ANTIFERTILITY ACTIVITY OF METHANOL EXTRACT OF *BASSIA LATIFOLIA* AND *CAJANUS CAJAN* IN MALE ALBINO MICE

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ABSTRACT

Bassia latifolia bud and *Cajanus cajan* seed were evaluated for antifertility activity in mature male mice . Male antifertility agent affects the count & motility of spermatozoa directly by inhibition of steroidogenesis. Methanol extracts of *Bassia latifolia* bud and *Cajanus cajan* seed decreased the sperm count and motility in mature male mice and this may be due to low concentration of fructose levels in the reproductive organs. The reduction in the weight of testis and accessory reproductive organs and the activities of Glucose-6-Phosphate Dehydrogenase (G-6-PDH) and Δ^5–3β–Hydroxysteroid Dehydrogenase (Δ^5–3β–HSD) inhibits the androgen biosynthesis in testis. The inhibition of steroidal biosynthesis was responsible for reduction of sperm count and motility. Therefore it may be concluded that the methanol extract of *Bassia latifolia* bud and *Cajanus cajan* seed produces antifertility effect on mature male mice.

Keywords: *Bassia latifolia*; *Cajanus cajan*; Δ^5-3β-HSD; G-6-PDH; Sperm count; Sperm motility.

INTRODUCTION

Male antifertility agents are those, which interfere with spermatogenesis or spermatozoa viability. In male mouse the formation and motility of spermatozoa totally depends on the biosynthesis of androgens. Any male antifertility agent affects the count & motility of spermatozoa directly by inhibition of steroidogenesis. *Bassia latifolia* [1] ((Family: Sapotaceae) commonly known as Madhuka, Indian Butter Tree, Mahua etc. It grows well in hot and dry, moist climate of central, western and eastern India. Fresh corollas of *Bassia latifolia* are used as contraceptive. Its seeds are used as abortifacient. Fresh root of it is used as an abortifacient agent. *Cajanus cajan* [2, 3] (Family: Fabaceae) is commonly known as Arhar. It is cultivated in West Bengal and all over India. The plant is used in the treatment of cough, fever, inflammation, pain, ulcer, wound, diabetes etc. and the seed of *Cajanus cajan* is a traditional tribal medicine for birth control. Methanol extract of *Bassia latifolia* bud and *Cajanus cajan* seed reduced sperm motility and density[4].

MATERIALS AND METHODS

Preparation of extract:

The bud of *Bassia latifolia* and seed of *Cajanas cajan* were collected from West Bengal and were authenticated by the division of Pharmacognosy, Department of Pharmaceutical Technology, Jadavpur University, Kolkata. Shade-dried, powdered, sieved in 40 x mesh plant material was soxhelet extracted first with petroleum ether and then with methanol. The methanol extract was evaporated to dryness. The trace amount of methanol which might be present within the solid mass of methanol extract was removed by vacuum pressure. For pharmacological testing, methanol extract (ME) of *Bassia latifolia* bud and

Cajanas cajan seed were dissolved in propylene glycol (PG). The yield of methanol extracts were 7.1% and 12.5% for *Bassia latifolia* and *Cajanas cajan* respectively on dry weight basis.

Animal experiments:

Adult male albino mice (18-22gm.body weight) of Swiss strain were acclimatized to normal laboratory conditions in the laboratory (25-30^{0}C, 75-80 % relative humidity, 12 hr light/dark cycle) for one week and given pellet diet (Hindustan lever limited, India) and water *ad libitum*. The experiment was performed under the guidance of The Institutional Ethical Committee, Jadavpur University, Kolkata. The LD_{50} [5] values of methanol extract of *Bassia laifolia* bud and *Cajanas cajan* seed are 451.88 mg/kg and 530.88 mg/kg body weight respectively .

The mice were then divided into 8 groups, each group containing 6 mice and given the following treatment:

Group-I: Normal saline as control (5 ml. /kg body weight, 0.9% NaCl, w/v, i.p.)

Group-II: Propylene glycol as vehicle control (PG, 5 ml. /kg body weight, i.p.)

