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ANTI-OXIDANT AND ANTI-PYRETIC ACTIVITY OF HYDROALCOHOLIC EXTRACT OF CASSIA AURICULATA

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Abstract:
The aim of present research work is evaluate antioxidant and antipyretic activity of hydroalcoholic extract of cassia auriculata. Cassia auriculata commonly known as Tanner's Cassia is an important medicinal shrub used in traditional systems of medicine. Subcutaneous injection of sterilized brewer's 20 % yeast suspended in 9.0 % in saline at the dose of 1 mL/100 g body weight in albino rat leads to pyrexia. The results conclude that the extract of Cassia auriculata exhibited significant antipyretic activity. While Antioxidant activity of hydroalcoholic extract of Cassia auriculata was used as a conventional compound with ascorbic acid. Free radical hydrogen peroxide scavenging activity (percent) has been calculated.

Keywords: *Pyrexia, Estimation, Phytoconstituents, Extraction, Percentage yield*

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INTRODUCTION:

Fever often occurs in response to infection, inflammation and trauma. However, this view of fever is merely an oversimplification as a growing body of evidencenow suggests that fever represents a complex adaptive response of the host to various immune challenges whether infectious or non-infectious. Although elevated body temperature is an indispensable component of the febrile response, it is not synonymous with fever. It is generally agreed that fever is a regulated rise in body temperature above normal daily fluctuations occurring in conjunction with an elevated thermoregulatory set point. To highlight the adaptive nature of the febrile response, the International Union of Physiological Sciences Commission for Thermal Physiology in 2001 defined fever as a state of elevated core temperature, which is often, but not necessarily, part of the defensive responses of multicellular organisms (host) to the invasion of live (micro-organisms) or inanimate matter recognised as pathogenic or alien by the host. Pyrexia or fever is caused as a secondary impact of infection, malignancy or other diseased states. It is the body's natural defense to create an environment where infectious agent or damaged tissue cannot survive [1]. Normally the infected or damaged tissue initiates the enhanced formation of proinflammatory mediator's (Cytokines like interleukin 1β, α, β and TNF- α), which increase the synthesis of prostaglandin E2 (PG E2) near peptic hypothalamus area and thereby triggering the hypothalamus to elevate the body temperature [2]. As the temperature regulatory system is governed by a nervous feedback mechanism, so when body temperature becomes very high, it dilate the blood vessels and increasing sweating to reduce the temperature; but when the body temperature become very low hypothalamus protect the internal temperature by vasoconstriction. High fever often increases faster disease progression by increasing tissue catabolism, dehydration and existing complaints, as found in HIV [3]. Drugs having anti- inflammatory activity generally possess antipyretic activity (e.g) non-steroidal anti-inflammatory drugs (NSAIDs). It has beensuggested that prostaglandin (PGE) mediates pyrogen fever; the ability of NSAIDs, to inhibit prostaglandin synthesis could help to explain their antipyretic activity.

Fever is one of the most common presenting signs of illness in office-based primary care pediatric practice, accounting for 19% to 30% of visits [4-5]. Infants and young children are particularly susceptible to fever because of their small body size, high ratio of body surface area to weight, and low amount of subcutaneous fat. Although most experts consider fever a beneficial physiologic response to the infectious process, it can lead to patient irritability and stress as well as high parental anxiety [6]. Therefore, physicians usually prefer to prescribe antipyretic agents in addition to nonpharmacologic, physical fever-reducing modalities [7].

Pyrexia or Fever is defined as an elevation of body temperature. It is a response due to tissue damage, inflammation, malignancy or graft rejection. Cytokines, interleukin, interferon and Tumor Necrosis Factor α (TNF- α) are formed in large amount under this condition, which increase PGE2 which in turn triggers hypothalamus to elevate body temperature [8].Fever is associated with symptoms of sickness behavior which consist of lethargy, depression, anorexia, sleepiness, & inability to concentrate.

