experimental group 1 (extract dose 100 mg/kg), experimental group 2 (extract dose 200 mg/kg). The activity was performed according to Eddy's hot plate method. Animals were positioned on Eddy's hot plate (LE740, Spain) and temperature was kept 55^oC±0.5^0C. The cut off time was set at 15 seconds to prevent any damage of rat's paw (21). The reaction time was recorded at different time intervals at 0 min, 30 min, 60 min and 90 min. Percentage inhibition was calculated by following formula (22).

$$\% \text{ inhibition} = \frac{\text{post-treatment latency} - \text{pre-treatment latency}}{\text{cut-off time} - \text{pre-treatment latency}}$$

Anti-diabetic activity

Fasting BGL of experimental rats was checked with glucometer (EG-101, Accuchek). Alloxan was chosen as a drug to induce diabetes in rats. Rats were divided into following five groups, positive control (Glibenclamide 0.66 mg/kg), normal control (distilled water 10 ml/kg), negative control (distilled water 10 ml/kg), experimental group 1 (extract dose 250 mg/kg), and

experimental group 2 (extract dose 500 mg/kg). According to the weight of each rat, Alloxan was weighed individually for them and dissolved in 0.9% w/v normal saline with continuous stirring just before injecting in rats. It was injected in overnight fasted rats through Intra-peritoneal (IP) administration. Animal's food and water were given to rats after 30 minutes of administration of Alloxan. To avoid any type of hypoglycemic shock and impermanence, rats were given 10% of glucose water for the next 24 hour. After injecting Alloxan, seven days were waited and after that BGL of each rat was checked with glucometer (EG-101, Accuchek Samples of blood were collected from the tails of rats. Rats containing BGL more than 200 mg/dl were selected. They were given extract doses of 250mg/kg and 500mg/kg on daily basis. BGL of rats was checked on the 7^{th}day then on 14^{th} day and at the end on 21^{st} day (20).

RESULTS AND DISCUSSIONS

Oral acute toxicity test

Ethanol and *n*-hexane extracts of *Roystonea regia* showed the mortality at the dose of 1800 mg/kg of body weight. However, chloroform extract showed the mortality when extract was given at the dose of 1400 mg/kg of body weight. All the rats were found dead at this dose.

Analgesic activity

n-hexane extract with both doses (100 mg/kg and 200 mg/kg) of *Roystonea regia* expressed increase in the latency time when compared with negative control (1% of tween 80 only). Both showed the percentage inhibition of 47.14% and 51.00% respectively. Results presented in Figure 1 showed that both doses produced their maximum effect at 120 min. Standard drug (Diclofenac sodium) revealed the maximum percentage inhibition of 87.62% at 120 min time interval. Statistical analysis after applying ANOVA showed that results of both doses were highly significant ($p<0.05$) as compared to negative control.

No analgesic effect was produced by chloroform extract at doses of 100 mg/kg and 200 mg/kg of body weight. Both doses did not show increase in the reaction time till 120 min and expressed the percentage inhibition of 0.60% and 0.11% respectively. None of both doses was found to be significant when ANOVA (along with Dunnett's test) was applied.

Ethanolic extract of *Roystonea regia* produced highly significant ($p<0.001$) dose dependent effect at the dose of 100 mg/kg and 200 mg/kg of body weight with percentage inhibition of 67.80% and 74.46% at 120 min respectively. Both results were comparable with standard drug which exerted protective effect on heat induced pain in rats. Similar effect was seen in the case

of aqueous extract of *Flos populi* when it was compared with Morphine (standard drug)as described by Qianqian Xu *et al* (23).