Group-III: Methanol extract of *Bassia latifolia* dissolved in Propylene glycol (55mg/ kg.,b.w.,i.p)

Group-IV: Methanol extract of *Bassia latifolia* dissolved in Propylene glycol (75mg/ kg.,b.w.,i.p)

Group-V: Methanol extract of *Bassia latifolia* dissolved in Propylene glycol(110 mg/ kg.,b.w.,i.p)

Group-VI: Methanol extract of *Cajanus cajan* dissolved in Propylene glycol (65 mg/kg., b.w.,i.p)

Group-VII: Methanol extract of *Cajanus cajan* dissolved in Propylene glycol(90 mg/kg, b.w.,i.p)

Group-VIII: Methanol extract of *Cajanus cajan* dissolved in Propylene glycol

(130mg/kg.,b.w.,i.p)

Low, medium and high dose of the extracts were approximately 1/8th ,1/6th and 1/4th of the LD_{50} value. Normal saline (0.9 % w/v), propylene glycol (5 ml./ kg, b.w.) and methanol extract (low, medium and high dose) were given intraperitoneally in alternate days for 14 days for all the groups.

The mice were weighed before & after the commencement of the experiment. All the animals were sacrificed 24 hours after the last injection and 18 hours of fasting. Testis, cauda epididymis and adrenal glands were immediately dissected out, trimmed off adherent fatty materials, weighed and kept on ice for biochemical estimation.

Biochemical estimations

Sperm from mouse cauda epididymis were released in Berhringh Whitter Whittingham (BWW) media. Assesment of sperm vitality was measured by eosin y and spermatozoa was counted by hemocytometer [6]. Fructose content in seminal plasma was measured by standard procedure[6].

The content of Cholesterol [7], ascorbic acid [8], Glucose-6-Phosphate Dehydrogenase [9], Δ^5 –3β– Hydroxysteroid Dehydrogenase [10] and Protein [11] of the testis were determined biochemically according to the standard procedures.

Statistical analysis

Statistical analysis was done by Student's-t-test [12].

RESULTS

Results are summarized graphically in Figure1-8. During the period of treatment, body weight of extract treated mice was similar to that of control animals. The weights of testis, epididymis had been reduced whereas that of adrenal glands increased significantly in case of methanol extract of *Bassia*

latifolia bud and *Cajanus cajan* seeds treated animals in comparison to saline and vehicle treated animals (figure 1-4). The sperm count and percentage of motile sperm was decreased markedly in case of methanol extract of *Bassia latifolia* bud and *Cajanus cajan* seeds treated animals in all dose level of treatment (figure 5 & 7). Protein content of epididymis and fructose content of seminal vesicles were significantly reduced in both the extract treated mice (figure 6 & 8).

Both the extract significantly elevated the cholesterol and ascorbic acid content in the testis in a dose dependent manner (Figure 5 and 7). The activities of two key steroidogenic enzymes, Glucose-6-Phosphate Dehydrogenase (G-6-PDH) and Δ^5 –3β–Hydroxysteroid Dehydrogenase (Δ^5 –3β–HSD) were significantly inhibited in extract treated mice (Figure 6 and 8).

DISCUSSIONS

The reduced sperm motility and sperm density may be due to low concentration of fructose levels in seminal vesicles. Fructose is the source of sperm motility [13]. The principal cells of epididymis synthesize different proteins, which have important role to play in the maturation of spermatozoa. Alteration in secretion and function of these proteins caused incomplete maturation of spermatozoa with a decline in sperm motility[14]. Protein content of epididymis was decreased in methanol extract of *Bassia latifolia* bud and *Cajanus cajan* seed treated animals and this may also decrease the sperm motility and sperm density.

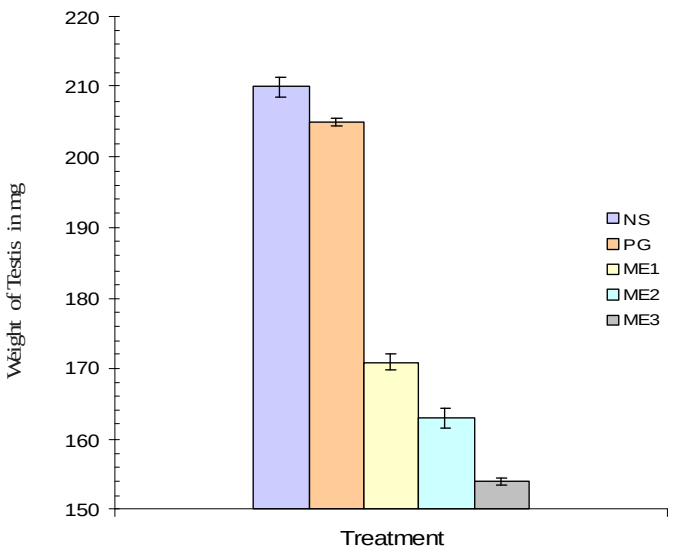
Role of cholesterol as precursor molecule in the synthesis of steroid hormones is well established[15]. Cholesterol content of testis in methanol extract *Bassia latifolia* bud and *Cajanus cajan* seed treated mice is significantly increased. This high accumulation of cholesterol may suggest the non utilization of lipid towards testosterone biosynthesis[16].