MATERIALS AND METHODS:

Collection of plant material:

Leaves of *Cassia auriculata* were collected in the month of January 2022, , from the botanical garden of SIRT-P Bhopal (M.P.). Leaves of *Cassia auriculata* were cleaned by tab water and a portion was dried at room temperature. The dried samples were ground and passed through a sieve.The powdered drugs were kept in sealed containers and protected from light until used. Another portion of root sample was used for maceration.

Extraction procedure:

Following procedure was adopted for the preparation of extracts from the shade dried andpowdered herb

Defatting of plant material:

78.2 gram of powdered leaves of *Cassia auriculata* were coarsely powdered and subjected to extraction with petroleum ether by Soxhlet extraction.

Extraction by soxhlet extractionprocess:

Defatted dried powdered of *Cassia auriculata* has been extracted with hydroalcoholic (ethanol: water: 75:25v/v) as a solvent using soxhlet extraction process for 48 hrs, filtered and dried using vacuum evaporator at 40°C. [62]

Determination of percentage yield: The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powdered drug}} \times 100$$

Phytochemical analysis: Photochemical examinations were carried out extracts as per the following standard methods [63].

Detection of alkaloids: By hager's Test
Detection of carbohydrates: By hehling's Test
Detection of saponins: By froth Test
Detection of phenols: By ferric chloride test
Detection of flavonoids: By lead acetate test
Detection of proteins: By Xanthoproteic test
Detection of diterpenes: By copper acetate test

Quantitative studies of phytoconstituents: In India, the ayurvedic system has features a numerous of such medicinal remedies on plants or plant products and the determination of their morphological, pharmacological or pharmacognostical characters can provide a better understanding of their active principle and mode of action.

Total phenol content estimation: The total phenol content of the extract was determined by the modified folin-ciocalteu method.

Total flavonoids content estimation: Determination of total flavonoids content was based on aluminum chloride method.

Preparation of standard: 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol.

Preparation of extract: 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids.

In-vitro antioxidant activity using H2O2 method: *In-vitro* antioxidant activity of hydro alcoholic extract of *Cassia auriculata* using hydrogen peroxide was performed. The percentage inhibition of free radical H2O2 was calculated from the following equation: % inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%.

In vivo antipyretic activity of extract using Brewer's yeast-induced pyrexia in rats: Laboratory Swiss albino rats (180–200 g) of either sex were housed at 25° ± 5 °C in a well-ventilated animal house under 12/12 h light/dark cycle.

Acute toxicity study (LD50): Acute oral toxicity test was carried out according to the Organization for Economic Co-operation and Development (OECD) guidelines for Testing of Chemicals number 420 (OECD, 2001). The LD50 of the extract as per OECD doses of 2000 mg/kg. Hence the biological evaluation of the extracts was carried out at a dose of 200 mg/kg body weight.

5.2. Brewer's yeast-induced pyrexia in rats:
The antipyretic properties of *Cassia auriculata* were tested in rats in which hyperthermia had been induced following the method of Teotino et al. (1963).

Animal grouping and administration of chemical compounds: Thirty albino rats were grouped into 5 consisting of 6 rats each as follows: Group A, the control, received orally 6.7 ml/kg body weight of physiological saline.

Group B, received orally 1 ml corresponding to 5 mg/kg body weight of the reference drug, indomethacin.
Groups C, D and E were orally administered with 1 ml each, corresponding to 100, 200 and 300 mg/kg body weight Of HydroalcoholiCextract of *Cassia auriculata* respectively, 17 h after induction of pyrexia

RESULT AND DISCUSSION:

Yield of plant material:
The moderately coarse powder of the leaves of *Cassia auriculata* was subjected to extraction by soxhletion method with hydroalcoholic as a solvent.

Preliminary phytochemical screening of *Cassia auriculata*
The crude extract so obtained after the maceration process, each extracts were further concentrated on water bath evaporation the solvents completely to obtain the actual yield of extraction.