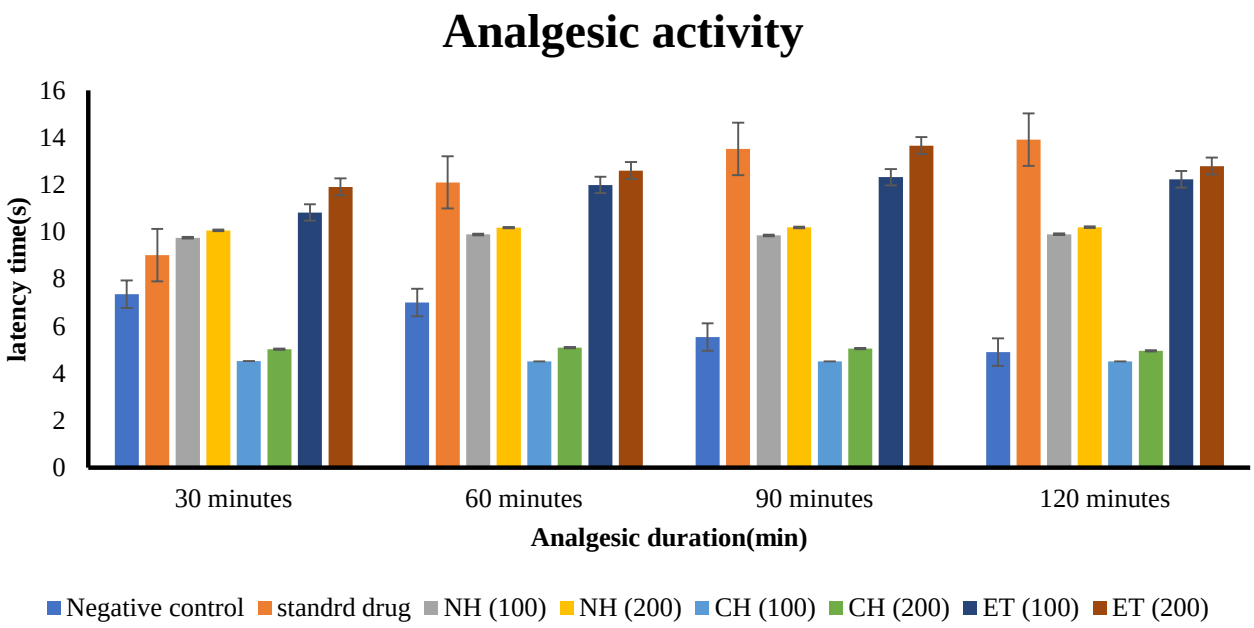

Figure 1. Analgesic potential of *Roystonea regia*

Anti-diabetic activity

Oral administration of *Roystonea regia n*-hexane extract (250 and 500 mg/kg of body weight) produced considerable *($p<0$*.021) reduction in BGL of Alloxan induced rats. Results presented in Figure 2 revealed that both doses possess little anti-diabetic activity when compared with negative control. 250 mg/kg reduced the BGL from 397.80 mg/dl to 390.20 mg/dl and 500 mg/kg dose reduced the BGL from 379.80 mg/dl to 342.60 mg/dl. Similarly, Standard drug (Glibenclamide) showed the significant decrease in BGL of rats after every 7th, 14th and 21st day. In the case of chloroform extract, fasting BGL of diabetic rats (negative control) was much higher than normal rats (normal control). Both doses expressed the significant reduction in the BGL (445.0 to 425.80 mg/dl and 389.6 to 366.0 mg/dl) of rats after every week similar to the effect shown by fruit extract of Karchure *chooranam*(24), and the results were comparable with standard drug. After applying ANOVA, chloroform extract showed the highly significant results (p<0.001) when compared with negative control (25-28). Dunnett's test analysis presented that

ethanol extract of 250 mg/kg dose triggered significant (p<0.001) drop of BGL of rats, while 500 mg/kg dose did not produce significant results till 21st day after the IP administration of Alloxan.

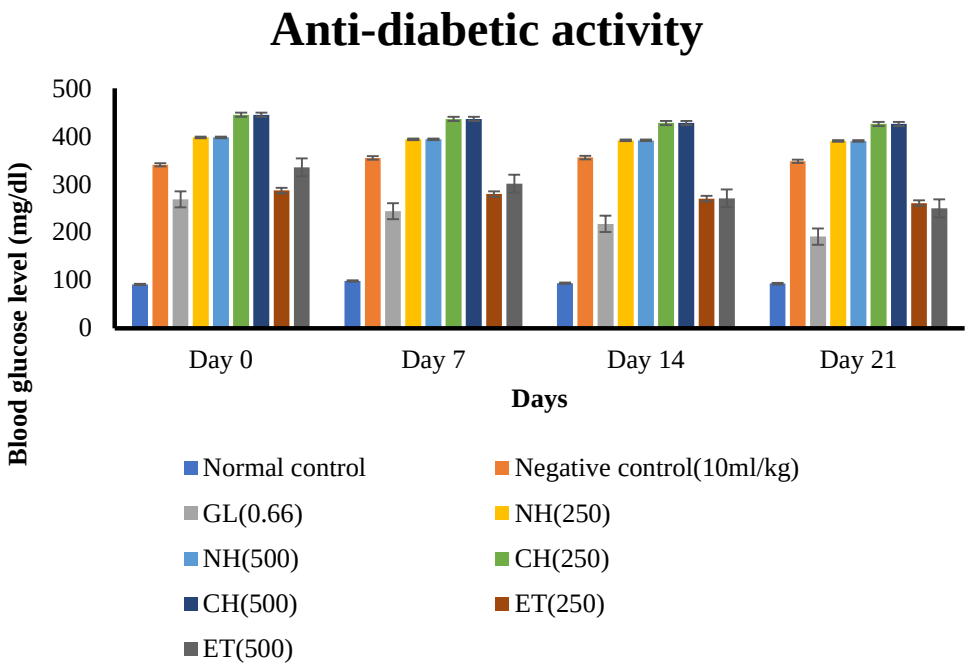

Figure 2. Anti-diabetic potential of *Roystonea regia*

CONCLUSION

Medicinal plants have always been the potential source of crude drugs and discovery of new biologically active molecules. Selection of medicinal plant depends upon its ethno-botanical importance and its traditional use for the treatment of different diseases. Current study revealed that shoot extracts of *Roystonea regia* possess highly significant analgesic and anti-diabetic potential. However, chloroform extract of *Roystonea regia* did not show any significant analgesic potential while anti-diabetic potential was highly significant as compare to negative control. *n*-hexane extract showed considerable results in both cases i.e., analgesic and anti-diabetic activity. Moreover, ethanolic extract produced dose dependent analgesic effects while its anti-diabetic potential was more pronounced at lower dose (250mg/kg) as compare to high dose (500mg/kg). However, further study is required to understand the complete mechanism involved in both activities. In future study and experiments, purified fractions and column chromatography can be conducted for their complete characterization and their toxic effects to establish a proper therapeutic dose for further trials.

Authors' contributions

MAS designed the complete project and described different methods for *in -vivo* study of plants. ZM contributed in collecting the data and the arrangement of materials required to complete the project. NA performed the literature survey and supervised the execution of the lab work. UF performed the statistical analysis and proof reading of the manuscript. MUM performed the experiment and wrote the manuscript. All the authors contributed equally and approved the final manuscript.

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Declarations

Ethics approval

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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