Ascorbic acid is easily diffusible water soluble compound. It is found abundantly in testis[17], where it plays an important role in testicular steroidogenesis[18]. Methanol extracts of *Bassia latifolia* bud and *Cajanus cajan* seed caused elevation of testicular cholesterol along with impairment of spermatogenesis by significant increase in ascorbic acid content.

The steroidogenesis in testis is under the physiological control of two dehydrogenase namely Glucose-6-Phosphate Dehydrogenase (G-6-PDH) and Δ^5 –3β–Hydroxysteroid Dehydrogenase (Δ^5 –3β–HSD) [19-22] . Reduced activities of these two steroidogenic enzymes in methanol extract of *Bassia latifolia* bud and *Cajanus cajan* seed treated mice indicated inhibition of steroidogenesis.

From the above study it may be concluded that methanol extract of *Bassia latifolia* bud and *Cajanus cajan* seed exhibited inhibition of steroidogenesis in male mice as a result inhibited the sperm motility and density. Hence both the plant extracts can be used as herbal drugs for male fertility control.

Figure 1: Effect of methanol extract of *Bassia latifolia* bud on the weight of testis of mature male mice



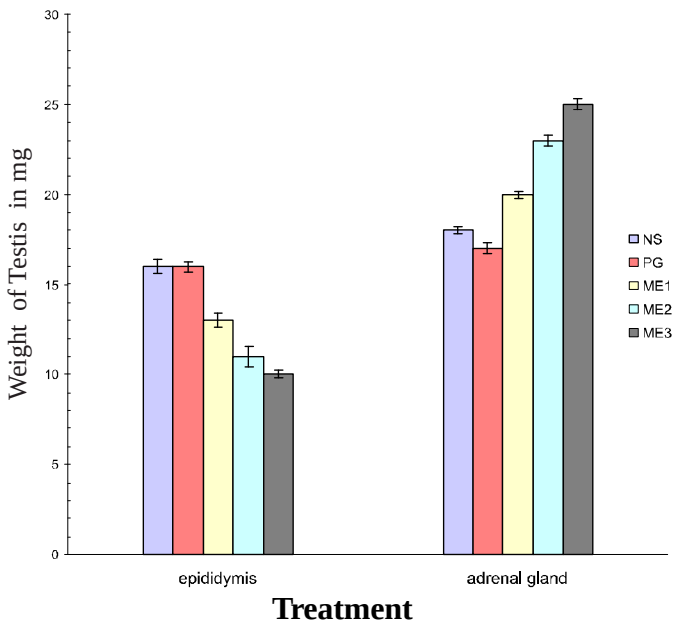
NS: Normal saline (5 ml/ kg,b.w., i.p.) PG: Propylene glycol (5 ml/kg,b.w., i,p.)

ME1: Methanol extract of *Bassia latifolia* bud (55mg/kg, b.w., i.p)

ME2: Methanol extract of *Bassia latifolia* bud (75 mg/kg, b.w., i.p)

ME3: Methanol extract of *Bassia latifolia* bud (110 mg/kg, b.w., i.p)

Figure 2: Effect of methanol extract of *Bassia latifolia* bud on weight of epididymis and adrenal glands in mature male mice



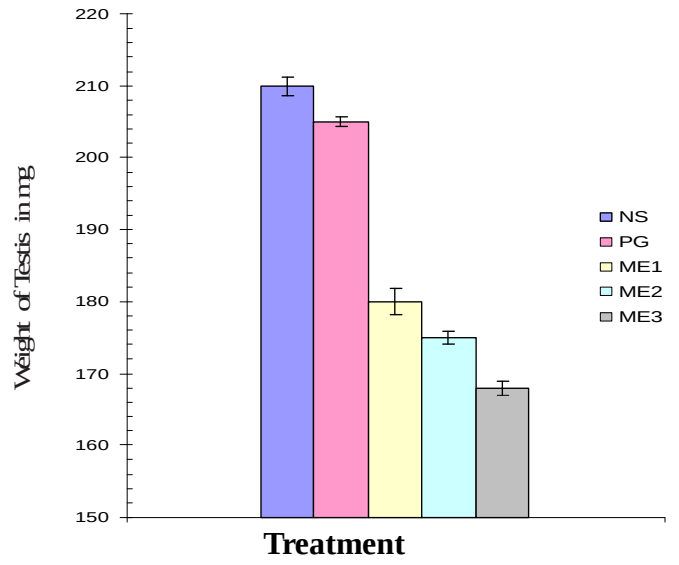
NS: Normal saline (5 ml/ kg,b.w., i.p.) PG: Propylene glycol (5 ml/kg,b.w., i,p.)