Table 3.3: Preliminary phytochemical screening of *Cassia auriculata*

S. No.	Phytoconstituents	Test Name	Hydroalcoholic extract
1	Alkaloids	Hager's Test	Absent
2	Glycosides	Legal's Test	Absent
3	Carbohydrates	Fehling's Test	**Present**
4	Flavonoids	Lead acetate	**Present**
5	Diterpenes	Copper acetate Test	Absent
6	Saponins	Froth Test	**Present**
7	Proteins	Xanthoproteic Test	**Present**
8	Phenols	Ferric Chloride Test	**Present**

Quantitative study of bioactivecompounds

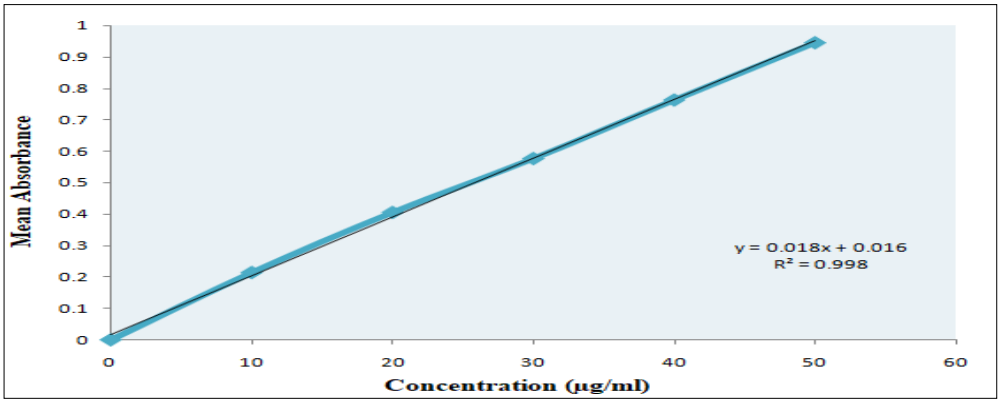
Estimation of total phenolic content:

Gallic acid is used as a standard compoundand the total phenols were expressed asmg/100mg gallic acid equivalent using the standard curve equation: y = 0.018x + 0.016, R^2 = 0.998, Where y is absorbance at 765 nm and x is total phenolic content inthe hydroalcoholic extract of *Cassia auriculata*.

Table 3.4: Preparation of calibration curve of Gallic acid

S. No.	Concentration (µg/ml)	Mean Absorbance
1	10	0.214
2	20	0.405
3	30	0.576
4	40	0.762
5	50	0.944

Figure 3.4 Graph of calibration curve of Gallic acid

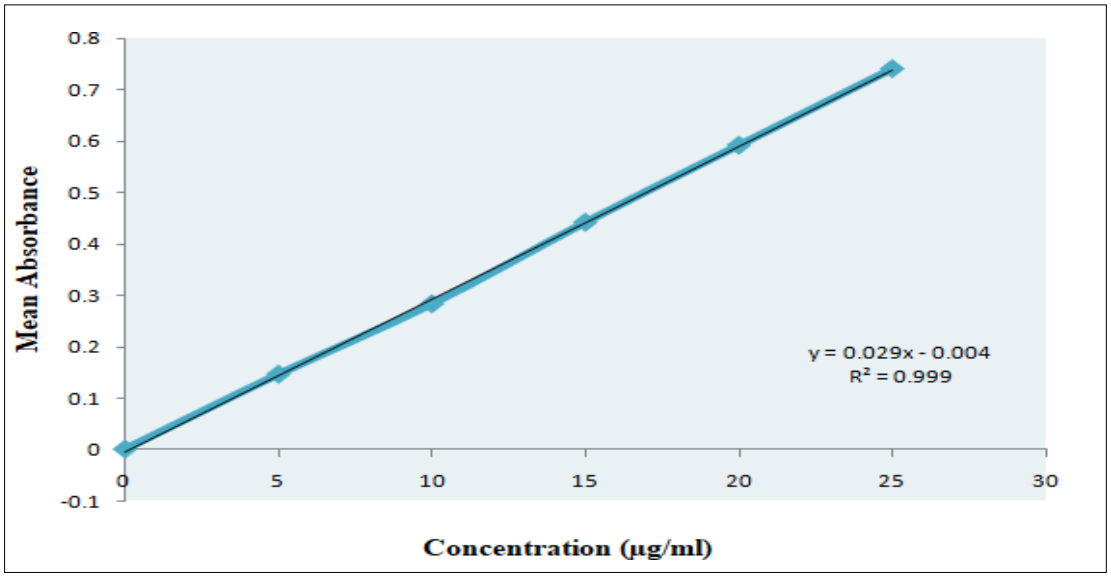


3.5:Estimation of total flavonoids content Flavonoids content was calculated from theregression equation of the standard plot y =0.029x - 0.004, R^2 =0.999) and is expressed asquercetin equivalents (QE) (fig. 7.2). Total flavonoids content was 0.784mg/100mgquercetin equivalent in hydroalcoholic extract of *Cassia auriculata*.

Table 3.5: Preparation of calibration curve of Quercetin

S. No.	Concentration (µg/ml)	Mean Absorbance
1	5	0.146
2	10	0.283
3	15	0.442
4	20	0.592
5	25	0.741

Figure 3.5: Graph of calibration curve of Quercetin



Results of antioxidant activity using hydrogen peroxide model