ME1: Methanol extract of *Bassia latifolia* bud (55mg/kg, b.w., i.p)

ME2: Methanol extract of *Bassia latifolia* bud (75 mg/kg, b.w., i.p)

ME3: Methanol extract of *Bassia latifolia* bud (110 mg/kg, b.w., i.p)

Figure 3: Effect of methanol extract of *Cajanus cajan* seed on the weight of testis of mature male mice



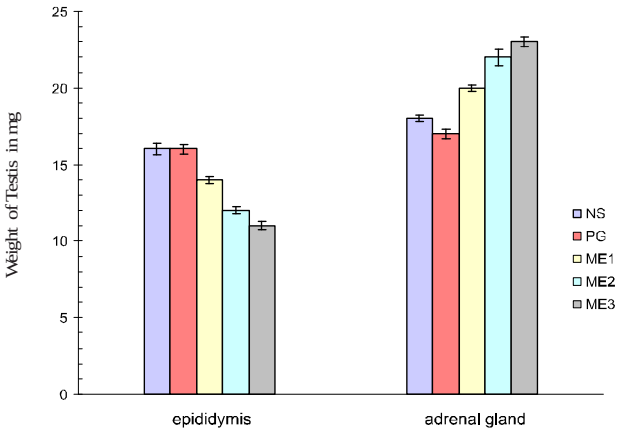
NS: Normal saline (5 ml/ kg,b.w., i.p.) PG: Propylene glycol (5 ml/kg,b.w.,i,p.)

ME1: Methanol extract of *Cajanus cajan* seed (65mg/kg,b.w., i.p)

ME2: Methanol extract of *Cajanus cajan* seed (90 mg/kg,b.w., i.p)

ME3: Methanol extract of *Cajanus cajan* seed (130 mg/kg,b.w., i.p)

FIGURE 4: Effect of methanol extract of *Cajanus cajan* seed on weight of epididymis and adrenal glands in mature male mice



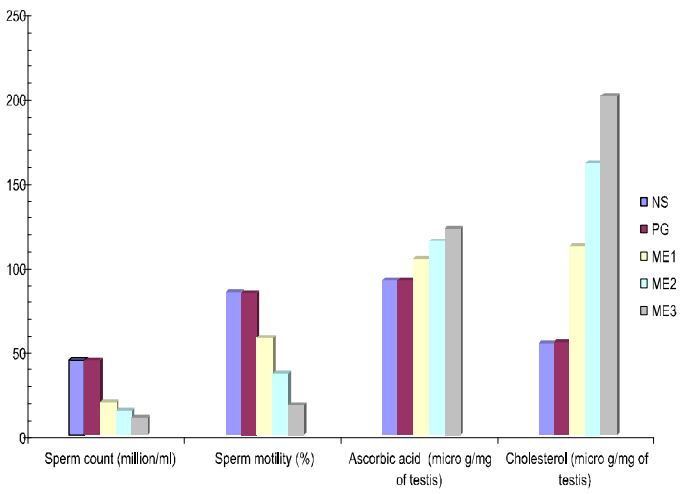
NS: Normal saline (5 ml/ kg,b.w., i.p.) PG: Propylene glycol (5 ml/kg,b.w.,i,p.)

ME1: Methanol extract of *Cajanus cajan* seed (65mg/kg,b.w., i.p)

ME2: Methanol extract of *Cajanus cajan* seed (90 mg/kg,b.w., i.p)

ME3: Methanol extract of *Cajanus cajan* seed (130 mg/kg,b.w., i.p)

Figure 5: Effect of methanol extract of *Bassia latifolia* bud on sperm count, sperm motility, content of ascorbic acid and cholesterol in mature male mice