Table 4: % Inhibition of ascorbic acid and extract of *Cassia auriculata* using hydrogen peroxide method

S. No.	**Concentration (µg/ml)**	**% Inhibition**	
		Ascorbic acid	**Hydroalcoholic extract**
1	20	40.57	24.75
2	40	49.86	30.96
3	60	65.63	45.88
4	80	69.57	50.32
5	100	75.24	61.57
IC 50		37.19	75.72

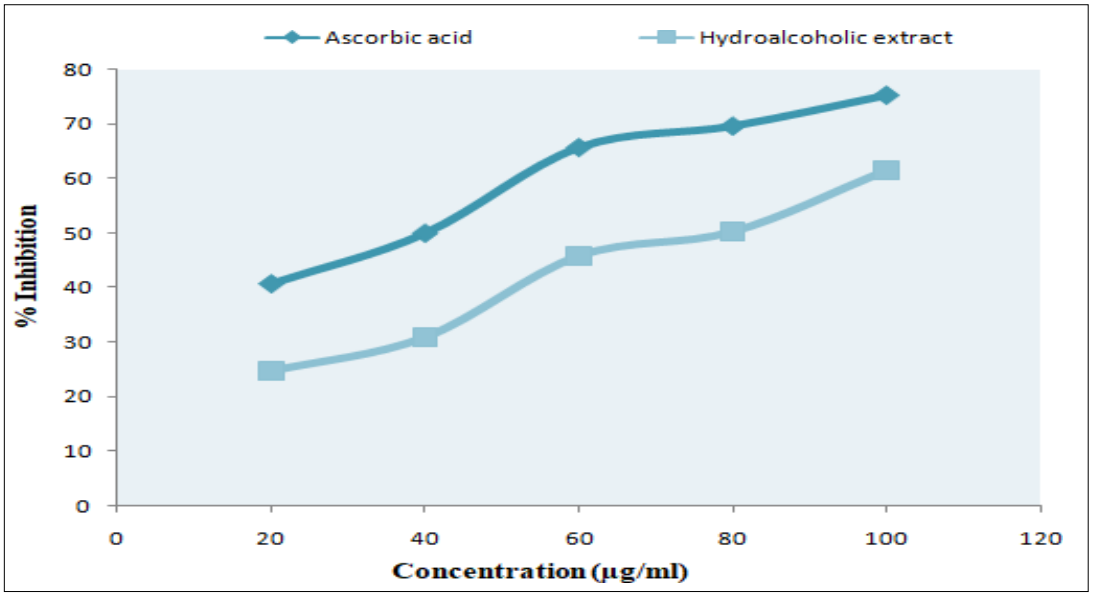


Figure 4.1: % Inhibition of ascorbic acid and extract of *Cassia auriculata* using hydrogen peroxide method

Results of in vivo antipyretic activity ofextract

Acute toxicity

The Hydroalcoholic extract of *Cassia auriculata* at a dose of 100 mg/kg does notshow any significance lowering in rectal temperature, but at the dose of 200 and 300 mg/kg caused a significant lowering inrectal temperature of hyperthermic rats on 120 min after the administration of Hydroalcoholic extract of *Cassia auriculata* was (36.84 ^{0}C and 36.56 ^{0}C) respectively.

Table 5.2: Effect of Hydroalcoholic extract of *Cassia auriculata* on brewer'syeast-induced hyperpyrexia in rats

Treatments	Initial Temp (^{0}C)	Temp (^{0}C) (17 h after induction of fever	Temp (^{0}C) (60 min after extract)	Temp (^{0}C) (90 min after extract	Temp (^{0}C) (120 min after extract)
Control (Physiological Saline)	36.94 ± 0.05	38.36 ±0.17	38.20 ± 0.18	38.26 ± 0.17	38.14 ±0.18
Indomethacin (5 mg/kg body weight)	36.96 ± 0.06	38.24 ±0.15	37.36 ± 0.18	37.04 ± 0.09	36.86 ±0.05
Extract (100 mg/kg body weight)	37.00 ± 0.04	38.46 ±0.13	37.92 ± 0.22	37.50 ± 0.19	37.14 ±0.17
Extract (200 mg/kg body weight)	36.56 ± 0.35	38.00 ±0.34	37.28 ± 0.31	37.26 ± 0.30	36.84 ±0.25
Extract (300 mg/kg body weight)	36.68 ± 0.21	38.20 ±0.22	37.22 ± 0.34	36.80 ± 0.38	36.56 ±0.37

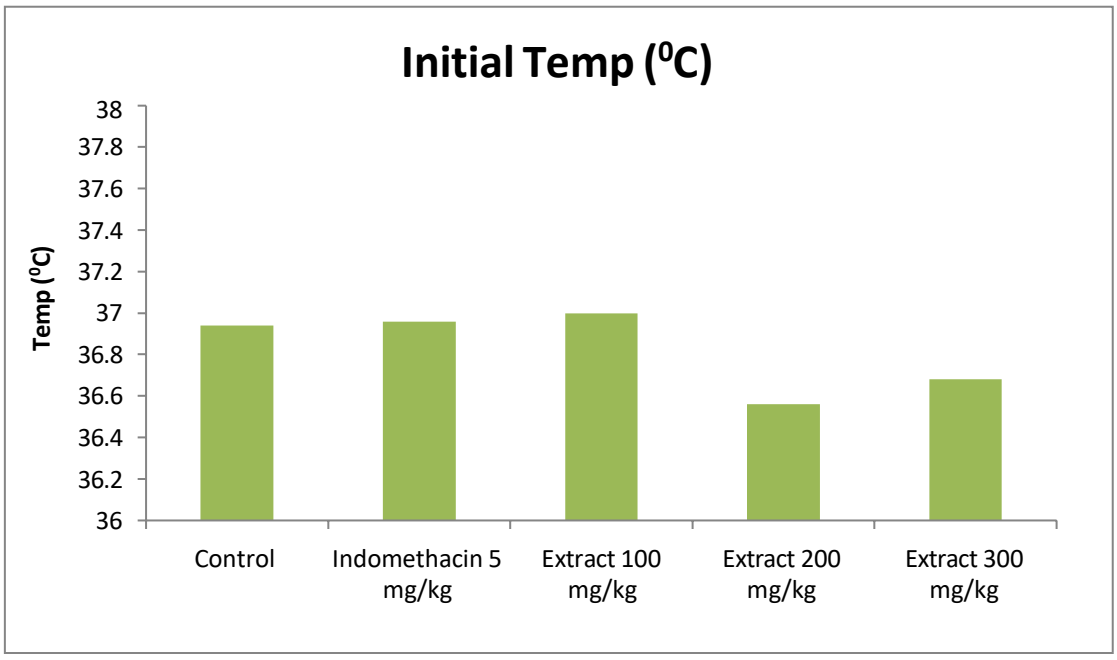


Figure 5.3: Mean Initial temperature of all groups

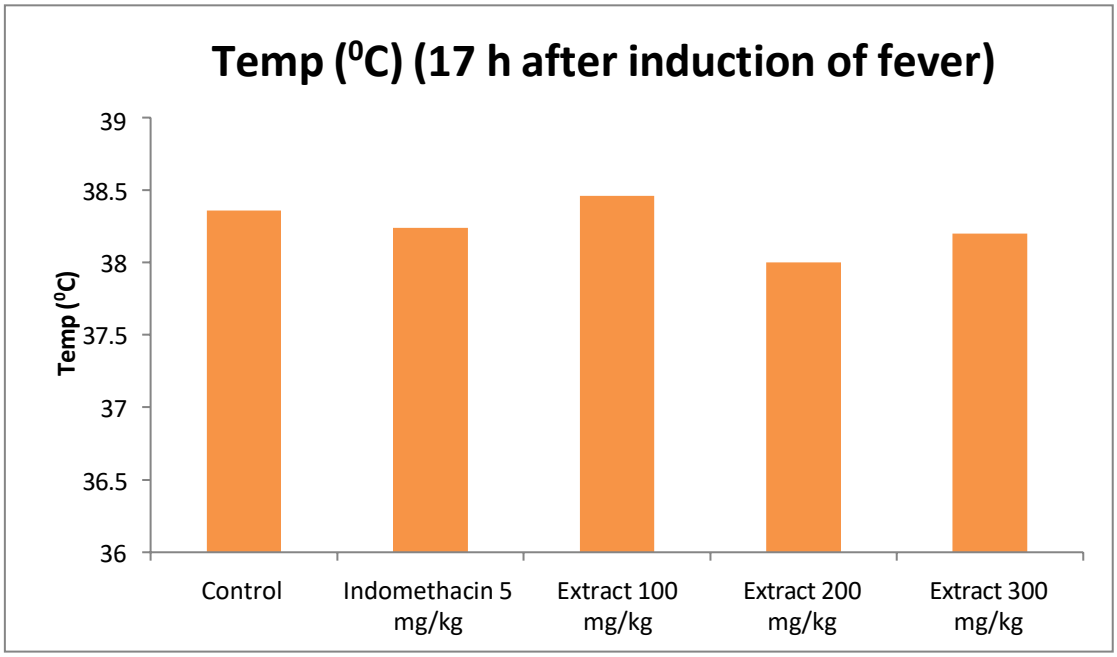


Figure 5.4: Mean Temperature (17 h after induction of fever) in all theexperimental groups