Treatment

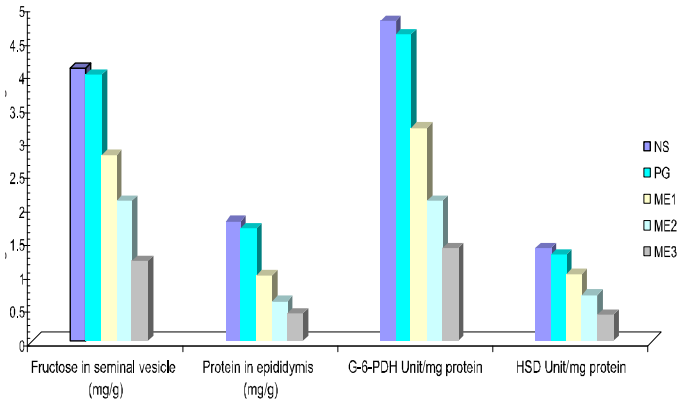
NS: Normal saline (5 ml/ kg,b.w., i.p.) PG: Propylene glycol (5 ml/kg,b.w., i,p.)

ME1: Methanol extract of *Bassia latifolia* bud (55mg/kg, b.w., i.p)

ME2: Methanol extract of *Bassia latifolia* bud (75 mg/kg, b.w., i.p)

ME3: Methanol extract of *Bassia latifolia* bud (110 mg/kg, b.w., i.p)

Figure 6: Effect of methanol extract of *Bassia latifolia* bud on fructose content in seminal vesicles, protein content in epididymis and the activities of g-6-pdh and Δ^5 –3β–Hsd in mature male mice



Treatment

NS: Normal saline (5 ml/ kg,b.w., i.p.) PG: Propylene glycol (5 ml/kg,b.w., i,p.)

ME1: Methanol extract of *Bassia latifolia* bud (55mg/kg, b.w., i.p)

ME2: Methanol extract of *Bassia latifolia* bud (75 mg/kg, b.w., i.p)

ME3: Methanol extract of *Bassia latifolia* bud (110 mg/kg, b.w., i.p)

G-6-PDH - Glucose-6-Phosphate Dehydrogenase,

HSD - Δ^5 –3β–Hydroxysteroid Dehydrogenase

Figure 7: Effect of methanol extract of *Cajanus cajan* seed on sperm count, sperm motility, content of ascorbic acid and cholesterol in mature male mice

Treatment

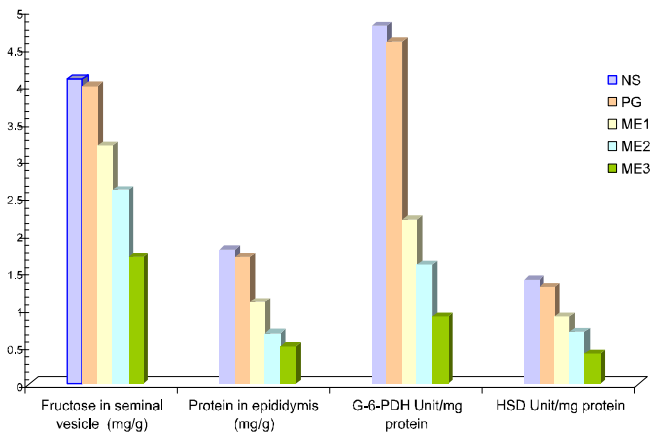
NS: Normal saline (5 ml/ kg,b.w., i.p.) PG: Propylene glycol (5 ml/kg,b.w.,i,p.)

ME1: Methanol extract of *Cajanus cajan* seed (65mg/kg,b.w., i.p)

ME2: Methanol extract of *Cajanus cajan* seed (90 mg/kg,b.w., i.p)

ME3: Methanol extract of *Cajanus cajan* seed (130 mg/kg,b.w., i.p)

Figure 8: Effect of methanol extract of *Cajanus cajan* seed on fructose content in seminal vesicles, protein content in epididymis and the activities of g-6-pdh and Δ^5 –3β–Hsd in mature male mice



Treatment

NS: Normal saline (5 ml/ kg,b.w., i.p.) PG: Propylene glycol (5 ml/kg,b.w.,i,p.)

ME1: Methanol extract of *Cajanus cajan* seed (65mg/kg,b.w., i.p)

ME2: Methanol extract of *Cajanus cajan* seed (90 mg/kg,b.w., i.p)

ME3: Methanol extract of *Cajanus cajan* seed (130 mg/kg,b.w., i.p)

G-6-PDH - Glucose-6-Phosphate Dehydrogenase, Δ^5 –3β–HSD - Δ^5 –3β–Hydroxysteroid Dehydrogenase

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