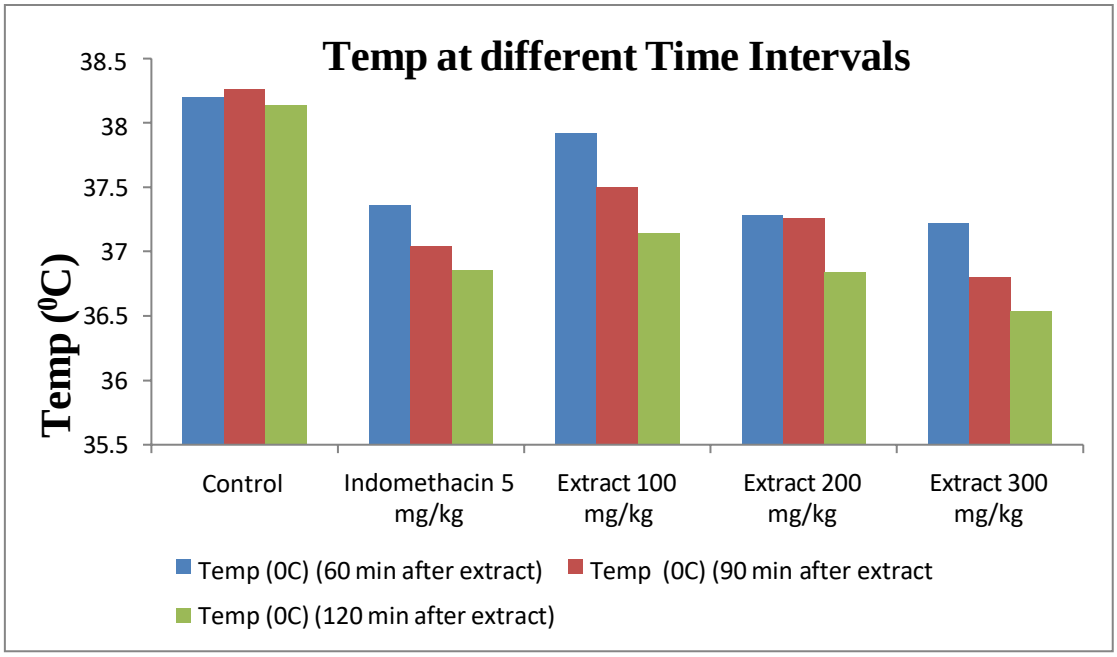


Figure 5.5: Mean Temperature (at different Time Intervals) in Control group and treated groups

CONCLUSION:

Percentage yield of extracts of hydroalcoholic of *Cassia auriculata* were found to be 8.63%. Hydroalcoholic extract of *Cassia auriculata* revealed the presence of flavonoids, proteins, carbohydrates, phenol and saponins. The total phenolic content of the crude extract calculated from the calibration curve, where we obtained linear regression y = 0.029x - 0.004, withR2 value = 0.999. The total plate count(TPC) of crude extracts was expressed as milligrams of gallic acid equivalents (GAE) per 100 milligrams crude extracts. The TPC was obtained from hydroalcoholic extract 0.842mg/ 100mg. Total flavonoids content was calculated from Quercetin calibration curve, where we obtained regression equation y = 0.018x + 0.016 with R2 value =0.998. Total flavonoids content of plant was statedin QE (Quercetin equivalent) or defined as an equivalent amount of mg quercetin per 100 milligrams sample. Result of flavonoids content indicated that hydroalcoholic extract has the flavonoids content (0.574mg QE/100mg extract). Hydroalcoholic extract of *Cassia auriculata* with different concentrations (20, 40, 60, 80, and 100 µg/ml) were screened for Hydrogen peroxide scavenging activity, and the results were shown in Table 7.6 and Fig 7.4. The absorbance was found to decrease with an increase in the dose of extract used. It indicates that hydroalcoholic leaf extractof *Cassia auriculata* (100 µg/ml) exhibited the maximum hydrogen peroxide scavenging activity of 61.57% which is comparable to the standard effect is 75.24%. The hydroalcoholic extract exhibited potent hydrogen peroxide radical scavenging activity in concentration dependent manner. The IC50 values of hydroalcoholic extract (IC50 75.72µg/ml) followed by Ascorbic acid (IC50 37.19µg/ml), respectively, using linear regression equation. Extract was administered orally. 30 min before the injection of the pyrogen. The rectal temperature of animals was recorded at 30 min intervals for 2 h following the administration of drug or plant extract. The hydroalcoholic extract of *Cassia auriculata* at a dose of 100 mg/kg does not show any significance lowering in rectal temperature, but at the dose of 200 and 300 mg/kg caused a significant lowering in rectal temperature of hyperthermic rats on 120 min after the administration of Hydroalcoholic extract of *Cassia auriculata* was (36.84 ^{0}C and 36.56 ^{0}C) respectively. This decrease persisted when an assessment was made 120 minutes after test drug administration and the efficacy was comparable to that of indomethacin at a dose of 5 mg/kg was (36.86 ^{0}C).This result seems to supportthe view that the plant has some influence on prostaglandin biosynthesis, since prostaglandin isbelieved to be a regulator of body temperature. Eskerud JRLaerum

EFagerthun HLunde PKNaess AA Fever in general practice, I:

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